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Profile Phenolic Compounds in Spanish-Style and Traditional Brine Black Olives ('Gemlik' Cv.) Provided from Different Regions of Türkiye

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Abstract: The aim of this study was to evaluate the effect of growing regions and processing methods on the composition and the quantity of phenolic compounds in 'Gemlik' variety table olives. Two different processing methods, Spanish-style and traditional brine (naturally processed) olives, were used in the processing of 'Gemlik' table olives. According to the data obtained in this study, the highest concentrations of phenolic compounds were observed for 3-hydroxytyrosol (4.58–168.21 mg/kg), followed by 4-hydroxyphenyl (0.76–97.58 mg/kg), luteolin 7-glucoside (0.32–58.64 mg/kg), tyrosol (1.57–47.24 mg/kg), and luteolin (0.17–53.56 mg/kg) in overall samples. The highest quantity of phenolic compounds was determined in raw olives, and the lowest phenolic compound content was determined in Spanish-style processed olives. Table olives which are produced by the natural process were observed to contain higher concentrations of phenolic compounds compared with the olives, which are produced in the Spanish style. In this sense, statistical results showed that region and processing methods have significant impacts on the phenolic compounds of table olives.

Keywords: Cv. 'Gemlik'; table olives; phenolic compounds; Spanish style; traditional processing

Citation: Demir, C.; Yildiz, E.; Gurbuz, O. Profile Phenolic Compounds in Spanish-Style and Traditional Brine Black Olives ('Gemlik' Cv.) Provided from Different Regions of Türkiye. *Processes* **2023**, *11*, 2412. https://doi.org/10.3390/pr11082412

Academic Editors: Sebastián Sánchez Villasclaras and Juan Francisco García Martín

Received: 21 June 2023
Revised: 1 August 2023
Accepted: 5 August 2023
Published: 10 August 2023

1. Introduction

Olea europaea L. (olive tree) is one of the oldest plants cultivated in the Mediterranean Basin [1]. The olive is known as the most important fruit among other fruits in Mediterranean countries such as Italy, Spain, and Greece (6). Table olive, a traditional Mediterranean food, is of great importance both economically and socially in Türkiye [2]. According to the International Olive Council (IOC, 2017), worldwide production of table olives is around 3020.500 tons [3]. Regarding the averages for the 2012/13–2015/16 seasons, Türkiye was considered the third largest producer of table olives, with more than 15.6% of the world's production (IOC, 2017) [2].

Olive fruit is known for being a rich source of phenolic compounds [4]. The phenolic compounds of table olives are of great importance in terms of nutritional, color, and flavor properties [5] and have strong antioxidant effects and improve the nutritional and organoleptic qualities of olives [6].

Phenolic compounds, which are bioactive components, have gained importance in recent years mainly due to their antioxidant, anti-inflammatory, and antitumor properties [6–9]. They also help preventing certain diseases, such as Alzheimer's and cancer [10]. The phenolic compounds are formed from the metabolism of primary products, including carbohydrates, fats, and amino acids [9]. Factors such as the

degree of ripening, growing conditions, fruit size, and processing method affect the quantity of phenolic compounds in olives [11]. Oleuropein, 3-hydroxytyrosol, and rutin are the most abundant phenolic compounds in olive fruit [7]. Oleuropein has a strong antioxidant activity, it is the main compound that gives characteristic bitterness to olives, and its content in fruit flesh is 2–4% [9]. While the oleuropein content of olives is quite high in the early fruiting period, the amount in some cultivars may decrease during the black ripening period [12]. Additionally, Salis et al. [9] detected 3-hydroxytyrosol, tyrosol, verbascoside, rutin, oleuropein, and luteolin in Spanish-style and Greek-style processed olive fruits of cv. 'Kalamata'. Irmak and Irmak [13] determined hydroxytyrosol, tyrosol, apigenin, and luteolin in the raw and processed olives of varieties 'Ayvalık' and 'Domat'. Hydroxytyrosol, rutin, oleuropein, tyrosol, luteolin-7-glucoside, apigenin, luteolin, verbascoside, and apigenin are determined phenolic compounds in olive fruit [9,13,14]. Also, Uylaser [15] found the highest contents of 3-hydroxytyrosol and vanillic acid in 'Gemlik' variety raw olives.

It has been reported that olive processing methods affect the taste of olives and can significantly change the health properties of olive fruit [9]. The processing method of the final products also has a significant effect on the phenolic compound content. It has been reported that olive processing methods affect the taste of olives and can significantly change the health properties of olive fruit [9]. Laruen et al. [14] determined that oleuropein, 3-hydroxytyrosol, verbascoside, tyrosol, luteolin 7-glucoside, and rutin were higher in processed olives.

Since olives contain a high level of bitterness caused by oleuropein after harvest, they must be processed. Three different commercial olive processing methods have international importance: (1) The Spanish style for green olives, (2) The Greek style for natural black olives, and (3) The Californian style for black olives [6,11,16]. Phenolic compounds are distinct for *Olea europaea* and have antioxidative, antimicrobial, antiviral, anti-inflammatory, and anticarcinogenic effects. Table olive processing methods cause severe losses in phenolic compounds, and as a result, the positive effects of table olives on health may change [16]. In addition, the most widely used table olive production processes in Türkiye are those that aim at the production of natural black olives (unprocessed, in brine, and dry-salted), Californian style for black olives, and Spanish style for green olives [12].

Ninety-three olive varieties are known to be present in Türkiye. The percentage of the distributions of olive varieties in all olive-producing regions of Türkiye in 2014 and 2015 was calculated, and it was determined that 48.71% of the total olive tree consists of cv. 'Gemlik' [17]. The 'Gemlik' variety is thin-skinned and adhered to the flesh and has a high flesh/stone ratio, small seeds, and aromatic features. In addition, higher quantities of aromatic compounds result in higher-quality table olive products [18,19].

'Gemlik' olive is the dominant variety of the Marmara region. This variety shows remarkable agronomic characteristics (such as not showing severe alternation, high adaptability, early yield, being a tree that is partially resistant to cold and diseases, and easy reproduction from steel) and dual-purpose technological advantages (such as black table and olive oil varieties). It is a variety that has spread rapidly in all olive-growing regions of Türkiye (including the Aegean region, East-West Mediterranean region, and even the Southeastern Anatolia region) except the Marmara (Bursa) region, which has been the place of origin for the last 25–30 years.

Due to its superior properties, the 'Gemlik' olive was protected by the Turkish Patent Institute on 23 September 2003 with the 'Geographical Indication Registration Certificate' and 'Name of Origin'. The geographical boundaries for 'Gemlik' olives are stated in the registration document as Gemlik, Iznik, and Orhangazi districts of Bursa, which shows that this variety is a local product [20].

'Gemlik' olives are obtained from Gemlik (Gem), Iznik (Izn), Mudanya (Mud), Orhangazi (Orh), and Erdek (Erd) regions in Bursa, where this variety is the most widely grown, and from the Akhisar (Akh) region in Manisa, where cultivation has grown rapidly in recent years. According to the Turkish Food Codex Table Olive Communiqué,

processing methods are divided into two as natural and chemical methods. In the table olive sector, some producers deceive consumers by selling chemically processed olives as naturally processed. In this study, in addition to regional differences, differences between processing methods were also determined. According to the phenolic compounds of the processed olives, it will be possible to identify which process was used. As a result, the deception of the consumer can be prevented.

