LC-DAD-ESI-MS/MS elucidation of health-promoting phenolic compounds and antioxidant properties of naturally fermented table olives from cv. Gemlik

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Received: June 11, 2020
Accepted: September 29, 2020

The main objective of this study was to compare phenolic compounds, antioxidant activity, and other important quality parameters of fresh and naturally fermented green table olives *cv.* Gemlik. The health-promoting phenolic compounds of fresh and fermented olives were identified by liquid chromatography coupled to diode array detection and electrospray ionisation tandem mass spectrometry (LC-DAD-ESI-MS/MS). A total of 21 phenolic compounds were identified in fresh and fermented table olive samples. The higher level of phenolic compounds was determined in the fresh olives rather than the fermented olives. Oleuropein was the principal phenolic component in the fresh olives while luteolin and hydroxytyrosol were the main phenolic components in the fermented olives. The amounts of luteolin, apigenin, quercetin, and hydroxytyrosol were higher in the fermented olives, when compared to the fresh olives. According to sugar analysis, sucrose, glucose, and fructose were determined in both fresh and fermented olives, and the total amount of these sugars decreased due to fermentation. Glucose was determined to be the dominant sugar in fresh and fermented olives. The results of this study showed that, although the fermentation caused significant decrement of phenolic content, the fermented table olives are still important sources of phenolic compounds and antioxidant capacity.

Keywords: Table olive, Fermentation, Oleuropein, Phenolics, Antioxidant capacity, LC-MS/MS.

INTRODUCTION

Olives are a unique and essential component of the Mediterranean diet in terms of their nutritious and sensorial properties. Olives have a functional value because of their health-promoting phenolic compounds and antioxidant potential. Phenolic compounds, which are minor components of olives, are water-soluble and have important roles due to their anticarcinogenic, antimicrobial, antioxidant, anti-inflammatory, antiviral, hypocholesterolemic and hypoglycemic properties. These compounds, together with their high anti-oxidant effects, add important structural and sensorial properties to olives [1-3].

Processing fresh olives into table olives significantly changes their phenolics profile. Among phenolic compounds, oleuropein is the dominant compound responsible for the high degree of bitterness in unprocessed green olives, whereas in processed olives, tyrosol, hydroxytyrosol, and elenolic acid are the dominant compounds. The concentrations of phenolic compounds in olives vary, depending on their processing techniques as well as according to their degree of ripening [1, 4, 5].

Fermentation is an application that has been used in the protection of fruits

and vegetables since very ancient times. Besides its protective effect on foods, fermentation has several other benefits, such as its positive effect on the nutritional values of foods, providing functional properties, unique sensory characteristics, and increasing food's economic value [6]. The main purpose of processing table olives is the removal of the bitterness related to oleuropein. For this purpose, several processes, mainly based on alkaline hydrolysis or diffusion in brine, are employed to lead to oleuropein decreases and hydroxytyrosol increases [6-7]. In Mediterranean countries, the production of table olives is mostly done by the brine method. The production of table olives, which is appreciated in the world market, is achieved by alkaline treatment with sodium hydroxide and then the fermentation in the brine with 5-6% of NaCl; this is known as the Spanish method. We did not find any comprehensive study on the changes of the antioxidant capacity and bioavailability of phenolic compounds for the olives processed with the brining method. Therefore, this study aims to determine the changes in health-promoting phenolic compounds, antioxidant capacity, bioavailability, and other important quality parameters in the production of table olives that use the brining method for *cv.* Gemlik olives. Analysis of the phenolic compounds of olives was investigated in detail by using the LC-DAD-ESI-MS/MS, and their antioxidant potentials were determined using DPPH and ABTS methods.

MATERIAL AND METHODS

STANDARDS AND CHEMICALS

HPLC standards of sucrose (57-50-1), glucose (50-99-7), fructose (57-48-7), hydroxytyrosol (10597-60-1), caffeic acid (331-39-5), elenolic acid (34422-12-3), verbascoside (61276-17-3), luteolin-7-glucoside (5373-11-5), ohty-eda (oleacein) (149183-75-5), rutin (153-18-4), oleuropein (32619-42-4), quercetin-3-rhamnoside (522-12-3), luteolin-4-glucoside (5373-11-5), apigenin-7-glucoside (578-74-5), quercetin (117-39-5), luteolin (491-70-3), apigenin (520-36-5) were obtained from Sigma-Aldrich (Steinheim, Germany). Moreover, acetonitrile, formic acid, 2,2′-azino-bis-(3-ethyl-benzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulphate, and 2,2- diphenyl-1-picryl hydrazyl (DPPH) were purchased from Merck (Gernsheim, Germany). All chemicals and solvents were analytical or HPLC grade.

SAMPLE PREPARATION AND FERMENTATION

Olive fruits of the Gemlik cultivar (50 kg) from the Adana province in Turkey were used in this study. Fruit samples were randomly obtained, and only healthy fruits were picked from the same tagged healthy trees; which were all over 10 years old. Olives were cracked manually using stones. Fermentation processing was carried out in duplicate at room temperature. The amount of salt (sodium chloride) concentration in brines was kept at 8%. The salinity of brines during the fermentation was controlled by Baume and if necessary, salt was added to the brines. The progress of fermentation was monitored by pH and total acidity determination. Fermentation was completed at the end of the 26th week. When the fermentation process was completed, table olives were packaged and stored at +4°C for 30 days prior to analysis.

