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ORIGINAL ARTICLE

Enrichment of green table olives by natural anthocyanins during fermentation

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Abstract The aim of this study is the enrichment of green table olives with anthocyanins by using beetroot and black carrot in the fermentation media and to improve functional properties of fermented olives. For this purpose, a full factorial design was constructed by considering the fermentation time, vegetable type and vegetable concentration as processing factors. The changes in the chemical and microbiological properties of both olive and brine samples were monitored. During fermentation, while phenolic components of olives were transferred to the brine, the anthocyanins originating from the black carrot and beetroot diffused into both olive and brine samples. The total monomeric anthocyanin content of fermented olives containing 20% percent of black carrot and beetroot was 149.87 and 154.05 mg/kg respectively. Moreover, the color of olives turned as fermentation progressed. Both ANOVA results ($p < 0.05$) and PCA model ($R^2 = 0.99$; $Q^2 = 0.93$) confirmed that reaction time is most important factor for the fermentation process. The sensorial analysis results indicated that the olives fermented with 20% vegetable for 10 days had been highly scored by panelists.

Keywords Oleuropein · Anthocyanins · Table olive · Beetroot · Black carrot · Lactic acid fermentation

Abbreviations

TPC	Total phenolic content
TMA	Total monomeric anthocyanin content
TAC	Total antioxidant capacity
SC	Salt content
TA	Total acidity
TBC	Total viable bacteria count
TYMC	Total yeast and mold count
BT	Beetroot
BC	Black carrot
PCA	Principal component analysis

Introduction

Table olives are considered as precious fermented products in terms of both nutritional and economic value in Mediterranean countries (Perpetuini et al. 2020). In 2010, table olives were added to the Healthy Eating Pyramid of the Mediterranean diet due to their higher contents of bioactive compounds, dietary fibers, fatty acids, and antioxidants. Spain, Italy, Greece, Turkey, Egypt, Algeria, and Portugal are the foremost countries earning remarkable incomes from table olive production (Talhaoui et al. 2015)

The olive fruit has a great interest due to its postulated health benefits which are related to its higher monounsaturated fatty acid content and the antioxidant capacity that derived from phenolic compounds (Malheiro et al. 2011). The phenolic profiles of olives are highly affected by the cultivar, cultivation area, climatic conditions and degree of ripening. Oleuropein is the most predominant phenolic compound of olive fruits and followed by hydroxytyrosol and

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tyrosol (Amanpour et al. 2019). The endogenous enzymes, especially β-glucosidases and esterases hydrolyze oleuropein into derivative compounds throughout ripening period (Ramírez et al. 2017).

Olives go through three stages of maturation on the tree: the green stage, the turning color stage, and the final purple stage. The color change is observed as a result of decline in chlorophyll and carotenoid contents which contribute to the green-yellow colorness in olives. Furthermore, the increases in the anthocyanin content are responsible for the transition from red to purple color at the final stages of maturation. (Khosravi et al. 2021). In particular, cyanidin-3-rutinoside and cyanidin-3-glucoside accumulate in olive fruits towards the end of the purple stage (Aprile et al. 2019).

Table olives are defined as "the sound fruit of varieties of the cultivated olive trees (*Olea europaea L.)* that are chosen for their production of olive whose volume, shape, flash-to-stone ratio, fine flesh, taste, firmness, and ease of detachment from the stone make them particularly suitable for processing; treated to remove their bitterness and preserved by natural fermentation; or by heat treatment, with or without the addition of preservatives; packed with or without covering liquid" (IOOC 2004). The most well-known commercial types of table olives are Spanish, Greek, and Californian (Rodríguez-Gómez et al. 2017). Table olive production starts with a debittering stage by removal of oleuropein and continues with fermentation process with applying different methods. The bitterness can be removed by different technics including alkaline treatment, brining/salting, and acidification (Rodrigues et al. 2019). The debittering stage and fermentation period result in some changes in the phenolic profile of olives. The amount of phenolics of fermented olives may decrease due to the diffusion of constituents and the hydrolysis of oleuropein to hydroxytyrosol or other phenolic compounds (Caponio et al. 2019).

The natural olive fermentation is a complex process that comprises the growth of various microorganisms which are naturally present on olive drupes, especially lactic acid bacteria (LAB) (e.g. *Lactobacillus plantarum* and *Lactobacillus pentosus*) and yeast (*Saccharomyces cerevisiae, Wickerhamomyces anomalus, Candida boidinii,* etc.) (Anagnostopoulos et al. 2020). Mostly, the LAB is in charge of the lactic fermentation of treated olives. The activity of LAB results in brine acidification via the formation of organic acids and decline of pH by consuming the fermentable substrates. On the other hand, yeast lead to the formation of some flavor and aroma compounds. It was also reported that yeast that also degradates the phenolic compounds (in particular oleuropein) exist in olive drupe (Anagnostopoulos et al. 2020).

Generally, the addition of salt (especially NaCI) into fermentation media as a preservative is desirable in order to prevent growth of pathogens and also contribute formation of some organoleptic properties of the final product (Bautista-Gallego et al. 2013). During fermentation, while water-soluble compounds are diffusing from olives to the brine, salts in brine penetrate to olives oppositely, until the equilibrium is achieved (Anagnostopoulos et al. 2020). The whole treatments applied for processing of table olives end up with several alterations in the both phenol profile and the level of valuable biophenols and reduce the biological functions of the end product (Kiai and Hafidi 2014).