2. Materials and Methods

2.1. Procedure and Collection Date of the Samples

'Gemlik'-type raw olive fruits were obtained from Marmarabirlik (S.S. Marmara Olive Agricultural Sales Cooperatives Union, Bursa, Türkiye) grown in 6 different regions in Türkiye. Gemlik (Gem), Iznik (Izn), Mudanya (Mud), Orhangazi (Orh), Erdek (Erd) and Akhisar (Akh), in the harvest period of 2019–2020 in November for this study. A total of 220 number/kg olives were hand-harvested at 5 maturity index from these regions, and 3 samples were taken from each region. The maturity index (MI) was determined according to the color changes in peel and pulp with the procedures of Vinha et al. (2005) [21] and varied between 0 and 7 in eight categories. Sampling codes are given in Table 1.

Table 1. The regions and olive processing methods of the samples.

Regions	Codes of Regions	Olive Processing Methods Codes		
		Raw	Natural Processing (NP)	Spanish Style (SS)
Gemlik	Gem	GemRaw	GemNP	GemSS
Iznik	Izn	IznRaw	IznNP	IznSS
Mudanya	Mud	MudRaw	MudNP	MudSS
Orhangazi	Orh	OrhRaw	OrhNP	OrhSS
Erdek	Erd	ErdRaw	ErdNP	ErdSS
Akhisar	Akh	AkhRaw	AkhNP	AkhSS

2.2. Chemicals

HPLC grade water, methanol, and orthophosphoric acid solutions, phenolic compound standards 3-hydroxytyrosol (3Hyt, CAS No: 10597-60-1), protocatechuic acid (Prt, CAS No: 99-50-3), keracyanin (Ker, CAS No: 18719-76-1), coumarin (Kum, CAS No: 41044-12-6), catechin (Kat, CAS No: 154-23-4), tyrosol (Tyr, CAS No: 501-94-0), 4-hydroxyphenyl (4Hdf, CAS No: 67914-60-7), epicatechin (Ep, CAS No: 490-46-0), syringic acid (Syr, CAS No: 530-57-4), oleuropein (Ole, CAS No: 32619-42-4), trans-cinnamic acid (Trs, CAS No: 140-10-3), luteolin 7-glucoside (L7g, CAS No: 5373-11-5), luteolin 4-glucoside (L4g, CAS No: 6920-38-3), luteolin (Lt, CAS No: 491-70-3), kaempherol (Kam, CAS No: 520-18-3), and apigenin (Apg, CAS No: 520-36-5) analytical standards were obtained from Merck (Darmstadt, Germany).

2.3. Table Olive Processing

Two types of olive processing are preferred in black table olive production. The first one is the natural fermentation known as traditional brine olive production, also traditionally called Gemlik style in Türkiye. For production, black table olives (17 kg) were washed with tap water to remove dust, placed in 40 L plastic vessels, and processed by natural processing (NP). Then, olive samples were brined (17% NaCl) and fermented for two months at room temperature (18–25 °C) in the dark. The second one is Spanish-style (SS) table olive production. Firstly, black table olives (17 kg) were washed with tap water to remove dust, placed in 40 L plastic vessels, and NaOH solution (1.5%) was added. The olives were kept in NaOH solution overnight, and the NaOH was allowed to penetrate 2/3 of the olive flesh. After the NaOH, olives were kept in water for 18 h, washed, brined (17% NaCl and 1% lactic acid solution), and fermented for two months at room temperature (18–25 °C) in the dark. After the fermentation, the olive samples were analyzed.

2.4. Phenolic Compound Characterization

Extraction

Phenolic compound analysis was performed using the HPLC-DAD technique with the modified method of Ramirez et al. [22]. For the extraction, firstly, olive pits were removed and homogenized with a blender. A total of 10 g of olive pulp was weighed into 250 mL flasks. Next, 50 mL of petroleum ether was added and shaken at 180 rpm for 2 h. The olive pulp and petroleum ether mixture were filtered through filter paper (pore size 22 µm). Afterward, the olive pulps remaining from the filtrate were taken into 50 mL falcon tubes. Petroleum ether was kept for a while with volatile nitrogen and evaporated. Then, 40 mL of methanol/water mixture prepared at 80:20 (v/v) ratio acidified with 0.001% orthophosphoric acid and kept in the refrigerator overnight was added to the olive pulp. After that, the mixture was kept in an ultrasonic bath for 30 min; then, the upper phase was separated by centrifugation at 3500 rpm (15 min). A total of 20 mL of the same mixture was added to the remaining olive pulp, kept in an ultrasonic bath for 30 min, and centrifuged at 3500 rpm (15 min). After the second phase was separated, 2 mL of each of the 1st and 2nd phases was taken and mixed.

The final mixture was filtered into Eppendorf tubes by 0.22 µm membrane filters, transferred to HPLC vials (Agilent, screw tap V9201911A), and injected into HPLC. The analysis of olive samples was carried out using HPLC equipped with ChemStation software, version A.02.14 (2016 Agilent Technologies, California, USA), a 250 × 4.6 mm C18 column, and a column oven (G1316A) using an Agilent 1260 Infinity II HPLC device and DAD. HPLC conditions were established by modifying the method stated by Ramirez et al. [22]. Solutions of methanol (C) and water (pH adjusted to 2.70 with orthophosphoric acid solution) (B) were used as mobile phase. The flow rate was 1 mL/min, the injection volume was 20 µL, and detection wavelengths were 280 nm and 330 nm. While defining the peaks of phenolic compounds, the wavelength at which the phenolic compounds give the maximum absorbance value is taken as a basis. The calibration curves of the standards were obtained by the intermediate stock solutions at the mg/kg levels. The evaluated phenolic compound codes and branch lengths were as follows: 3-hydroxytyrosol (3Hyt), protocatechuic acid (Prt), keracyanin (Ker), coumarin (Kum), catechin (Kat), tyrosol (Tyr), 4-hydroxyphenyl (4Hdf), epicatechin (Ep), syringic acid (Syr), oleuropein (Ole), and trans-cinnamic acid (Trs) were determined at 280 nm; luteolin 7-glucoside (L7g), luteolin 4-glucoside (L4g), luteolin (Lt), kaempherol (Kam), and apigenin (Apg) determined at 330 nm.

The gradient elution program was planned as follows: to 90% (B) and 10% (C) for 0 min, 95% (B) and 5% (C) in 10 min, 70% (B) and 30% (C) in 20 min, 60% (B) and 40% (C) in 40 min, 60% (B) and 40% (C) in 45 min, 50% (B) and 50% (C) in 45.10 min, 40% (B) and 60% (C) in 50 min, 30% (A) and 70% (B) in 55 min, 0% (B) and 100% (C) in 60 min, 0% (B) and 100% (C) in 68 min, 90% (B) and 10% (C) in 73 min. The identification of phenolic compounds was enabled by the analysis of retention times of standards. The linear calibration curves were obtained from the standards (R^2 = 0.98).

3. Statistical Analysis

Analysis of variance was carried out using JMP 7.0 to determine the significant differences at a level of confidence of $p < 0.05$. The degree of differences in the means was compared using the Student's t-test. Among the chemometrics methods in the classification of olives, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were utilized, and the obtained data were evaluated with Minitab (Minitab 16 Statistical Software, Minitab, Inc., New York, NY, USA).

4. Results and Discussion

HPLC Identification and Quantification of Phenolic Compounds

Various factors are known to affect the presence and content of the phenolic compounds of table olives. These are variety, agronomic process, ripening level, and processing steps of the table olive processing methods. Also, there are some significant differences in

the phenolic composition of processed table olives, identified as chemical and enzymatic changes in certain phenolics compounds during several processing methods [12].