GENERAL ANALYSIS

Total acidity, salt content, pH, and colour were analysed in fresh and fermented olives [8, 9]. Total acidity was measured by titration with sodium hydroxide (0.1 N). pH was assessed by using a digital pH-meter (Mettler-Toledo, Schwerzenbach, Switzerland). The colour analysis was determined by instrumental measurements by using Minolta Chroma Meter CM-5 (Osaka, Japan). The data revealed by this instrument were given in terms of the CIE Lab colour profile as L*, a* b*, C, h. The total phenolic assay was performed using a Folin–Ciocalteau reagent according to the modified method described by Saafi et al. [10]. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/kg of extracts.

SUGAR ANALYSIS

Sugar content of olives was determined according to the method described by Legua et al. [11]. For analysis, 2 g sample of freeze-dried and ground olive sample was taken and then homogenised with 20 ml of 80% ethanol/water for 60 minutes, then centrifuged at 7000 rpm at 4°C. The supernatant was collected and filtered through 0.45 µm membrane filters (Whatman Inc., Clinton, NJ, USA). Then, the extract was directly injected into HPLC to determine the sugar content of the samples. Analysis of the sugar content was performed by HPLC (Agilent 1260 HPLC system, CA, USA). Sugars were analysed using Aminex HPX-87C column (150 × 4.6 mm, 5 µ; Bio-Rad, CA, USA). A flow rate and an eluent were determined to be 0.5 mL/min, 5 mM H_2SO_4, respectively. The chromatographic peak corresponding to each sugar was identified by comparing the retention time with that of a standard (sucrose, glucose and fructose). An external calibration curve was prepared using standards to determine the relationship between the peak area and concentration.

LC-DAD -ESI-MS/MS ANALYSIS OF PHENOLIC COMPOUNDS

The extraction of phenolic compounds was carried

out according to Sonmezdag et al. [12] with some modifications. A sample of 1 g of freeze dried and powdered olives was weighed into a centrifuge tube, and 10 ml of methanol-water (80:20, v/v) was added. The mixture was vortexed, shaken in an orbital shaker for 30 min at 200 rpm (IKA KS 3000 Staufen, Germany). Then, the tube was centrifuged at 5500 rpm for 10 min at 4°C. The extraction was repeated and then the extract was filtered through a 0.45 µm pore size membrane filter before injection [13].

Phenolic compounds of the samples were eluted with the following parameters: the flow rate and temperature were set to 0.5 ml/min and 25°C, according to Kelebek [14]. All peaks were detected in the UV-VIS spectra (between 200 and 600 nm). To identify the phenolics, the relative retention times and UV spectra were compared to authentic standards and subsequently approved by an Agilent 6430 LC-MS/MS spectrometer having an electrospray ionisation source. The negative ion mode was employed with the following optimised parameters: capillary temperature of 400°C, drying gas of N_2 12 L/min, nebuliser pressure of 45 psi electrospray ionisation mass spectrometry detection. Mass spectra (over the range of *m/z* 100–2000) were simultaneously acquired in the negative ionisation mode. Identification and quantification of mass spectrum data of phenolic compounds were collected in negative ion and MRM mode. Each phenolic compound was quantified using the calibration curves of the standard phenolic compounds. The standard curves were acquired utilising the commercial standards at concentrations normally existing in fresh and table olives (nearly 1-100 mg/kg) and getting regression coefficients (R squared, R^2) above 0.995 in all cases. Phenolic content was calculated as described previously [12, 13].

The quantification of individual compounds was calculated with a calibration curve of the standard compound. Instead the absence of reference compounds, the calibration of structurally related substances was used considering the molecular weight correction factor. Thus, hydroxytyrosol glucoside (peak 2) was quantified using hydroxytyrosol calibration curve; elenolic acid glucoside (peak 5) was quantified in terms of elenolic acid; demethyloleuropein (peak 6), oleuropein aglycon (peak 11), 6′-β-glucopyranosyl oleoside (peak 15) and 6′-rhamnopyranosyl oleoside (peak 17) were quantified by using oleuropein as a reference standard. The limits of detection (LOD) and quantification (LOQ) under the existing chromatographic conditions were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

ANTIOXIDANT ASSAYS

Antioxidant activity was measured using two well-known radical scavengers, ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl). Assays were carried out with respect to the methodology mentioned in an earlier study [15]. In order to obtain a standard curve, a Trolox standard solution was used at different concentrations. The absorbance of the solution was measured by a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

STATISTICAL ANALYSIS

The results of the analysis of fresh olives and fermented table olives were analysed with variance analysis using SPSS 20 package program (SPSS Inc., Chicago, Illinois, USA) at 95% confidence interval ($p<0.05$). According to the Duncan multiple comparison test, significant differences were evaluated.