The main purpose of this research was to manufacture green table olives enriched by anthocyanins using beetroot and black carrot in the fermentation media in order to widen product diversity and improve functional properties of table olives. It was also targeted to evaluate the effects of processing parameters on some chemical and microbiological properties of both table olive and brine samples during fermentation process and to compare these data with the regular fermented samples. In this work, the diffusion of anthocyanins originated from beetroot and black carrot to green olives was achieved with the aim of enhancing functionality of table olives and developing a new product.

Materials and methods

Olive samples and reagents

Kalamata type of green olives were used in the fermentation process and obtained from Sultanhisar village of Aydın, Turkey. The beetroot and black carrot were purchased from a local market. All other reagents and solvents are of analytical or chromatographical grade and were provided from Sigma (Sigma-Aldrich, Germany).

Table olive fermentation

A full factorial experimental design was employed to evaluate the effects of fermentation time (5, 10,15, 20 days), vegetable type (beetroot and black carrot) and vegetable concentration (10–20%w/w) on chemical and microbiological properties of table olives (Table 1), as a result 19 different fermentation media including 3 central points were prepared. Before starting fermentation process, a debittering step was applied to olive samples for a month in brine. After debittering stage, approximately 500 g of olive samples were mixed with sliced vegetables at certain ratios given in design table (Table 1) and put into sterile glass jars. The brine used in the fermentation was prepared by addition of 8%NaCI (w/v) and 0.5%citric acid (w/v) into drinking water and pH of the brine was setted up 4.5. After addition of brine, the fermentation was carried out at 25 °C for specified times given in the design table (Table 1). Moreover, the fermentation processes were also performed without additon of vegetables into fermentation media as control groups under same conditions.

Table 1 The experimental design table for fermentation process of table olives

Experiment no	Sample code	Fermentation time (days)	Vegetable type	Vegetable ratio (%)
1	BT51	5	Beetroot	10
2	BT52	5	Beetroot	20
3	BC51	5	Black Carrot	10
4	BC52	5	Black Carrot	20
5	BT101	10	Beetroot	10
6	BT102	10	Beetroot	20
7	BC101	10	BlackCarrot	10
8	BC102	10	Black Carrot	20
9	BT151	15	Beetroot	10
10	BT152	15	Beetroot	20
11	BC151	15	Black Carrot	10
12	BC152	15	Black Carrot	20
13	BT201	20	Beetroot	10
14	BT202	20	Beetroot	20
15	BC201	20	Black Carrot	10
16	BC202	20	Black Carrot	20
17	CP1	10	Beetroot	15
18	CP2	10	Beetroot	15
19	CP3	10	Beetroot	15

*BC: black carrot, BT:beetroot, CP:central point

Chemical property analyses

Determination of total phenolic content

The concentrations of total phenolic compounds (TPC) of olive and brine samples were determined with the Folin–Ciocalteu assay (Aktas et al. 2014). The absorbances of the samples were measured at 765 nm with a UV spectrophotometer (Optima SP3000, Japan). Total phenol content of the samples was calculated using the gallic acid standard curve and the results were expressed in milligrams of gallic acid equivalent (GAE) per 100 g or ml of samples.

Determination of total monomeric anthocyanin content

The pH differential method was used for the measurement of total monomeric anthocyanin content (TMA) of olive and brine samples (Cemeroglu 2010). Olive samples were kept at 4 °C in ethanol:HCl (85:15 v/v) solution overnight and then filtered. The centrifugated brine samples were diluted with deionized water. The aqueous buffer solutions having pH of 1.0 (potassium chloride, 0.025 M) and 4.5 (sodium acetate, 0.4 M) were mixed with olive extract and brine individually. The maximum absorbance value was determined by using deionized water as the blank (Optima SP3000, Japan). The anthocyanin content (mg/kg, mg/l) of samples was expressed as cyanidine-3-glucoside equivalent.

Determination total antioxidant capacity

DPPH Radical Scavenging method was used to measure total antioxidant capacity (TAC) of olive and brine samples (Akbas et al. 2017). The analyses were performed twice. TAC was expressed as mg DPPH per g or ml sample.

Color measurements

The color of olive samples was determined according to CIE XYZ color space model by measuring L* (lightness), a* (redness), and b* (yellowness) with a color measurement device (Minolta, Japan) and total color change (ΔE) values were calculated with Eq. (1). The color parameters including color density (IC), and percent color components (yellow (OD420%), red (OD520%) and blue (OD620%)) of centrifuged brine samples were measured by a spectrophotometer (Optima SP3000, Japan) at 420, 520, and 620 nm (Karaoglan et al. 2019) and calculated by using the Eqs. (2)–(5). The all color measurements were conducted twice.

$$\Delta E = \sqrt{(\Delta L^2) + (\Delta a^2) + (\Delta b^2)} \tag{1}$$

$$\text{Color density (IC)} = \text{OD}_{420} + \text{OD}_{520} + \text{OD}_{620} \tag{2}$$

$$OD420\% = \frac{OD420}{IC} * 100 \tag{3}$$

$$OD520\% = \frac{OD520}{IC} * 100 \tag{4}$$

$$OD620\% = \frac{OD620}{IC} * 100 \tag{5}$$

Physicochemical analyses

The percentages of salt content of olive and brine samples were analyzed by titration with a 0.1 N silver nitrate solution (Nielsen 2017). The pH of brine samples during fermentation were measured with a pH meter (Hanna HI2211, Germany). The total acidity of brine samples were determined by titration with 0.1 N NaOH solution and expressed as percentage of lactic acid (Cemeroglu 2010).