In this study, phenolic compounds were evaluated in raw, naturally processed (traditional brine olives), and Spanish-style olives in terms of 3Hyt, Ker, Kum, Kat, Tyr, 4Hdf, Trs, L7g, L4g, Lt, Kam, and Apg.

A chromatogram of fourteen phenolic compounds of the standard mixture solution as well as the internal standard is shown in Figure 1. As presented in the chromatogram, all the studied phenolic compounds had responses at 280 nm and 330 nm, where they were efficaciously separated. The peak chromatograms of raw, traditional brine, and Spanish-style olive extracts are given in Figure 2a–f, respectively. The comparison of the retention times of those compounds with the retention times of authentic standards led to their identification.

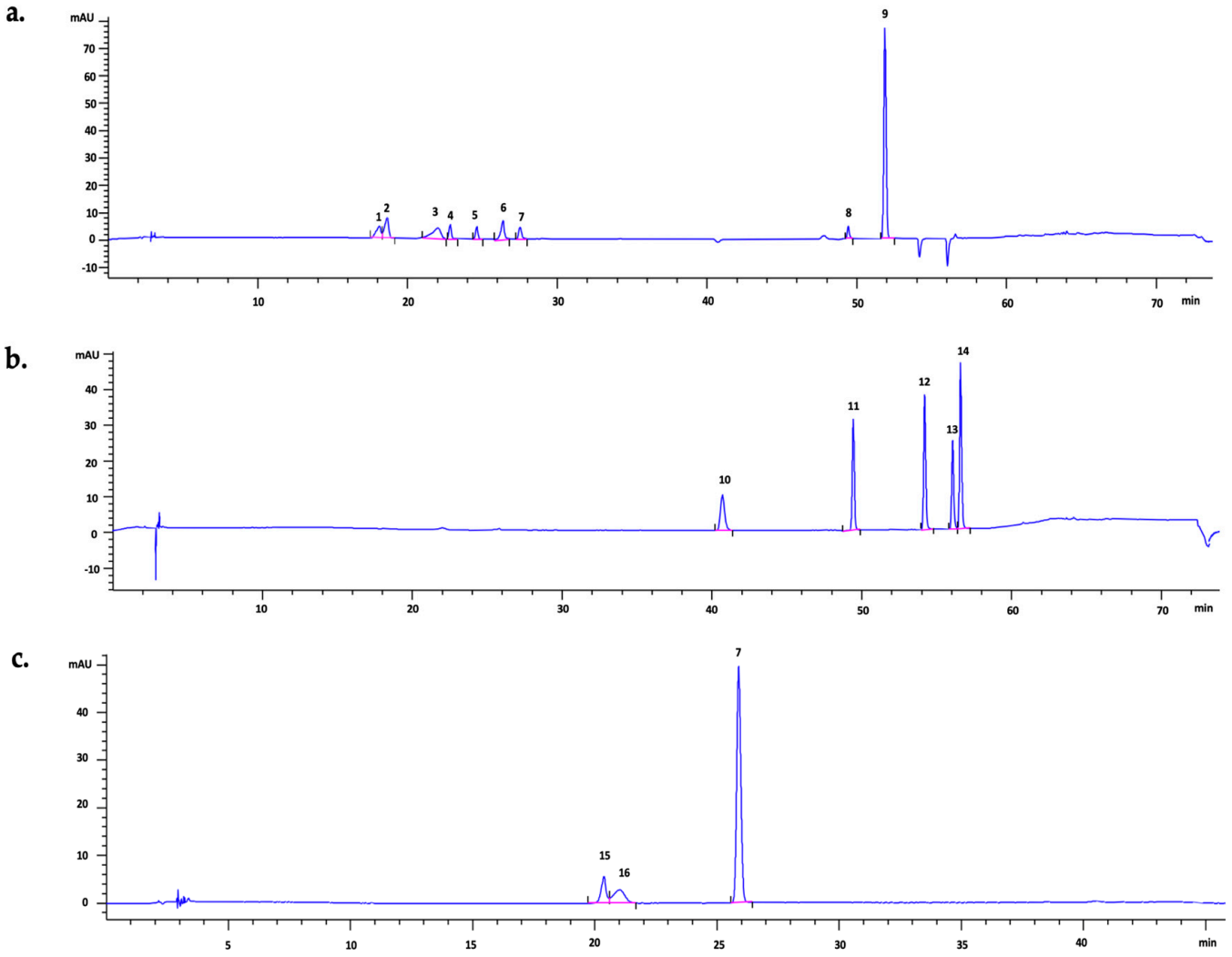

Figure 1. HPLC-UV chromatograms of the standard mixture solutions: (**a**,**b**) 1: 3-hydroxytyrosol; 2: protocatechuic acid; 3: coumarin; 4: tyrosol; 5: 4-hydroxyphenyl; 6: epicatechin; 7: syringic acid; 8: oleuropein; 9: trans-cinnamic acid; 10: luteolin 7-glucoside; 11: luteolin 4-glucoside; 12: luteolin; 13: kaempherol; 14: apigenin; (**c**) 15: catechin; 16: keracyanin.

The high and low concentration calibration curves determined for each phenolic compounds showed a linear response with correlation coefficients of 0.989–0.999. The recovery values of the phenolic compounds ranged from 82.16% to 93.53% with RSD varying from 3.44% to 8.02%.

The quantity of phenolic compounds of raw and processed olives (traditional brine-natural processed olive, NP; Spanish-style processed, SS) obtained from six different regions (Gem, Izn, Mud, Orh, Erd, and Akh) is given in Table 2.