RESULTS AND DISCUSSION

GENERAL COMPOSITION OF FRESH AND FERMENTED TABLE OLIVES

Total acidity is a significant component related to the quality and stability of table olives. It is also associated with the generation of organic acids throughout the fermentation of olives and influences the sensory characteristics (taste and aroma) of the end products. Acidity in fresh olives was determined to be 0.09% and 0.10% in the fermented table olives. In the related literature, total acidity values were reported to be between 0.35% and 1.41% [16]. The pH values in fresh and fermented samples were 5.85 and 5.60, respectively. The ability of lactic acid bacteria to carry out a healthy fermentation is directly related to the pH of the medium. When the pH is below 4.5 in brine fermentation, the formation of unwanted changes in the environment is prevented and the desired result in olive flavouring is obtained. The amount of salt in pickled olives was determined to be 5.79%. According to Turkish table olive standards, salt content in table olives must be a maximum of 7% and pH must be a maximum of 4.3. The results of this study agree with the Turkish table olive standard [17]. Compared to the IOC olive standards, it was noticed that the pH is comparable, but salt concentration was slightly below the minimum limit of 6%. Different studies are carried out to eliminate the negative effects of high salt concentrations in the production process of table olives and specially to shorten the ripening period. Among these studies, 0.5% $CaCl_2$ and 150 mg/kg iron gluconate were added to the brine in order to ensure the desired firmness of the olive fruits and to prevent softening [19]. In another study, it was reported that the fermentation step was completed in a shorter time by treating

Table I - General properties, color parameters and antioxidant capacities of olives

Analysis	Fresh Olives	Table Olives	Significance
TA (%)	0.09 ± 0.01	0.10 ± 0.00	ns
pH	5.85 ± 0.75	5.60 ± 0.50	ns
Salt (%)	0.37± 0.01	5.79± 0.02	*
L	41.14 ± 0.21	44.93 ± 0.03	*
a*	0.52 ± 0.02	3.37 ± 0.01	*
b*	8.53 ± 0.24	17.98 ± 0.01	*
C*	8.54 ± 0.24	18.29 ± 0.01	*
H	86.50 ± 0.17	79.37 ± 0.03	*
Total phenolic content (mg GAE/kg)	1473.04 ± 1.34	923.00 ± 1.89	*
DPPH (µmol Trolox/kg)	10.74 ± 0.54	10.34 ± 0.65	ns
ABTS (µmol Trolox/kg)	9.67 ± 0.48	9.30 ± 0.58	ns
Sugars compositions (g/kg)			
Sucrose	0.61 ± 0.00	0.18 ± 0.03	*
Glucose	2.89 ± 0.00	1.42 ± 0.00	*
Fructose	2.30 ± 0.01	1.43 ± 0.20	*

TA: Total acidity, ns = not significant, * Statistical signifance at $p<0.05$.

olives with 1-2% NaOH solution in order to remove oleuropein contained in the olives [20].

The total phenolic content of the fresh olives was determined to be 1473.04 mg/kg. A significant ($p<0.05$) decrease in the total amount of phenolic compounds was observed due to fermentation. Boskou et al [21] reported that the total amount of phenolic compounds in table olives ranged from 820 to 1710 mg/kg (Caffeic acid equivalent-CAE). Kadakal [22] reported that the total phenolic content of black table olives is in the range of 298.74 to 783.75 mg CAE/100g. Susamci et al. [23] determined the total phenolic amount in Gemlik and Ayvalik cultivars to be 274.91 mg CAE/100g and 250.80 mg CAE/100g, respectively. The total amount in fermented olives has been reported to be 244.10 mg CAE/100g and 133.20 mg CAE/100g.

COLOUR PARAMETERS

The L, a * and b * values of the fresh and table olives analysed are given in Table I. L values show brightness (whiteness or lightness/darkness ratio); + a* is red; -a* is green; '+ b*' represents yellow and '–b' represents blue. L* values in fresh and fermented olives were 41.14 and 44.93 respectively. Due to fermentation, L* values increased. The a* value, which is indicative of redness in colour, was 0.52 in the fresh olives and 3.37 in the table olives. Depending on the fermentation method, a* values increased. The green colour of fresh olives turned yellow due to brining, as seen in the processing of other fresh vegetables into fermented products. The b* value, which is indicative of yellowness, was 8.53 in the fresh olives and increased to 17.98 in the table olives. Depending on the method of fermentation, b* values also increased. Chroma value indicating the colour intensity of the olives was found to be 8.54 in fresh olives and 18.29 in fermented olives. Because of fermentation, C values increased by 26.31% in table olives when compared to the fresh olives. As can be seen, the C value increased more than 2 times due to fermentation. López-López et al. [24] stated that the L* in fresh and brine olive varies between 61.70 and 50.86, a* value between 9.56 and 5.44, b* value between 37.54 and 38.81 and C* value between 39.05 and 39.24. L* and a* values decreased after the fermentation of olives, and b* and C* values increased. The data we obtained in our study agree with this study.