Microbiological property analyses

The microbiological properties of olive samples were analyzed at regular time intervals of fermentation. Total

viable bacteria count (TBC), total yeast and mold counts (TYMC) were calculated by using the standard pour and spread plate methods (Halkman 2005). The results were expressed as log cfu (colony forming unit) per g sample.

Sensorial analysis

The olives samples were served in individual white plates, codified with a three-digit number, with a glass of water to 20 untrained volunteer panelists (10 men and 10 women). The samples were evaluated using a preference test based on a nine-point hedonic scale (9 ¼ like extremely and 1 ¼ dislike extremely). The following characteristics were measured: color, sourness, saltiness, bitterness, odor and aroma characteristics. The sensorial analysis results were explained by analysis of variance (ANOVA) (Minitab 19 software, UK).

Statistical analyses

The data were analyzed by the ANOVA in order to investigate the effects of fermentation time, vegetable type, vegetable concentration and their interaction ($p < 0.05$) on chemical and microbiological properties of the olive and brine samples (Minitab 19 software, UK). Moreover, the principal component analysis (PCA) was also used (SIMCA 14.1, MKS Umetrics, Umea, Sweeden) to investigate the effects of processing parameters on table olive fermentation and also for classification of samples.

Results and discussions

Total phenolic content of olive and brine samples

The TPC of the olive and brine samples are given in Table 2 and 3, respectively. The TPC of the initial olive (only debittered olive) was calculated as 358.47 mg GAE/100 g. The TPC of fermented olive samples ranged from 97 to 843 mg

Table 2 Chemical and microbiological properties of olives fermented with vegetables, initial olive (IO) and control groups (CO)

	TPC (mg GAE/ 100 g)	TAC (mg DPPH/ g)	ΔE	SC%	TBC (log kob/g)	TYMC (log kob/g)	TMA (mg/kg)
BT51	161.81	30.57	12.70	8.06	7.58	7.57	5.93
BT52	127.98	26.33	26.08	7.13	7.60	7.58	8.43
BC51	187.92	30.11	17.94	6.43	7.58	7.59	5.43
BC52	97.15	29.98	16.63	4.44	7.52	7.53	15.86
BT101	643.22	24.86	11.49	6.31	6.67	6.72	124.82
BT102	632.92	16.63	21.14	5.61	6.50	6.34	149.87
BC101	842.68	20.18	11.14	6.19	6.48	6.36	93.93
BC102	870.55	18.82	15.86	4.79	6.33	6.28	154.05
BT151	159.29	10.11	11.46	8.06	6.30	6.27	31.31
BT152	147.61	8.12	12.12	5.61	6.04	6.18	91.84
BC151	131.41	10.13	12.42	5.84	6.45	6.46	66.80
BC152	195.48	7.52	9.71	5.14	6.75	6.53	96.02
BT201	314.05	2.01	18.58	6.90	6.16	6.27	106.46
BT202	204.26	3.27	24.36	6.55	6.23	6.19	110.21
BC201	176.49	9.67	10.66	7.25	6.47	6.25	112.72
BC202	253.24	8.30	13.40	6.43	6.71	6.59	106.46
CP1	548.25	31.04	15.83	5.49	6.52	6.46	164.48
CP2	618.39	30.38	17.26	5.96	6.35	6.46	136.51
CP3	599.77	32.29	12.22	6.55	6.05	6.06	119.40
IO	358.47	17.41	9.14	1.91	6.64	5.53	2.50
CO5	345.16	29.20	9.02	10.05	7.06	7.04	3.34
CO10	749.28	25.29	12.66	6.55	6.73	6.67	14.82
CO15	151.63	11.50	9.99	8.53	6.64	6.47	11.44
CO20	193.16	16.19	10.35	7.71	6.36	6.42	24.21

*Standard deviations (SD) $SD_{TPC} = 36.33$; $SD_{TAC} = 0.96$; $SD_{\Delta E} = 2.60$; $SD_{SC} = 0.53$; $SD_{TBC} = 0.24$; $SD_{TYMC} = 0.23$; $SD_{TMA} = 22.26$

(standard deviation for each measurement was calculated from three replicates of central points in experimental design)

Table 3 Chemical properties of brines of fermented olives, control groups (CB) and initial brine (IB)

Sample code	TPC (mg GAE/100 ml)	TAC (mg DPPH/ml)	TMA (mg/L)	IC	OD420 %	OD520 %	OD620 %	TA %	pH	SC %
BT51	69.33	9.56	213.75	1.61	0.09	0.29	0.02	0.58	3.39	6.66
BT52	74.81	9.69	259.67	3.13	0.07	0.29	0.02	0.47	4.19	8.42
BC51	58.65	9.98	288.06	1.65	0.10	0.28	0.02	0.28	3.17	7.48
BC52	70.53	9.13	358.19	3.93	0.09	0.29	0.01	0.23	4.37	8.53
BT101	296.45	14.04	458.80	0.98	0.07	0.30	0.01	0.41	2.84	6.31
BT102	352.31	10.53	483.85	1.82	0.06	0.30	0.01	0.42	3.62	5.14
BC101	430.35	18.89	361.11	0.54	0.10	0.30	0.00	0.22	3.48	3.39
BC102	564.24	15.60	479.68	1.47	0.10	0.29	0.01	0.25	3.59	4.56
BT151	526.04	21.42	480.93	1.62	0.10	0.21	0.09	0.53	3.53	5.96
BT152	492.78	21.64	510.15	2.63	0.08	0.30	0.01	0.50	3.62	6.90
BC151	545.35	18.13	507.65	1.90	0.11	0.29	0.01	0.21	3.33	5.14
BC152	712.51	19.51	584.46	2.76	0.10	0.29	0.01	0.23	3.40	5.96
BT201	911.70	47.87	880.03	0.80	0.07	0.30	0.02	0.45	3.47	4.44
BT202	1071.06	47.69	1182.28	1.77	0.06	0.30	0.01	0.40	3.84	5.14
BC201	1255.06	40.13	1127.17	0.52	0.05	0.25	0.06	0.26	4.02	3.74
BC202	1745.86	44.80	1221.52	0.97	0.09	0.25	0.05	0.22	4.52	3.97
CP1	364.22	20.58	522.26	1.22	0.06	0.30	0.01	0.46	3.14	4.68
CP2	426.65	18.84	512.24	1.52	0.07	0.29	0.02	0.50	3.16	3.62
CP3	341.22	17.78	453.37	0.93	0.06	0.30	0.02	0.51	3.23	4.09
CO5	21.95	1.87	3.34	0.22	0.46	0.11	0.07	0.53	2.64	7.60
CB10	35.35	1.93	5.64	0.67	0.14	0.14	0.14	0.42	2.37	8.88
CB15	47.97	1.98	5.59	0.44	0.45	0.12	0.07	0.49	3.22	7.95
CB20	54.69	2.29	7.51	0.17	0.25	0.14	0.12	0.45	4.71	7.25
IB	0.00	0.00	0.00	0.29	0.01	0.01	0.00	0.61	4.50	8.00