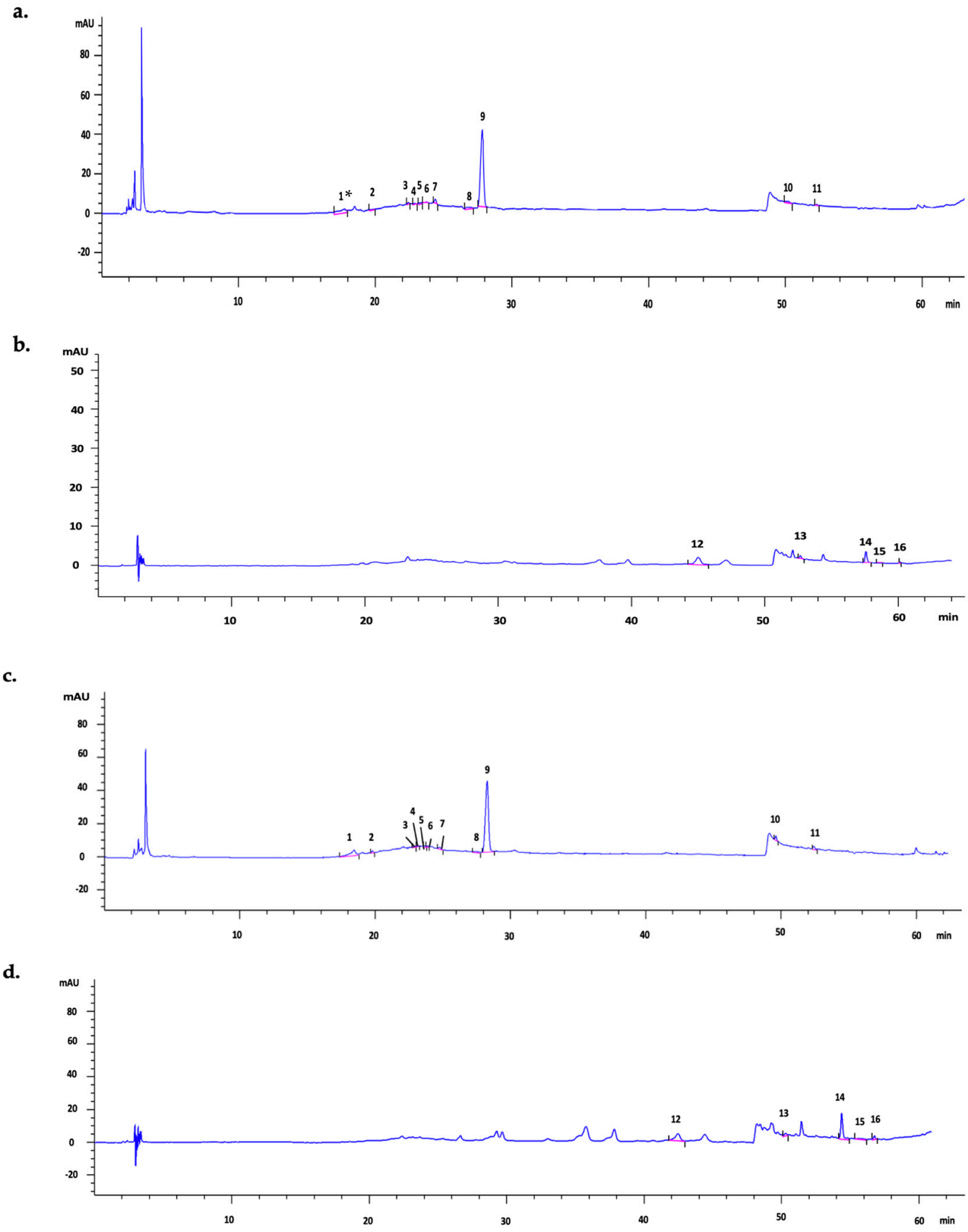

Figure 2. *Cont.*

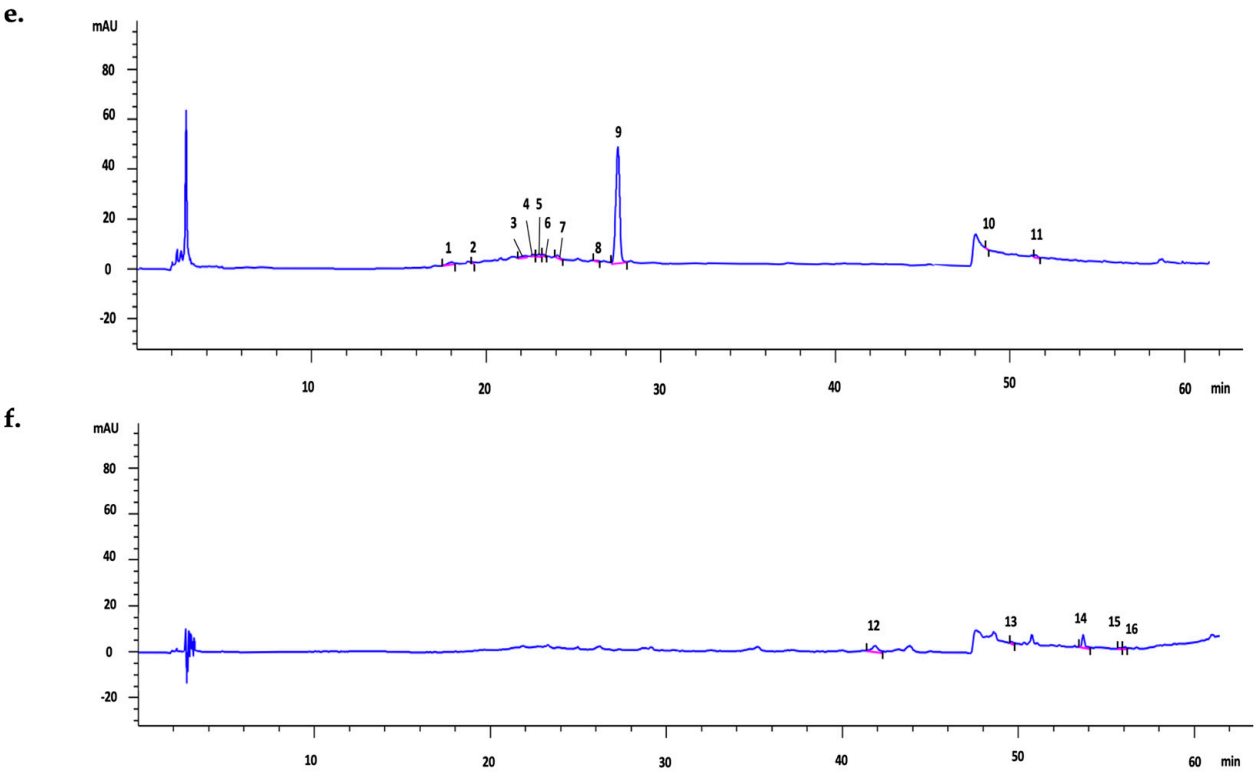

Figure 2. HPLC chromatograms of samples belongs to Gemlik region: (**a**,**b**) Typical HPLC chromatograms of raw olives from Gemlik region; (**c**,**d**) Typical HPLC chromatograms of traditional brine olives from Gemlik region; (**e**,**f**) Typical HPLC chromatograms of Spanish-style olives from Gemlik region. * 1: 3-hydroxytyrosol; 2: protocatechuic acid; 3: keracyanin; 4: coumarin; 5: catechin; 6: tyrosol; 7: 4-hydroxyphenyl; 8: epicatechin; 9: syringic acid; 10: oleuropein; 11: trans-cinnamic acid; 12: luteolin 7-glucoside; 13: luteolin 4-glucoside; 14: luteolin; 15: kaempherol; 16: apigenin.

3Hyt, Tyr, 4Hdf, Lt, and Apg contents were affected by the processing methods, while there was no statistically significant change for L7g and Kam by region ($p < 0.05$, Table 2). The results obtained from previous data confirmed that Hyt and Tyr were identified as the main phenolic compounds of table olives [4,19,23–25].

The main phenolic compound was determined as 3Hyt in raw olives; the content ranged from 104.11 mg/kg (Gem) to 168.21 mg/kg (Erd). These values are much lower than those reported by Uylaser [15] for Hyt ranging from 1.20 mg/kg to 891.80 mg/kg.

In terms of processing, the highest 3Hyt content was observed in SS (49.71 mg/kg); additionally, the content was lower than the results of Sahan et al. (264.56 mg/kg) [12]. The processing methods significantly affected the presence and the content of 3Hyt, which was decreased by the processing. The determined decrease in the SS olives was found to be higher than the NP olives. Contrary to our findings, Salis et al. [9] reported higher amounts of Hyt in processed olives (367.83 μg/g) and determined an important increase in the Hyt content by NP and SS olive processing.

Tyr is a phenolic alcohol, usually present in olives, in lower amounts than Hyt [12]. Try content ranged from 3.66 mg/kg to 8.12 mg/kg in raw olives and from 1.64 mg/kg to 47.24 mg/kg in NP olives. Ozkan et al. [20] reported that Tyr was determined between 33.31 mg/kg and 85.69 mg/kg in raw olive fruits. Accordingly, the amount of Tyr was lower than the findings of Ozkan et al. [20]. Also, higher amounts of Tyr were determined in previous studies [12,26]. These significant differences could be explained by the variety and the applied processes on the olives, especially the use of brine and lye. According to the results of our study, Tyr showed a significant decrease in the SS processing method and might be due to the oxidation of *o*-diphenol during NaOH debittering. Similarly, Salis et al. [9] determined a statistically significant decrease in Tyr.