SUGAR CONTENTS

Three different sugars were determined as existing in fresh and table olives; sucrose, fructose, and glucose. The total amount of sugars was 5.80 g/kg in fresh and 3.03 g/kg in table olives. Fermentation caused a significant ($p<0.05$) decrease in the sugar content of the olives. Among the sugars, glucose was found to be the most dominant in the samples. The glucose content was found to be 2.89 g/kg in the fresh olives and 1.42 g/kg in the table olives. Due to the fermentation process, glucose contents were significantly reduced. Depending on fermentation, sugar contents decreased from 12.6 g/kg to 2.6 g/kg in Sevillana olives, 10.7 g/kg to 2.6 g/kg in Ascolana olives and 1.4 g/kg to 6.7 g/kg in Moroccan olives [25]. In another study, the total amount of sugar in table olives was found to be 3.18-2.81 g/L [26]. Yıldız and Uylaser [27] determined that the amount of reducing sugar in Gemlik table olives obtained from different regions is between 0.85 and 1.20 g/100 g. Irmak et al. [28] reported that Gemlik olives have a reduced sugar content of 0.53-0.54% in brine.

Table II - Phenolic compounds of fresh and fermented table olives by HPLC-DAD-ESI-MS/MS including relative retention time, molecular ion $[M-H]^-$ and main fragment ions detected (m/z)

Peak n.	Rt (min)	RRT[b]	Pseudo molecular ion $[M-H]^-$	Product ions (*m/z*)	Quantitative transition (*m/z*)	Compounds	Fresh Olive	Table Olive	Significance
1	18.47	0.33	153	123, 122, 107	153→123	Hydroxytyrosol[a]	80.65 ± 1.34	119.89 ± 2.08	*
2	16.21	0.29	315	179, 153, 135, 89	315→153	Hydroxytyrosol glucoside	116.10 ± 2.27	12.43 ± 0.96	*
3	52.74	0.95	241	209,165, 139, 127, 101, 95	241→139	Elenolic acid[a]	246.71 ± 0.72	337.10 ± 5.66	*
4	34.85	0.62	179	135	179→135	Caffeic acid[a]	0.00 ± 0.00	17.60 ± 1.24	*
5	34.04	0.61	403	371, 359, 241, 223, 179, 119	403→179	Elenolic acid glucoside (oleoside methyl ester)	48.11 ± 1.09	87.69 ± 2.09	*
6	46.35	0.83	525	389, 319, 183	525→389	Demethyloleuropein	257.12 ± 3.78	12.72 ± 0.55	*
7	46.95	0.84	623	461, 315, 135, 161	623→161	Verbascoside[a]	589.25 ± 4.82	5.56 ± 0.27	*
8	47.96	0.86	447	285	447→285	Luteolin-7-glucoside[a]	519.51 ± 6.44	32.16 ± 0.88	*
9	58.34	1.04	319	301, 275, 249, 195, 183, 165, 59	319→195	OHTY-EDA (Oleacein) [a]	54.87 ± 0.66	4.61 ± 0.40	*
10	51.69	0.92	609	301, 179	609→301	Rutin[a]	297.24 ± 3.55	84.63 ± 1.04	*
11	36.65	0.65	377	307, 275	377→275	Oleuropein aglycon	64.24 ± 0.93	24.80 ± 0.55	*
12	56.08	1.00	539	377, 307, 275, 225	539→377	Oleuropein[a]	1141.42 ± 2.94	95.46 ± 0.84	*
13	52.35	0.93	447	301	447→301	Quercetin-3-rhamnoside[a]	252.63 ± 5.44	36.11 ± 0.79	*
14	53.45	0.95	447	285	447→285	Luteolin-7-glucoside[a]	256.27 ± 3.59	28.43 ± 0.60	*
15	51.35	0.92	551	507, 341, 389, 281	551→389	6'-β-Glucopyranosyl oleoside	85.54 ± 1.26	25.40 ± 0.55	*
16	52.91	0.94	431	269	431→269	Apigenin-7-glucoside[a]	63.61 ± 1.97	14.13 ± 0.37	*
17	55.95	1.00	535	491, 325	535→325	6'-Rhamnopyranosyl oleoside	758.23 ± 8.88	16.54 ± 0.48	*
18	58.03	1.03	301	151, 121	301→151	Quercetin[a]	29.47 ± 0.43	65.42 ± 0.78	*
19	59.34	1.06	523	361, 291, 259, 101	523→361	Ligstroside	49.73 ± 1.84	11.65 ± 0.52	*
20	62.63	1.12	285	151	285→151	Luteolin[a]	269.10 ± 4.18	396.92 ± 5.71	*
21	72.59	1.29	269	117, 151, 149	269→117	Apigenin[a]	50.91 ± 1.66	79.94 ± 1.06	*
						Total	5230.71 ± 43.01	1509.19 ± 12.57	*

[a] Identification confirmed by comparison with standards. * Statistical significance at $p<0.05$.
[b] RRT: Relative retention time obtained by dividing for retention time of oleuropein

ANTIOXIDANT CAPACITY

The antioxidant capacity of fresh and table olives was evaluated by DPPH and ABTS methods. The antioxidant capacity of the olives analysed by the DPPH method was found to be 10.74 µM Trolox/kg in fresh olives and 10.34 µM Trolox/kg in the table olives. It was observed that the levels of the antioxidant potential were reduced by the fermentation process. The antioxidant capacity obtained by the ABTS method was found to be 9.67 µM Trolox/kg in fresh olive samples and 9.30 µM Trolox/kg in the table olive samples. The total antioxidant capacities of fresh and table olives showed a similar trend of decline also seen in their phenolic contents.