*Standard deviations (SD). $SD_{TPC}=44.2$; $SD_{TAC}=1.41$; $SD_{IC}=0.29$; $SD_{SC}=0.53$; $SD_{OY420}=0.005$; $SD_{OY520}=0.005$; $SD_{OY460}=0{,}003$; $SD_{pH}=0.04$;$SD_{TA}=0.02$

$SD_{TMA}=37.22$ (standard deviation for each measurement was calculated from three replicates of central points in experimental design)

GAE/100 g (Table 2). The TPC results of the control groups agreed with the findings of a study of Kalamata type table olives (Blekas et al. 2002). The TPC of fermented olives decreased on the 5th day of fermentation when compared to the initial olive (Fig. 1). The decline of TPC could be associated with the diffusion of phenolics from the olive to

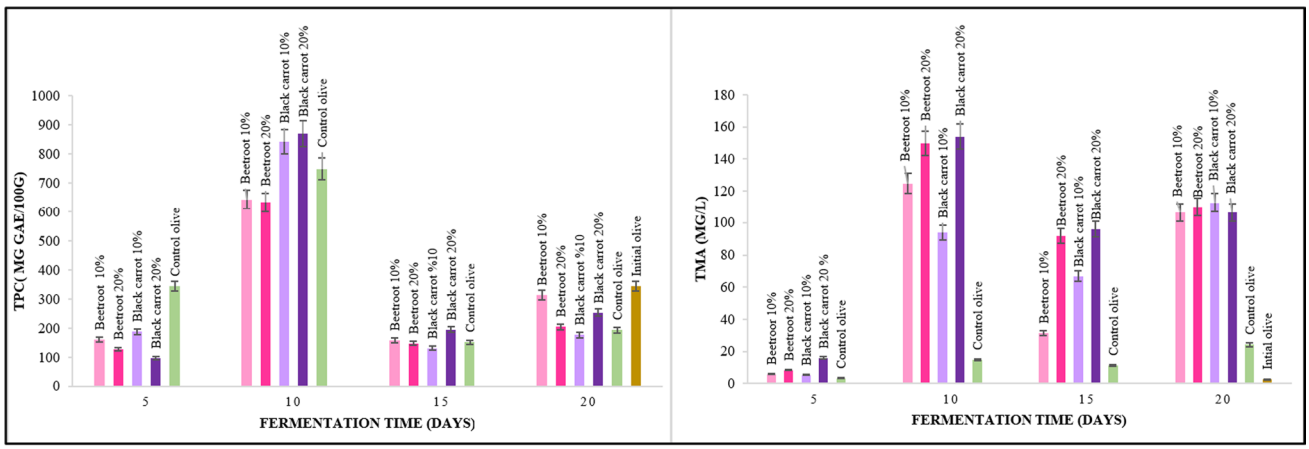

Fig. 1 The TPC and TMA content of olive samples during fermentation

the brine. However, as indicated by the main effect plot, a significant increase in TPC was observed in all olive samples on the 10th day of fermentation regardless of vegetable type or ratio (Fig. 2). In particular, the fermented olives that containing black carrot in the fermentation media had higher TPC values at the 10th day of fermentation (Fig. 1). This increase in TPC indicated that anthocyanins were properly diffused from black carrot to olives. However, TPC of olives diminished after the 10 days of fermentation regardless of vegetable type and ratio (Figs. 1 and 2). In a study about fermentation of Chétou type of green olives, there was a noticeable decrease in TPC during the first 10 days of fermentation process and the TPC of olives remained constant after the 20th day of fermentation (Othman et al. 2009). Another study that monitored the fermentation of four different types of green olives found out significant decreases in TPC during the first 20 days of fermentation (Kiai and Hafidi 2014). However, in this current work, phenolic compound loss could be limited by diffusion of anthocyanins derived from beetroot and black carrot on the 10th day of fermentation.