Table 2. Composition of the phenolic compounds of 'Gemlik' table olives.

	Gemlik Region			Mudanya Region			Iznik Region		
Phenolic Compound (mg/kg)	**Raw**	**Natural Processing**	**Spanish-Style**	**Raw**	**Natural Processing**	**Spanish-Style**	**Raw**	**Natural Processing**	**Spanish-Style**
3Hyt *	104.11± 2.66 ^{bcd}**	64.54 ± 1.73 ^{def}	40.86± 1.61 ^{efg}	131.69 ± 1.72 ^{abc}	80.32± 1.42 ^{de}	42.91 ± 1.36 ^{efg}	145.58 ^{a} ± 2.75	49.71 ±3.95 ^{ef}	49.71± 1.59^{ef}
Ker	0.48 ± 0.01	0.29 ± 0.05	0.29 ± 0.05	1.46 ± 0.09	nd ***	1.28 ± 0.07	nd	nd	nd
Kum	1.44 ± 0.04	1.92 ± 0.13	2.13 ± 0.10	5.36 ± 0.55	1.60 ± 0.12	3.36 ± 0.12	2.63 ± 0.12	2.54 ± 0.35	0.23 ± 0.05
Kat	1.44 ± 0.04	1.87 ± 0.12	1.87 ± 0.11	5.36 ± 0.55	5.53 ± 0.35	3.66 ± 0.11	2.63 ± 0.12	4.51 ± 0.66	1.63 ± 0.12
Tyr	8.09 ± 1.28 ^{bc}	4.43 ± 0.60 ^{c}	4.68± 0.50 ^{c}	7.26 ± 1.25 ^{c}	3.14± 0.28 ^{c}	3.36 ± 0.10 ^{c}	6.87± 1.89 ^{c}	16.02 ± 1.21 ^{b}	15.89 ± 1.22 ^{b}
4Hdf	38.89 ± 2.02 ^{bc}	42.77 ± 1.78 ^{bc}	42.77 ± 1.28 ^{bc}	48.12 ± 1.37 ^{bc}	50.95 ± 1.25 ^{bc}	34.63 ± 1.16 ^{bc}	26.86 ± 2.12 ^{cd}	34.03± 1.35 ^{bc}	34.04 ± 1.41 ^{bc}
L7g	39.25 ± 2.75	21.46 ± 0.82	0.32 ± 0.05	34.82 ± 1.22	26.30 ± 1.13	14.18 ± 1.55	8.59 ± 1.54	6.80 ± 0.36	8.02 ± 0.93
L4g	3.56 ± 0.02	0.72 ± 0.02	0.09 ± 0.01	0.52 ± 0.01	0.17 ±0.01	nd	nd	0.96 ± 0.08	0.96 ± 0.09
Trs	0.59 ± 0.03	0.10 ± 0.01	0.32 ± 0.04	3.73 ± 0.20	0.54 ± 0.03	1.05 ± 0.06	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Lt	1.60 ± 0.12 ^{lj}	11.28 ± 0.59 ^{efgh}	4.50 ± 0.52 ^{hlj}	13.09± 1.14 ^{efg}	0.17± 0.01 ^{j}	5.63 ± 0.12 ^{ghlj}	21.60± 1.53 ^{cd}	0.96 ± 0.05 ^{lj}	5.62 ± 0.70 ^{ghlj}
Kam	1.60 ± 0.12	0.63 ± 0.02	0.15 ± 0.01	0.79 ± 0.02	0.48 ± 0.01	0.08 ± 0.01	0.32 ± 0.01	0.23 ± 0.08	0.23 ±0.01
Apg	0.23 ± 0.05^{b}	nd	nd	0.52± 0.08 ^{b}	0.57± 0.01 ^{b}	nd	0.88 ± 0.03 ^{b}	nd	nd
	Orhangazi Region			**Erdek Region**			**Akhisar Region**		
Phenolic Compound (mg/kg)	**Raw**	**Natural Processing**	**Spanish-Style**	**Raw**	**Natural Processing**	**Spanish-Style**	**Raw**	**Natural Processing**	**Spanish-Style**
3Hyt *	155.04 ± 3.42 ^{a}**	32.76 ± 1.43 ^{fg}	4.58 ± 0.12 ^{g}	168.21 ± 2.55 ^{a}	131.01 ± 2.73 ^{abc}	44.97 ± 2.08 ^{ef}	141.38 ± 2.13 ^{ab}	94.32 ± 3.03 ^{cd}	42.52 ± 2.05 ^{efg}
Ker	nd ***	0.20 ± 0.01	nd	nd	nd	nd	nd	0.15 ± 0.01	0.15 ± 0.03
Kum	0.89 ± 0.03	4.17 ± 0.08	1.90 ± 0.08	7.01 ± 0.70	5.90 ± 1.87	4.71 ± 0.17	10.64 ± 1.46	2.30 ± 0.44	1.55 ± 0.44
Kat	7.32 ± 1.21	1.86 ± 0.12	0.09 ± 0.01	5.51 ± 0.55	nd	nd	5.83 ± 0.85	nd	2.85 ± 0.39
Tyr	4.19 ± 0.65 ^{c}	47.24 ± 2.45 ^{a}	1.64 ± 0.04 ^{c}	3.66 ± 0.65 ^{c}	1.95 ± 3.05 ^{c}	1.57 ± 0.44 ^{c}	8.12 ± 1.29 ^{bc}	2.21 ± 0.85 ^{c}	4.50 ± 0.64 ^{c}
4Hdf	30.17 ± 1.84 ^{bc}	27.92 ± 1.84 ^{cd}	4.04 ± 0.07 ^{de}	40.40 ± 1.57 ^{bc}	37.17 ± 11.94 ^{bc}	0.76 ± 0.05 ^{e}	49.41 ± 2.78 ^{bc}	97.58 ± 2.36 ^{a}	54.29 ± 1.80 ^{b}
L7g	15.48 ± 0.57	4.17 ± 0.07	8.02 ± 0.17	19.65 ± 0.93	48.99 ± 3.42	11.26 ± 0.51	40.24 ± 1.11	58.64 ± 1.70	26.21 ± 1.63
L4g	2.39 ± 0.02	4.28 ± 0.09	nd	nd	1.36 ± 0.04	0.39 ± 0.03	nd	1.63 ± 0.29	0.18 ± 0.08
Trs	0.29 ± 0.01	7.37 ± 0.42	0.01 ± 0.00	0.83 ± 0.03	1.36 ± 0.05	3.70 ± 0.64	0.47 ± 0.09	0.66 ± 0.72	nd
Lt	18.92 ± 1.08 ^{cde}	9.09 ± 0.21 ^{fghl}	1.60 ± 0.11 ^{lj}	30.33 ± 1.05 ^{b}	0.60 ± 0.03 ^{j}	14.76 ± 1.74 ^{def}	53.56 ± 2.31 ^{a}	27.18 ± 1.12 ^{bc}	7.38 ± 0.25 ^{fghlj}
Kam	0.39 ± 0.01	nd	0.10 ± 0.01	0.56 ± 0.04	0.66 ± 0.05	0.10 ± 0.01	0.24 ± 0.01	0.73 ± 0.05	0.13 ± 0.01
Apg	0.55 ± 0.02 ^{b}	nd	nd	0.56 ± 0.01 ^{b}	0.42 ± 0.01 ^{b}	0.15 ± 0.03 ^{b}	5.12±0.25 ^{a}	0.46 ± 0.0 ^{b}	nd

* 3Hyt: 3-hydroxytyrosol; Ker: keracyanin; Kum: coumarin; Kat: catechin; Tyr: tyrosol; 4Hdf: 4-hydroxyphenyl; Ep: epicatechin; L7g: luteolin 7-glucoside; L4g: luteolin 4-glucoside; Trs: trans-cinnamic acid; Lt: luteolin; Kam: kaempherol; Apg: apigenin; ** Different letters in each column indicate differences between regions and processing methods ($p < 0.05$). The data are reported as the average of triplicate measurements (n = 3); *** nd: not detected.