PHENOLIC COMPOSITIONS OF FRESH AND FERMENTED TABLE OLIVES

The data revealed by LC-DAD/ESI-MS/MS showed the phenolic profiles of fresh and table olives; given in Table II. The chromatograms of identified compounds and total ion chromatograms are presented in Figure 1a and Figure 1b. In the current study, a total of 21 phenolic compounds were identified and quantified (Table II). The total amount of total phenolic compounds was 5230 mg/kg in the fresh olives and 1509 mg/kg in the table olives. The health-promoting phenolic compounds are important as they have high antioxidant capacity and increase the oxidative stability and nutritional properties of olives. However, it was observed that the amounts of the phenolic compounds were sharply reduced by the applied fermentation process in olives. It was detected that the phenolic profiles of the fresh and table olives were similar. When evaluated in general, it was found that the total amount of phenolic compounds in fermented table olives decreased by 71%. Similarly, Montano et al. [16] reported that the phenolic compounds in fresh olives were very sensitive to fermentation treatment resulted in a significant reduction in their total amounts.

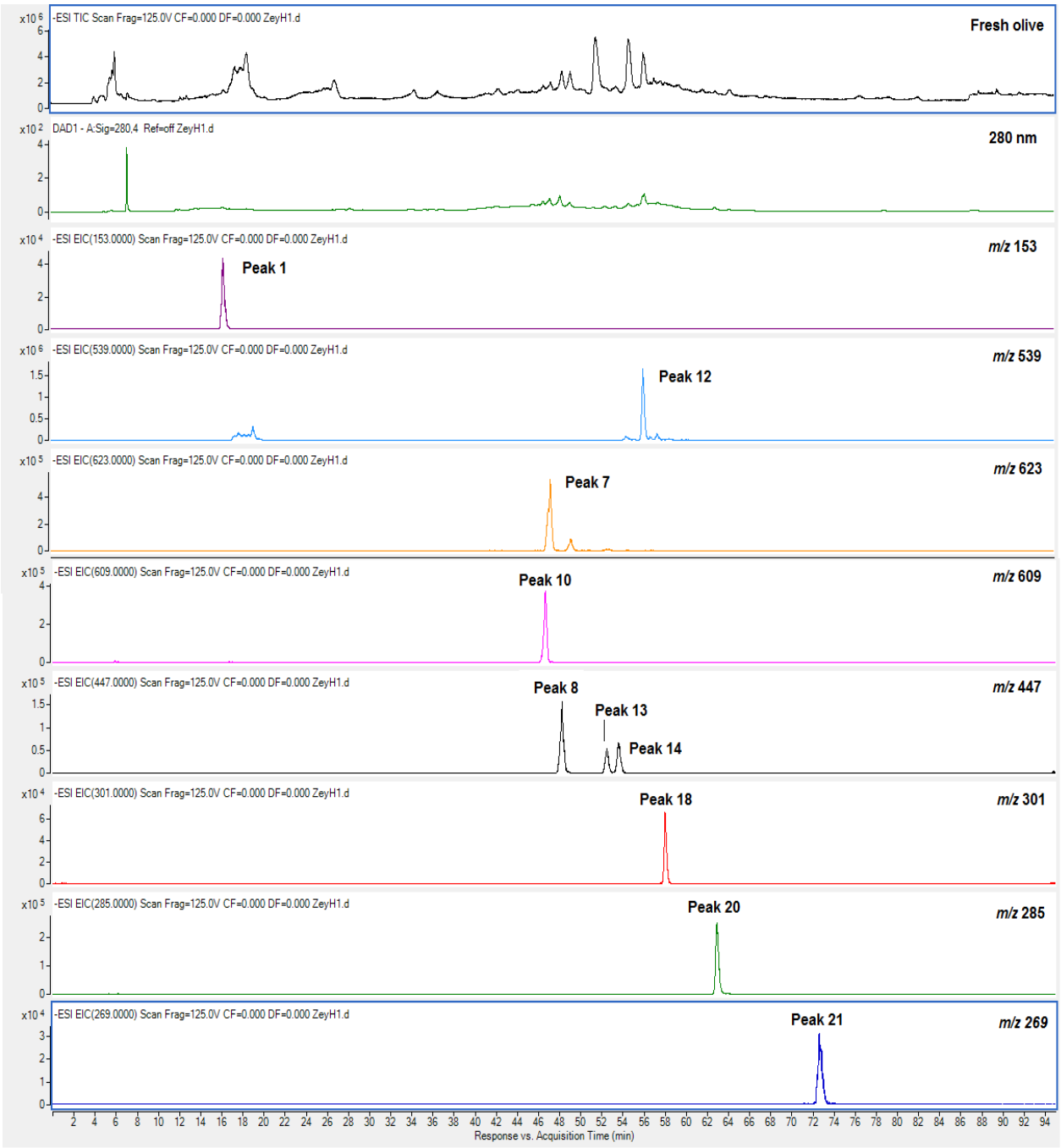

Figure 1a - LC-DAD-ESI-MS/MS chromatograms of some phenolic compounds identified in fresh olive on negative ionization mode. Peaks correspond to compounds in Table II.