The TPC of brine samples significantly increased since the fermentation process was proceed (Table 3). The addition of black carrot and beetroot into the fermentation media turned out with the increases in TPC. The TPC of all samples were quite low on the 5th day of fermentation. The TPC of the brine samples increased and reached to maximum concentrations at the end of fermentation period, respectively. These increases in TPC could be induced by the diffusion of phenolic substances of olives and vegetables present in the fermentation media. The TPC was higher in brine samples containing 20% black carrot than in the others emphasizing the effect of vegetable concentration on diffusion. ANOVA results indicated that the TPC models constructed for both olive and brine samples were significant at the 95% confidence interval (Supp. Mat. 1 and 2). Fermentation time, the interaction between fermentation time and vegetable type were the prominent factors. The vegetable type was also found to be important for the TPC model of brine samples ($p < 0.05$).

Total monomeric anthocyanin content of olive and brine samples

The TMA contents of the olive and brine samples are shown in Table 2 and 3. Although green olives are rich in terms of phenolic components, their anthocyanin content is quite low. The TMA content of the initial olive was determined as 2.5 mg/kg (Table 2). While the TMA values of the olives were quite low on the 5th day of fermentation, the amount of monomeric anthocyanins of samples increased considerably by 10th day of fermentation (Fig. 1). This result demonstrated that anthocyanins from vegetables were successfully absorbed by olives. It was also discovered that as the vegetable concentration in the fermentation medium increased, so did the TMA values of olives. On the 10th day of fermentation, the samples fermented with 20% black carrot had the highest TMA content (Fig. 1). The TMA content of olives could be detected after the fourth stage of ripening, according to a study that examined the amount of anthocyanins during the ripening period in Cellina di Nard type olives. At the 7th stage of ripening, the TMA content of olives increased to 4.62 g/kg. The accumulation of cyanidin-3-glucoside and cyanidin-3-rutinoside compounds, which cause the color of olives to turn purple-black during ripening, was found to be responsible for the increase in TMA content. Otherwise, after fermentation, the TMA content of green olives decreased to 1.16 g/kg. (Aprile et al. 2019). The TMA content of the control group olives in our study was also compatible with this work. As a result, by integrating these vegetables into fermentation media, the TMA content

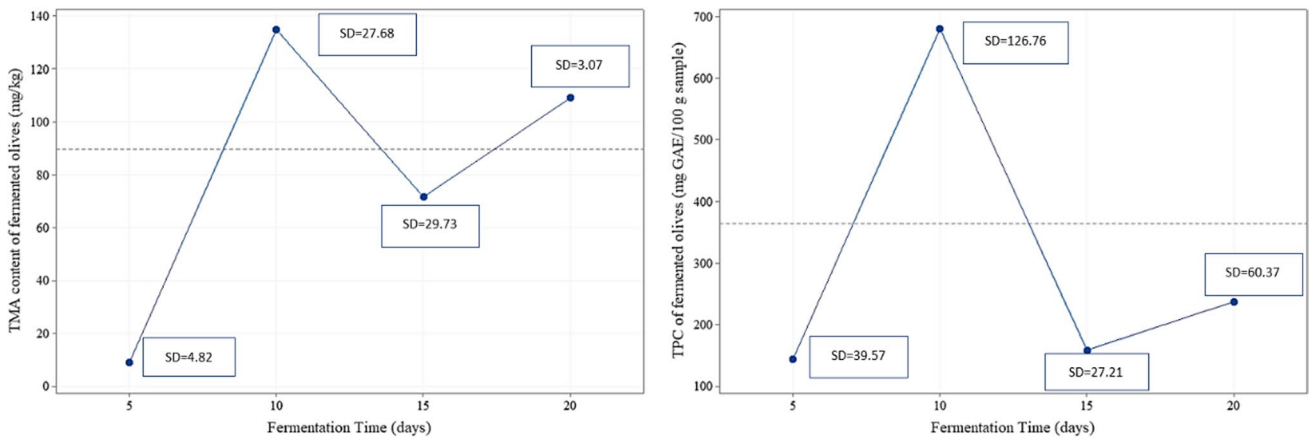

Fig. 2 The main effect plots of fermentation time on TMA and TPC of fermented olive samples

of fermented olives could be increased to 5.93–154.05 mg/kg.

Since the TMA values of the brine samples were examined during fermentation, it was discovered that the higher vegetable ratio in fermentation media, the higher the TMA content (Table 3). These increases in TMA contents could be attributed to the diffusion of anthocyanins from beetroot and black carrot into the brine during fermentation. A Pearson correlation was established between the TMA data of brine and olive samples to better explain anthocyanin diffusion during fermentation, and the 'r' value was calculated as 0.75. The Pearson correlation value highlighted the fact that the TMA content of both the olive and brine samples increased during fermentation. It has been revealed that the major anthocyanins in black olives were cyanidin-3-glucoside and cyanidin-3-rutinoside (Rocha et al. 2020). Furthermore, previous research has shown that these anthocyanins are generally degraded, particularly during the debittering, brining, and fermentation processes (Kiai et al. 2020; Durante et al. 2018; Gandul-Rojas and Gallardo-Guerrero 2018). In the current study, however, enrichment of fermentation media with anthocyanin-containing vegetables resulted in increases in the TMA content of both brine and olive samples. ANOVA table demonstrated that the models for TMA (Supp. Mat 1 and 2) were important and fermentation time was the major factor that effecting TMA content of samples ($p < 0.05$). The vegetable concentration was also significant for the TMA model of brine samples (Supp. Mat. 2).

Total antioxidant capacity of olive and brine samples

The TAC values of samples are presented in Tables 2 and 3. While the TAC of all olive samples was higher at the beginning of fermentation, the TAC values gradually decreased as fermentation progressed (Table 2). In the first 10 days of fermentation, the TAC values of olives fermented with 10% beetroot were higher than those of the other samples. The TAC values of olives fermented with beetroot decreased significantly by the 20th day of fermentation, regardless of the vegetable ratio. The TAC values decreased on the 15th day of fermentation, which was also observed in the TPC of olives. The previous studies have also revealed a relationship between the TAC and TPC of fermented olives (Malheiro et al. 2011; Pereira et al. 2006).