4Hdf was the second most abundant phenolic compound in raw olives; contents ranged from 26.86 to 49.41 mg/kg for Izn and Aks, respectively. By the natural processing, the amount of 4Hdf was generally increased, and the highest content was detected in AkhNP (97.58 mg/kg). But SS processing decreased the content of 4Hdf, and the lowest content was detected in ErdSS (0.76 mg/kg).

Apg was detected in NP and raw olives. The highest Apg content was determined in AkhRaw olives (5.12 mg/kg). Content of Apg was decreased dramatically after processing and found under quantification limits in SS olives. Ghorbal et al. [27] reported the A7g content as 1.25–7.79 mg/kg in Gemlik-style processed olives. Our findings are in good agreement with these results.

Additionally, the Lt content varied between 0.17 and 53.56 mg/kg, and after processing, the content was generally decreased by processing. The Lt content in raw and NP olives were found to be higher than in SS-processed ones. Salis et al. [9] reported content of Lt between 92.40 µg/g ft and 118 µg/g in their study, which was higher than our results.

As shown in Table 2, L7g amounts varied between 0.32 and 58.64 mg/kg in olive samples. The lowest L7g content was determined in SS processed olives, while the highest content was obtained in raw and NP olive samples. After processing, the L7g amount was decreased significantly in NP and SS processed olives. In general, L7g amounts were found to be higher than the Lt content (except Orh and Erd). This determined difference may arise from the glucosidase activity of L7g during the processing. Also, our findings are in good agreement with previous studies [28].

Kam was detected in all olive samples except from the OrhNP. The Kam content in raw and NP olives was found to be higher than SS processed ones. Additionally, Kam content (0.08–1.60 mg/kg) was lower than that of Ozcan et al. [29], who reported 1.31 and 4.18 mg/100 g in olive fruit. Ker was only determined in olives obtained from the Gemlik region (both raw and processed). Also, Kum content was determined higher (0.23–7.01 mg/kg) than reported by Ozcan et al. [29] (0.06–0.89 mg/100 g). Protocatechuic acid (Prt) and epicatechin (Ep) were not detected in any of the samples.

For statistical evaluation, the phenolic compounds of raw and processed olives were classified with chemometrics methods (principal component analysis, PCA) according to regions and processing methods. The data matrix of variables (table olive samples and their phenolic compounds) was processed in PCA. Olive samples obtained from six regions were classified according to raw, NP, and SS. The PCA model was constructed with two principal components as 45% of the total variance. PC1 described 28.23% of total variance; PC2 explained 16.21% of total variance, Figures 3–7.

As can be seen from scree plot of eigenvalues, there is a sharp decrease in eigenvalue from PC1 to PC2 and then a relatively flat appearance from PC2 to PC4. This is the possible reason for the low total variance explained with only two PCs (PC1 and PC2). Table 3 shows the eigenvalues along with percent explained variance (% Exp. Var.) and cumulative percent explained variance (Cum. % Exp. Var.).

Table 3. Eigenvalues along with percent explained variance (% Exp. Var.) and cumulative percent explained variance (Cum. % Exp. Var.).

No	Eigenvalues	% Exp. Var.	Cum. % Exp. Var.
1	3.90	28.23	28.23
2	2.24	16.21	44.44
3	2.15	15.59	60.03
4	1.79	12.93	72.95
5	1.16	8.38	81.33
6	1.06	7.67	89.00
7	0.65	4.73	93.73
8	0.47	3.41	97.13
9	0.23	1.67	98.80
10	0.17	1.20	100.00

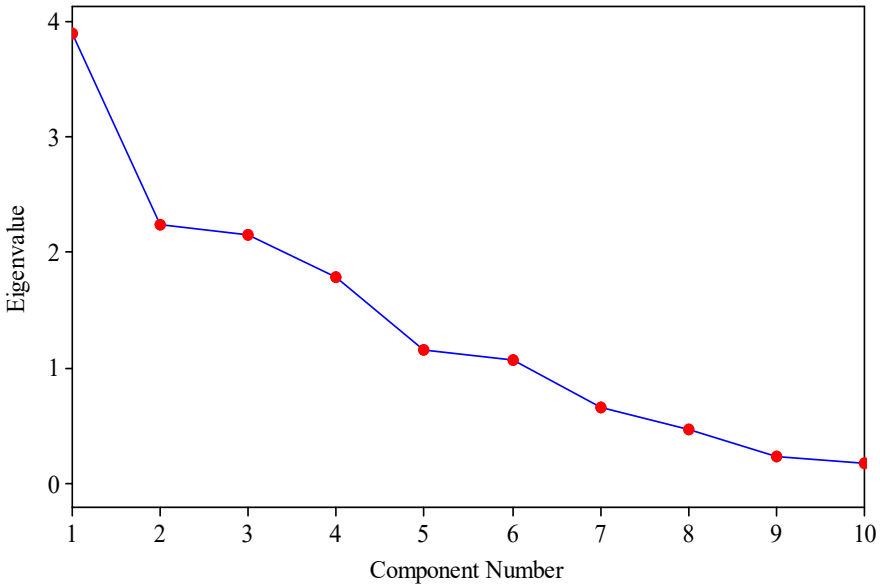

Figure 3. Plot of eigenvalues vs. number of principal components from PCA.

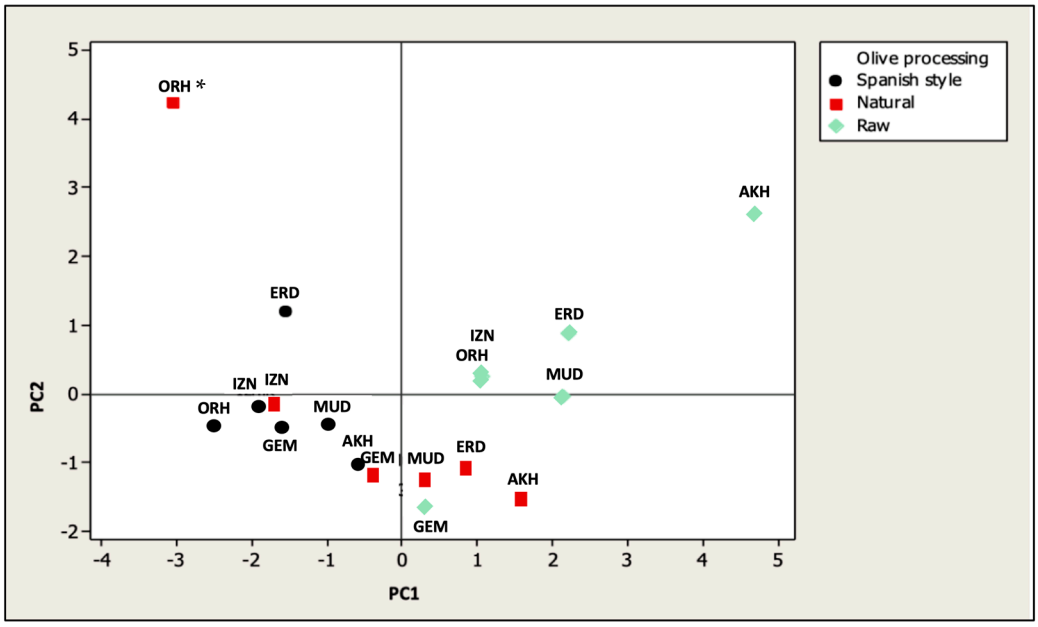

Figure 4. Score plot of PC1 vs. PC2 from principal component analysis. * GEM: Gemlik region; IZN: Iznik region; MUD: Mudanya region; ORH: Orhangazi region; ERD: Erdek region; AKH: Akhisar region.