PHENOLIC ALCOHOLS

Hydroxytyrosol (Peak 1; *m/z* 153 → 123), hydroxytyrosol glucoside (Peak 2; *m/z* 315 → 153), OHTY-EDA (Oleacein) (Peak 9; *m/z* 319 → 195) were the main simple phenols (phenolic alcohols) found in fresh and fermented samples (Fig. II). Hydroxytyrosol is predominant among these compounds, and its amount increased (49%) significantly ($p<0.05$) due to the fermentation process. The antioxidant effects of this compound have been proven by model studies. It has been determined that there is a relationship between phenolic compounds and oxidative stability, and the phenolic compounds in olives are powerful antioxidants. The amounts of peak 2 and peak 9 compounds decreased significantly due to the fermentation process.

These changes are in accordance with the data from similar studies [29]. Hydroxytyrosol is a hydrolytic derivative of oleuropein, and its amount increased significantly due to fermentation. Increases in the hydroxytyrosol concentration can be attributed to the increased activity of hydrolytic enzymes that catalyse oleuropein hydrolysis, particularly glucosidase and esterase, by the production of oleuropein aglycon and elenolic acid [30]. The amount of hydroxytyrosol

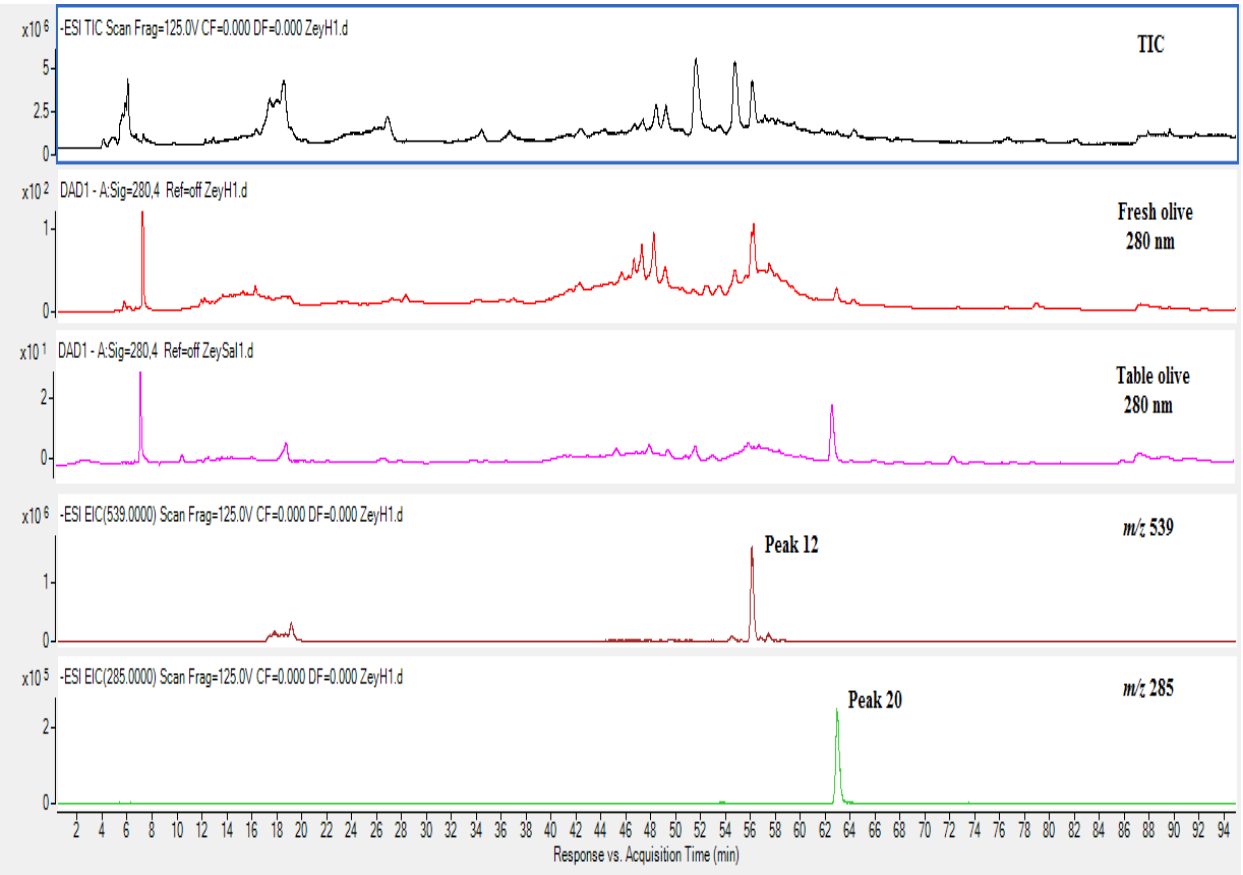

Figure 1b - LC-DAD-ESI-MS/MS chromatograms of fresh and table olive extracts recorded at 280, nm with MRM spectrum of olueropein and luteolin.

was determined to be 80.65 in the fresh olives and 119.89 mg/kg in the table olives. The fermentation process increased the amount of this compound by about 49%. Boskou et al. [21] reported that the amount of hydroxytyrosol ranges from 7 to 1140 mg/kg in Greek table olives.