In contrast to the TAC results of the olives samples, the TAC values of the brine samples were raised by proceeding fermentation process (Table 3). Since the effect of the vegetable types was considered, the brine samples containing black carrot had higher TAC values on the 10th day of fermentation. However, the TAC of brine samples containing beetroot in the fermentation media increased significantly after the 10th day. It was clear that the presence of various vegetables in the fermentation medium allowed for the diffusion of phenolic compounds into the brine and increased the TAC of brine samples. According to ANOVA tables, the models for TAC were significant. Fermentation time and vegetable ratio ($p < 0.05$) had remarkable effects on the TAC values of brine and olive samples. It was also statistically revealed that the interaction between vegetable type and fermentation time was also important for TAC values of brine samples (Supp. Mat 1 and 2).

Color of olive and brine samples

Some color changes in fermented olives were observed during the fermentation process as a result of anthocyanin diffusion (Table 2). The olives fermented with 20% beetroot produced the most noticeable color change. The color of the samples varied between pink and red depending on the diffusion of anthocyanins to green olives. The a* values of fermented olives were positive and ranging from 6.85 to 21.42. An increase in a* values was also observed in another study that examining green fermented olives colored with turnip juice (Erbay et al. 2010). Although the b* values of fermented olives varied during fermentation, they were generally lower than the b* values of control group olives. The L, a*, and b* values of the olive samples were used to calculate the ΔE values. The ΔE values of the fermented olives corresponded to the ΔE values of colored Memecik olives (Erbay et al. 2010). According to previous studies, the color change could be caused by either the degradation of the chlorophyll pigment or the activity of the polyphenol oxidase enzyme in fermented olives (Gallardo-Guerrero et al. 2013; Ramírez et al. 2015). The ANOVA table presented that constructed model for ΔE values of fermented olives was insignificant ($p > 0.05$) (Supp. Mat 1).

The color density (IC) and percent color components (OD) were used to evaluate the color changes in brine samples during fermentation (Table 3). As the ratio of vegetables in the fermentation medium increased, the IC values of brine samples improved. The changes in IC are induced by the diffusion of anthocyanins from vegetables into the brine. The OD values of the brine samples fluctuated during fermentation. The ANOVA table revealed that only the models created for IC and OD420% were significant ($p < 0.05$). It was determined statistically that the fermentation time and vegetable ratio had a prominent effect on IC and vegetable type was the only factor affecting OD420% of brine samples (Supp. Mat. 2).

Physicochemical results of olive and brine samples

The pH values of the brine samples are given in Table 3. Before initiating the fermentation process, the pH of the brine was adjusted to 4.5 to allow for the growth of LAB. The pH of the brine samples changed in the range

of 2.64–4.71 since the fermentation progressed (Table 3). The previous study has shown that the organic acids produced by LAB cause decreases in the pH values of the brine samples (García-Serrano et al. 2020). Therefore, a Pearson correlation between the total acidity and pH values of the brine samples was established. Since the 'r' value was determined to be -0.4, there was a negative and moderate correlation between the pH and total acidity values of brine samples. The Pearson correlation demonstrated that pH values decreased as total acidity increased during fermentation. The ANOVA table presented that the model constructed for the pH was significant at the 95% confidence interval (Supp. Mat 2). The fermentation time and the vegetable concentration were statistically important factors in terms of pH ($p < 0.05$).

The percentages of total acidity of brine samples are expressed in Table 3. The total acidity values of the brine sample varied between 0.21 and 0.58%. Generally, the brine samples containing black carrot had lower acidity. There was a decrease in the total acidity of samples on the 10th day of fermentation. The total acidity increased during the first 20 days of fermentation in a study of three different Spanish green olives (Garca-Serrano et al. 2020). The activity of homofermentative and heterofermentative LAB, which play important roles in olive fermentation and have the ability to synthesize different acids, could explain the ups and downs in total acidity of brine samples. The LAB are able to produce organic acids including lactic, acetic, and propionic acids during fermentation resulting in some increases in acidity of the brine samples (Rodríguez-Gómez et al. 2017). The model developed for total acidity of brine samples was found to be statistically significant in the 95% confidence interval and all factors were significant in terms of total acidity (Supp. Mat. 2).

The salt ratio of the initial olive was 1.91%. The salt content of fermented olives increased regardless of vegetable type or ratio (Table 2). However, the salt content of olives fluctuated during fermentation. This result could be associated with NaCl diffusion into olives. The olives fermented with different vegetables had lower salt content than the regular fermented samples. In a study, Memecik olives were colored with turnip juice, lycopene solution and dye, and the percentage salt content of the olive samples ranged from 3.22 to 5.36 (Erbay et al. 2010). The salt content results of this current work were comparable to the findings of this study. In general, olives fermented with 20% black carrot had lower salt content, which may be preferred by consumers with high blood pressure. The model developed for the salt content of fermented olives was found to be statistically significant at the 95% confidence interval. The vegetable type and ratio, as well as the interaction between fermentation time and vegetable ratio were important factors ($p < 0.05$) (Supp. Mat. 1). The salt content of the initial brine was 8% (Table 3). The previous researches recommended using brine containing 4–8% salt for the growth of LAB in table olive fermentation (Pino et al. 2019). Moreover, the presence of salt in the fermentation medium is critical for inhibiting the growth of pathogenic microorganisms as well as providing desirable sensory properties (Bautista-Gallego et al. 2013). The addition of vegetables into the fermentation medium reduced the salt content of the brine. The brine containing black carrot had lower salt content than the other samples, especially on the 10th and 20th days of fermentation. The model developed for salt content of brine was statistically significant, and all factors were prominent ($p < 0.05$) in terms of brines' salt content (Supp. Mat. 2).