As seen from Table 3, the sum of the four PC reaches about 73% cumulative explained variance. Figure 4 shows the score plot of PC1 vs. PC2 from principal component analysis. Figure 5 shows the score plot of PC1 vs. PC3 from principal component analysis. Figure 6 shows the three-dimensional score plot of PC1, PC2, and PC3 from principal component analysis.

As can be seen from Figures 4–6 additional third PC is also important to differentiate samples from three processing methods. Especially, the three-dimensional score plot indicates that samples with natural processing are located at the center of the score plot, while raw and Spanish-style samples are on the either side of the natural processed samples.

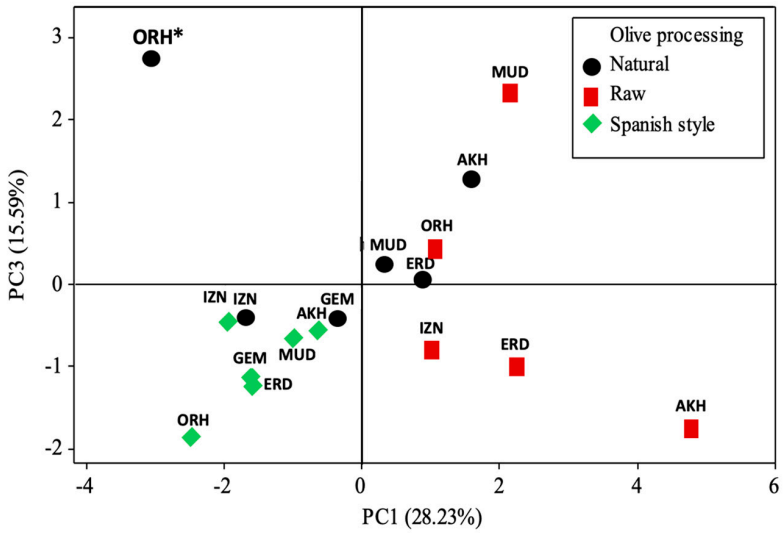

Figure 5. Score plot of PC1 vs. PC3 from principal component C. * GEM: Gemlik region; IZN: Iznik region; MUD: Mudanya region; ORH: Orhangazi region; ERD: Erdek region; AKH: Akhisar region.

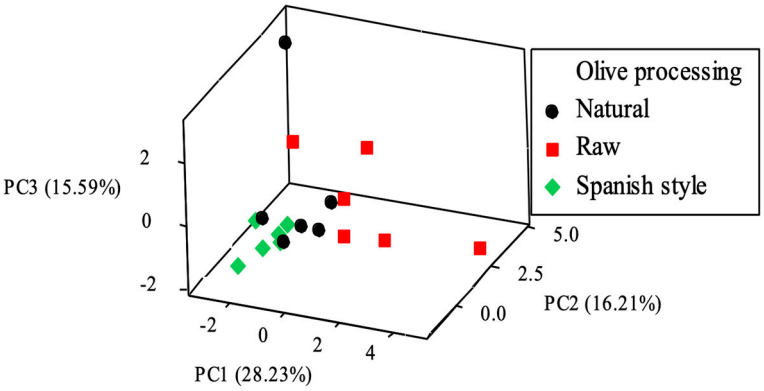

Figure 6. Three-dimensional score plot of PC1, PC2, and PC3 from principal component analysis.

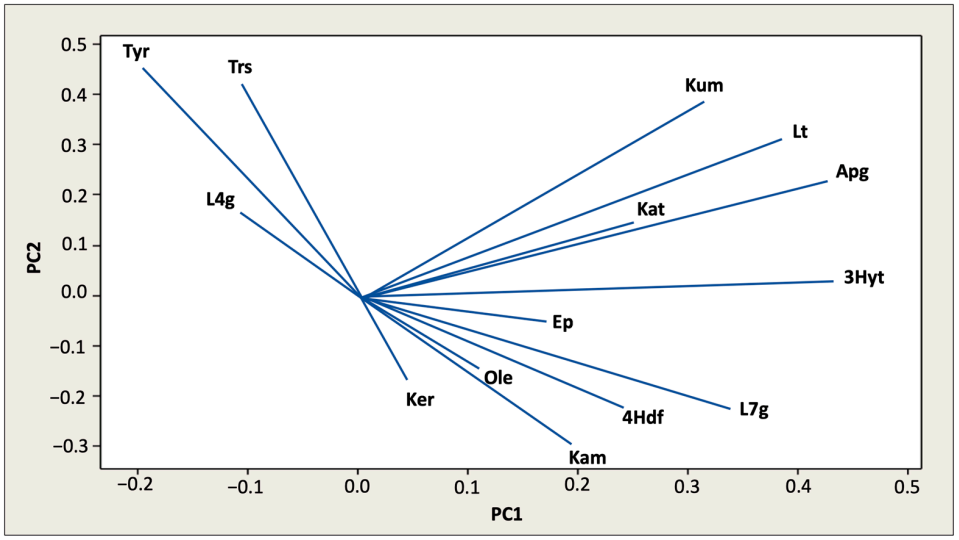

Figure 7. Loading plot of PC1 vs. PC2 from PCA. 3Hyt: 3-hydroxytyrosol; Ker: keracyanin; Kum: coumarin; Kat: catechin; Tyr: tyrosol; 4Hdf: 4-hydroxyphenyl; Ep: epicatechin; L7g: luteolin 7-glucoside; L4g: luteolin 4-glucoside; Trs: trans-cinnamic acid; Lt: luteolin; Kam: kaempherol; Apg: apigenin; Ole: oleuropein.

Olives taken from the Orh are distinguished in the classification of natural processing and are characterized by Trs, Tyr, and L4g phenolic compounds (Figures 4 and 7). Kum and Lt are included in the characterization of raw olives obtained from Orh, Mud, and Gem regions, and 3Hyt, L7g, Kam, Kat, and 4Hyd were characterized in raw olives from Akh, Erd, and Izn (Figures 4 and 7).

According to PCA biplot analysis, the 3Hyt, L7g, Kam, Kat, and 4Hdf were effective in the characterization of 'Gemlik' type black table NP olives obtained from the Akh, Erd, Mud, and Gem regions and processed with natural processing.

On the other hand, raw, NP, and SS processed olives obtained from Erd and Mud were the most similar samples in phenolic compounds. The dendrogram based on the HCA results (Euclidian method) of olive samples could be divided into three main groups of olive processing methods based on their phenolic compounds (Figures 8 and 9).