In the study, only caffeic acid (Peak 4; *m/z* 179→135) was determined among phenolic acids. While caffeic acid levels are not determined in fresh olives, the amount in table olives was determined to be 17.60 mg/kg. Boskou et al. [20] reported that the amount of caffeic acid ranged between 0-40 mg/kg in a study in which they examined the phenolic compounds found in green olives.

In the structure of elenolic acid and its derivative, two compounds, peak 3 (*m/z* 241→139) and peak 5 (*m/z* 403→179) were determined. The amount of peak 3 increased by 37%, and peak 5 increased by 45% due to fermentation. Elenolic acids are formed by hydrolysis of oleuropein. However, unlike oleuropein, elenolic acids are not judged as being bitter [31].

FLAVONOLS AND SECOIRIDOID GLUCOSIDES

Verbascoside (peak 7, *m/z* 623→161), luteolin-7-glucoside (peak 8, *m/z* 447→285), rutin (peak 10, *m/z* 609→301), oleuropein aglycon (peak 11, *m/z* 377→275), oleuropein (peak 12, *m/z* 539→377), quercetin-3-rhamnoside (peak 13, *m/z* 447→301), luteolin-7-glucoside (peak 14, *m/z* 447→285), 6′-*β*-glucopyranosyl oleoside (peak 15, *m/z* 551→389), apigenin-7-glucoside (peak 16, *m/z* 431→269), 6′-rhamnopyranosyl oleoside (peak 17, *m/z* 535→325), quercetin (peak 18, *m/z* 301→151), ligstroside (peak 19, *m/z* 523→361), luteolin (peak 20, *m/z* 285→151), apigenin (peak 21, *m/z* 269→117) were identified on the basis of their retention time, absorbance spectrum, MS fragmentation pattern using LC-DAD–MS/MS and by authentic standards (Tab. II) (Fig. 1a, 1b). An analysis of the chemical structure of individual aglycones showed fragmentation for sugars at *m/z* 162 (glucose or galactose), *m/z* 146 (rhamnoside), and *m/z* 308 (rhamnohexosyl as rutinoside).

Two compounds (Peak 11, and 12) were identified as oleuropein; based on their ultraviolet spectral data and MS fragmentation, leading to the oleuropein aglycone at *m/z* 377 in negative mode (Fig. 2). The mass spectrum obtained for oleuropein, a glycosylated secoiridoid, showed a pseudo molecular ion at m/z 539 and ionic fragments at *m/z* 307 and 275. These two characteristic ionic fragments originate from the ion at m/z 377 (a molecule resulting from the breakdown of the glycosidic bond of