Sensorial analysis

The sensorial analysis was carried out by 20 panelists, ten of who were women and ten of who were men. Color, sourness, salinity, bitterness, odor, and aroma criteria for fermented olive samples were scored from 1 to 9. The panelists tasted the fermented olive samples on specific days of fermentation (5, 10, 15 and 20 days). ANOVA table is used to express the sensorial analysis results (Supp. Mat. 3). The panelists were given the option of granting hedonic evaluation scores ranging from 1 to 9 for the general acceptance of fermented olives and the average scores of the general acceptance are shown in Supp. Mat. 4.

The ANOVA table revealed that there were significant differences in terms of criterias including general taste, color, sourness, bitterness, aroma and odor on the different days of fermentation ($p < 0.05$). The salinity was the only criteria that not affected by fermentation days. Except for salinity and bitterness, the vegetable type and ratio resulted in significant differences across all criteria (Supp. Mat. 3). Since the sensory analysis results were evaluated in terms of general acceptance on the 10th day of fermentation, it was found out that the fermented olives containing 20% black carrot had the highest score (7.9) and followed by the olive samples fermented with 20% beetroot (7.3) (Supp. Mat. 4). The addition of vegetables into the fermentation media improved chemical properties of olive samples and these results were supported by the panelists' scores. The sensorial analysis results agreed with PCA models and ANOVA tables as well.

Microbiological properties of olives

The results of TBC and TYMC of olive samples are shown in Table 2. The TBC of fermented olives varied between 6.05 and 7.58 log cfu/g (Table 2). The TBC of the samples increased slightly on the 5th day of fermentation when compared to the initial olive samples. This increase in TBC indicated that the desired lactic acid fermentation

for table olive production had started. However, there were some ups and downs in the TBC of olives during fermentation period. These fluctuations could be associated with the drops in pH and the accumulation of secondary metabolites produced by the LAB throughout fermentation. TBC were also not very high in a study that monitored the microbiological properties of six different Italian olives during fermentation, with values ranging from 4.88 to 5.85 log cfu/g (Tofalo et al. 2012). In other research, it was discovered that the TBC of fermented olives was also not very high (Pereira et al. 2006). The lower TBC of fermented olives could be explained by the presence of NaCl in the medium, the decrease in pH and the antimicrobial effects of the phenolic compounds found in olives, beetroot, and blackcarrot. The TYMC of olives ranged from 6.06 to 7.59 log cfu/g (Table 2). Similar to our findings, the TYMC were found in the range of 4.24–5.78 log cfu/g in a study of the microbiological properties of Italian olives during fermentation process (Tofalo et al. 2012). The number of yeast-mold decreased slightly on the 5th day of fermentation, and there were no significant differences in TYMC of olives until the end of fermentation. A similar trend was observed in a study on Halkidiki type olives, in which microbial change of fermented olives was detected (Blana et al. 2014). In general, the decline in TYMC is satisfying by means of the product's shelf life and long-term consumption. Moreover, the presence of yeast and mold in higher counts in the fermentation medium may induce the synthesis of pectolytic enzymes which have negative effect on the textural properties of table olives (Golomb et al. 2013).

According to the ANOVA table, both the models for TBC and TYMC were significant and the fermentation time ($p < 0.05$) was the only factor affecting microbiological properties of the olive samples statistically (Supp. Mat. 1).

In order to better understand the anthocyanin diffusion process during the fermentation period and to classify the olive samples a principal component analysis (PCA) was applied. The number of components of the PCA model was 6 and $R^2 = 0.99$, $Q^2 = 0.93$. The olive samples were colored with respect to fermentation time (Fig. 3a). Since the scatter plot is examined, it was observed that the olive samples were grouped together according to the fermentation time (Fig. 3a). While the olives fermented for 5 days were in the upper right part of the plot, the 10 day fermented olives were clustered in the upper left part of the ellipse. The olives fermented for 15 and 20 days did not separate from each other and placed together at the bottom part of the score plot. The 10th day samples were differentiated from other olives in terms of TPC and TMA (Fig. 3a). It has been previously discussed that the TPC and TMA of the fermented olive samples were higher on the 10th day of fermentation period compared to the other days. Microbiological characteristics distinguished 5th day olive samples from other olive samples since TBC and TYMC were found to be higher on the 5th day of fermentation.