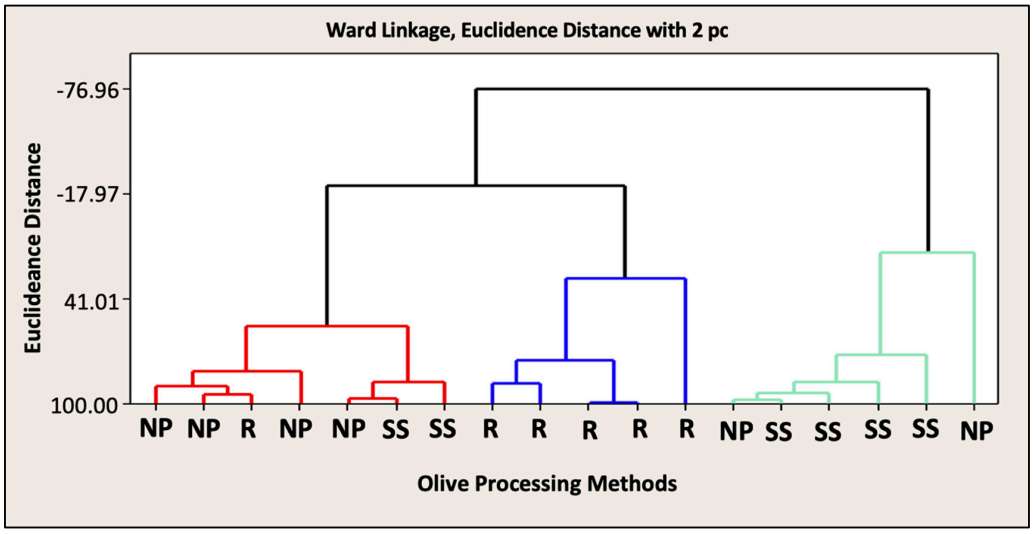

Figure 8. Dendrogram of the samples from hierarchical cluster analysis (HCA) by using two PCs from PCA; NP: natural processing (traditional brine olives); R: raw; SS: Spanish style.

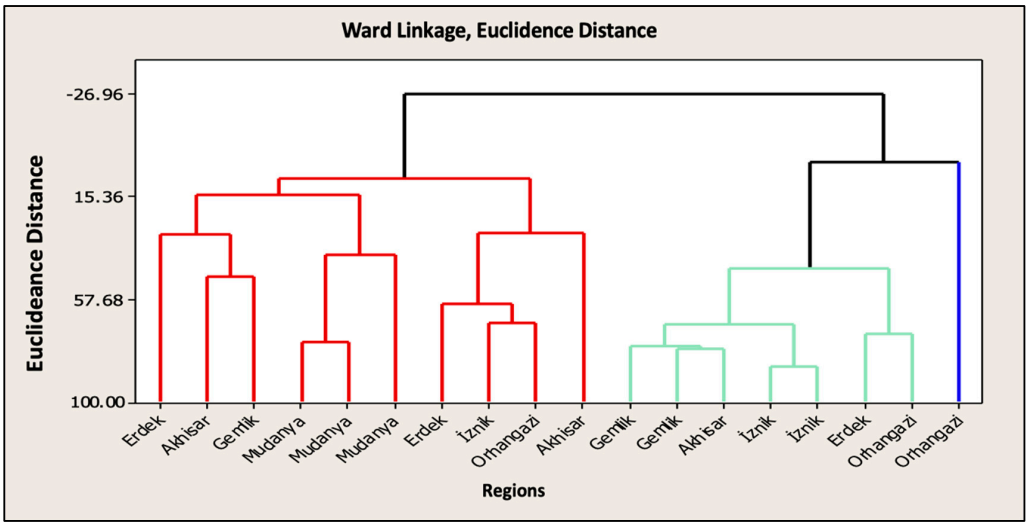

Figure 9. Dendrogram of the samples from hierarchical cluster analysis (HCA) by using standardized data.

A dendrogram was generated by using the Word Linkage method and Euclidean distance measure. As shown in Figure 9, it is made up of three subgroups and includes natural processing, natural processing, raw, and natural processing (subgroup 1); raw, raw, raw, and raw (subgroup 2); and natural processing, Spanish style, Spanish style, Spanish

style, and natural processing (subgroup 3) according to the olive processing methods. According to the HCA analysis, given in Figure 9, the study consisted of three subgroups. These are Erd, Mud, Gem, Akh, Gem, Akh, and Mud (subgroup 1) Erd, Mud, Izn, Orh, and Akh (subgroup 2); and Izn, Izn, Gem, Orh, Erd, and Orh (subgroup 3).

There are similar chemometric (including PCA and HCA) investigations based only on fruit profiles (especially total or individual phenolics) data for Türkiye [30] and Algeria olives [31].

The olives obtained from the GemNP and the AkhRaw had the most similar phenolic compound profile to each other. On the other hand, raw olives obtained from Erd and Mud regions and NP and SS processed olives obtained from Izn were the most similar samples. As seen in the dendrogram, Tyr, Trs, and L7g were prominent phenolic compounds in samples such as ErdRaw and OrhNP.

Chemometric analysis of phenolic compound data (PC1, PC2) provided some important indications about the characterization and classification of 'Gemlik' varieties (Figures 4 and 7).

Comparing the changes in the phenolic content of olives after treatment, significant differences were observed between raw and treated olives. After fermentation, changes in the profile and amount of simple phenolic compounds are mainly due to the diffusion of substances from the olives into the brine and vice versa. When alkali is used, sodium hydroxide and components with carboxyl and hydroxyl groups react, and hydrophilic derivatives are washed away [32].

Variations between the amounts of phenolic compounds may vary depending on some factors. Similarly, previous studies have shown that the composition and content of phenolic compounds in olives depend on many factors such as the variety, region, climate, and development conditions of the fruit, degree of maturity, type of harvest, pests, olive processing, and storage method.

It has been observed that traditional brine olive (NP) methods are the most important method for preserving the content of phenolic compounds. It has been determined that olives with the highest content of phenolic compounds were produced by NP olive samples. There are certain disadvantages of natural processes, such as longer processing time, being less applicable in the industry, high sensory bitterness, and therefore less appeal to consumers than SS olives with high salt content [24]. In addition, in NP methods, the fermentation does not take place in a standardized way, so it has adverse effects on food safety, sensory properties, and health effects [4].

5. Conclusions

Among the phenolic compounds identified in 'Gemlik' olives, 3-hydroxytirosol was the most abundant one. The natural (traditional brine) and Spanish-style processing methods differently decreased phenolic content of black table olives. The loss in phenolic compounds was found to be lower in the natural processing method. These results may indicate that natural processing is a more efficient processing method than Spanish-style processing for obtaining phenolic-compound-rich 'Gemlik' table olives and developing healthier fermented foods.

As they grow outside the geographical boundaries, the phenolic profile of the Akhisar-grown 'Gemlik' variety olives was found to be similar to Gemlik, Iznik, and Orhangazi-grown ones.

Additionally, according to chemometric analysis, it was determined that natural and Spanish-style processed 'Gemlik' olives can be identified according to some phenolic compounds (3-hydroxytyrosol, luteolin 7-glicoside, kaempherol, catechin, and 4-hydroxyphenyl). In other words, it will be possible to determine the processing method according to the phenolic compound profile of the 'Gemlik' variety. In this way, deception of the consumer can be prevented by the phenolic compound determination of the olives supplied from markets.

Author Contributions: C.D.: General planning, sample collection; experiments, analysis, tabulation and interpretation of the data, and writing of the manuscript. E.Y.: Writing of the manuscript, review of the manuscript. O.G.: General planning, review of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank to TAGEM (General Directorate of Agricultural Research and Policies, Ankara, Türkiye) for the financial support (Project No: TAGEM/HSGYAD/18/A3/P1/1158) and Marmarabirlik for technical support of the project.

Data Availability Statement: Data will be available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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