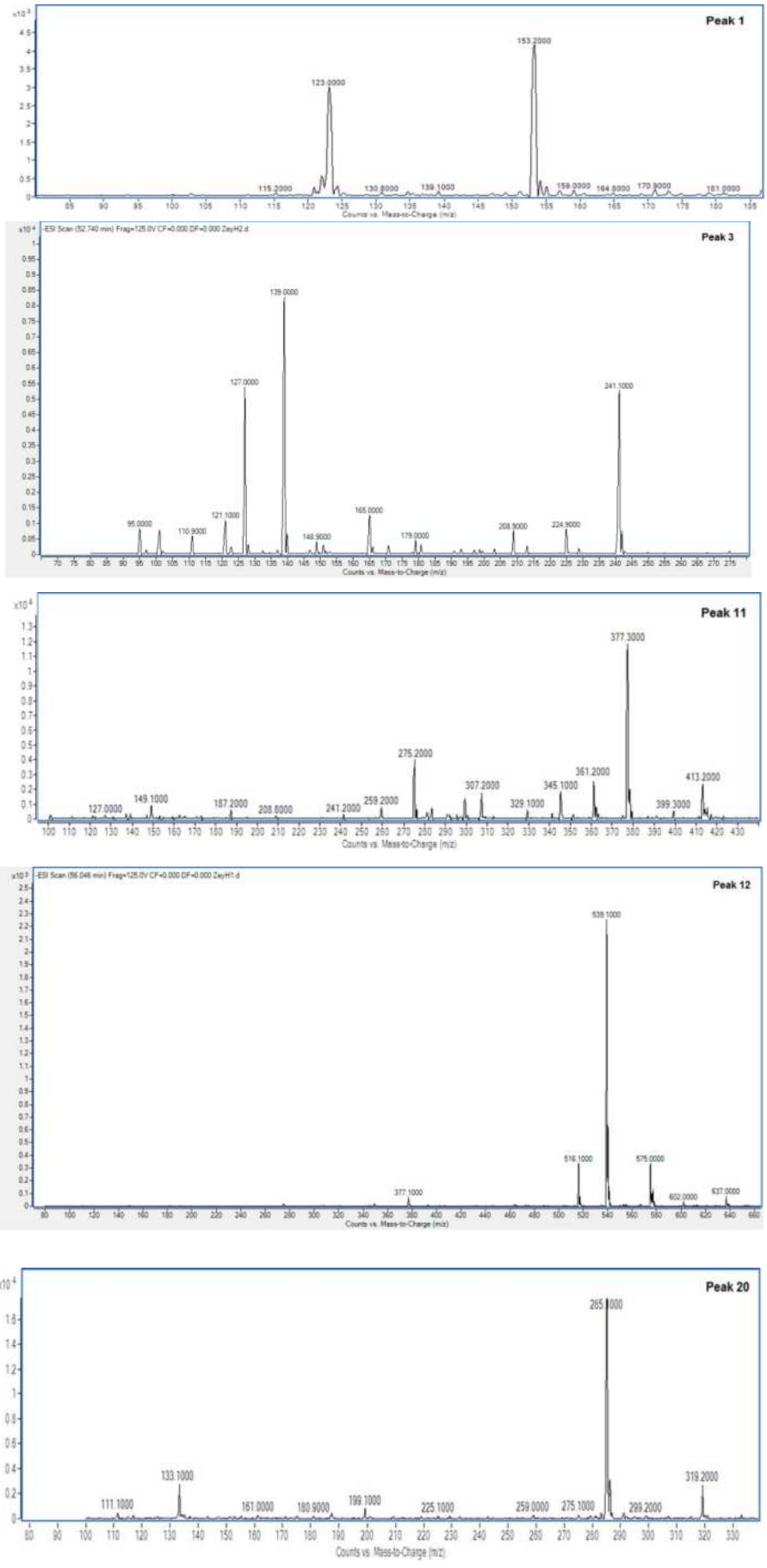

Figure 2 - Mass spectrum of hydroxytyrosol (Peak 1); elenolic acid (peak 3); oleuropein aglycon (Peak 11); oleuropein (Peak 12) and luteolin (Peak 20).

oleuropein which in ESI-MS experiments produces the ion at *m/z* 307 from the loss of a C_4H_6O fragment), and the ion at *m/z* 275 (derived from a rearrangement of other fragments). As seen in Table II, oleuropein is predominant in fresh olives (1141.42 mg/100 g), and the amount of this compound significantly decreases (95.46 mg/100 g) due to fermentation. The amount of peak 11 decreased from 64.24 mg/kg to 24.80 mg/kg, depending on fermentation. Oleuropein is the main glycoside in olives and is responsible for the bitter taste of immature and unprocessed olives. Chemically, oleuropein is the ester of elenolic acid and 3,4-dihydroxyphenyl ethanol that has beneficial effects on human health, such as antioxidant, antiatherogenic, anti-cancer, anti-inflammatory and antimicrobial properties.

Three compounds (Peak 8, Peak 14, and Peak 20) were identified in the structure of luteolin derivatives according to their ultraviolet spectral data and MS fragmentation based on the luteolin aglycone at *m/z* 285 in negative mode. As can be seen in Table II, there are two peaks with the same $[M-H]^-$. Peaks 8 and 14 had the same $[M-H]^-$ at *m/z* 447 (Fig. 1a). The *m/z* 447 ion yielded MS_2 fragment ion at *m/z* 285, corresponding to the loss of a 162 Da fragment (corresponds to the loss of glucose). Cleavage of this luteolin glycoside gave the anion aglycone at *m/z* 285. While the amount of luteolin compounds with glycoside structure decreased, the amount of aglycon luteolin increased by 47%.

When evaluated in general, the amount of flavonols decreased significantly due to the fermentation process. However, the amount of flavonol in the aglycon structure such as quercetin, luteolin and apigenin increased. In addition, it was found that the glycoside structure was dominant in fresh olives and aglycone structure was dominant in brined olives. Overall, it was found that the total amount of phenolic compounds decreased significantly due to fermentation, but the change in antioxidant capacity was not statistically significant ($p > 0.05$). This is thought to result from the conversion of glycoside compounds into aglycone compounds with a higher antioxidant potential.

CONCLUSIONS

In this study, it was found that the fermentation process had a significant effect on the colour, sugars and phenolic compounds of *cv*. Gemlik olives. It was observed that the sugar content and colour values of the fermented samples changed significantly as compared to the fresh sample. A total of 21 phenolic compounds have been identified in olives. The most dominant phenolic compound was found to oleuropein in the fresh sample. The phenolic compounds determined in the fresh olive samples decreased significantly with the fermentation process. In fermented table olives, hydroxytyrosol and luteolin were the predominant compounds. It was also found that the antioxidant potential assessed by the DPPH and ABTS methods was higher in fresh olive samples and decreased depending on the fermentation process. General evaluation of the results revealed that the bitter taste originating from the oleuropein of olives decreased after fermentation, as preferred by the consumers. It has been shown that the bioactive properties of table olives are preserved highly in the brining method. Nevertheless, an attentive selection of commercial strains is needed to enhance the contents and compositions of health-promoting phenolic compounds during the fermentation process.

Acknowledgments

We wish to thank the Adana Alparslan Turkes Science and Technology University Research Found (No: LTP-18332009) for financial support. We also wish to thank Andrew Russell Walle from Adana Alparslan Turkes Science and Technology University-Turkey for his outstanding editing and proofreading.

Compliance with Ethical Standards

Conflict of interest

All authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals carried out by any of the authors.

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