A PCA model was also constructed for the brine samples in order to better explain the changes in brine during the fermentation period. The number of components of the PCA model was 6 and $R^2 = 0.99$, $Q^2 = 0.81$. As in the PCA model of olive samples, the brine samples were also grouped together based on fermentation time (Fig. 3b). While the

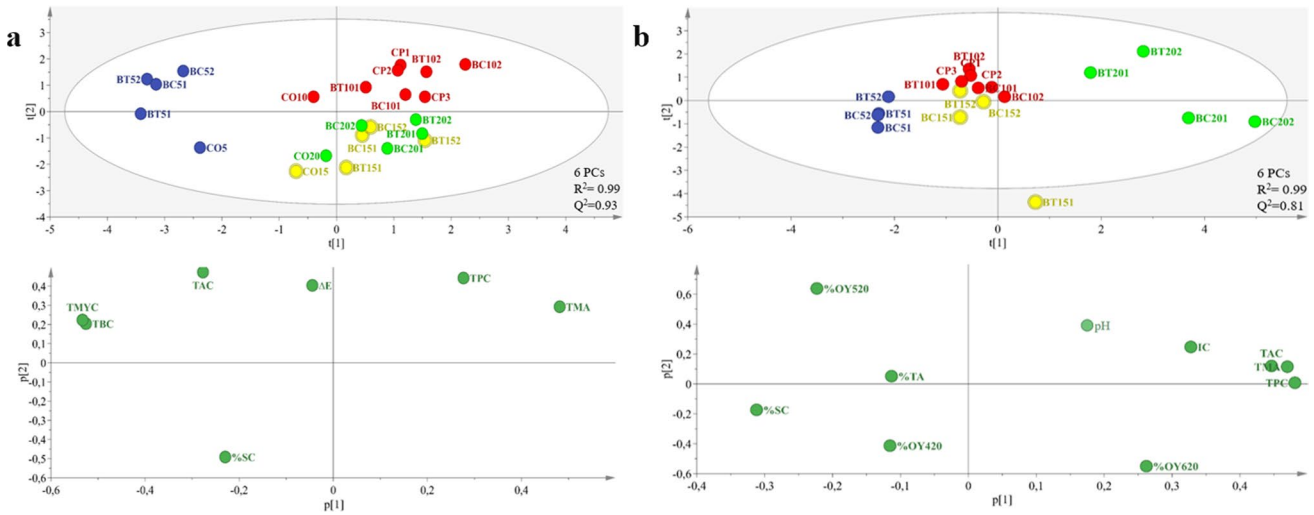

Fig. 3 **a** Score plot (coloring is done with respect to fermentation time. Blue: 5 days, red: 10 days, yellow:15 days, green:20 days) and loading plot obtained from principal component analysis for chemical and microbiological parameters of olive samples. **b** Score plot (coloring is done with respect to fermentation time. Blue: 5 days, red: 10 days, yellow:15 days, green:20 days) and loading plot obtained with principal component analysis for chemical parameters of brine samples (color figure online)

samples fermented for 5 days were in the upper left part of the plot, the brine samples belonging 10 days fermentation placed in the left bottom of the ellipse. The brine samples collected on the 15th day of fermentation took place in between the samples obtained on the 5th and 10th days of fermentation. The brine samples fermented for 20 days were distributed around the right part of the score plot (Fig. 3b). The 5th day brine samples differentiated from others in terms of SC% (Fig. 3b). The salt content of the brine samples were higher on the 5th day of fermentation compared to the other days. The 20th day brine samples grouped together in terms of TPC, TAC and TMA content. It has also explained that TPC, TAC and TMA values of brine samples increased since fermentation progressed.

Conclusion

The development of a new production technique for green table olives was achieved. The functional properties of green table olives were improved by the addition of different vegetables in the fermentation media. The diffusion of anthocyanins was carried out and confirmed by the changes in TMA, TPC and color values of olive and brine samples. The fermentation time was the important factor that affects the end product. It was observed that 10 days of fermention time had positive effects on the chemical properties of the table olives. The TPC and TMA content of olives increased at the 10th day of fermentation. Since, the fermented olives have lower salt content, they could be preferred by patients with high blood pressure. The microbiological properties of table olives were reasonable. Sensorial analysis results indicated that the olive samples fermented for 10 days with 20% vegetable concentration received the highest scores. As a result, it is suggested that anthocyanin enrichment of table green olives could be accomplished by lactic acid fermentation with 20% vegetable concentration for 10 days under the specified conditions. The production of anthocyanin-enriched green table olives on a larger scale could be enhanced in the food industry. A future study could focus on the changes in chemical and microbiological properties of green table olives enriched with anthocyanins under different storage conditions.

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References

Akbas E, Kilercioglu M, Onder ON, Koker A, Soyler B, Oztop MH (2017) Wheatgrass juice to wheat grass powder: Encapsulation, physical and chemical characterization. J Func Foods 28:19–27

Aktas AB, Ozen B, Tokatli F, Sen I (2014) Phenolics profile of a naturally debittering olive in comparison to regular olive varieties. J Sci Food Agri 94(4):691–698

Amanpour A, Kelebek H, Selli S (2019) Characterization of aroma, aroma-active compounds and fatty acids profiles of cv. Nizip Yaglik oils as affected by three maturity periods of olives. J Sci Food Agri 99(2):726–740

Anagnostopoulos DA, Goulas V, Xenofontos E, Vouras C, Nikoloudakis N, Tsaltas D (2020) Benefits of the use of lactic acid bacteria starter in green cracked cypriot table olives fermentation. Foods 9(1):17

Aprile A, Negro C, Sabella E, Luvisi A, Nicolì F, Nutricati E, De Bellis L (2019) Antioxidant activity and anthocyanin contents in olives (cv cellina di nardò) during ripening and after fermentation. Antioxid 8(5):138

Bautista-Gallego J, Rantsiou K, Garrido-Fernández A, Cocolin L, Arroyo-López FN (2013) Salt reduction in vegetable fermentation: reality or desire? J Food Sci 78(8):R1095–R1100

Blana VA, Grounta A, Tassou CC, Nychas GJE, Panagou EZ (2014) Inoculated fermentation of green olives with potential probiotic *Lactobacillus pentosus* and *Lactobacillus plantarum* starter cultures isolated from industrially fermented olives. Food Microbiol 38:208–218

Blekas G, Vassilakis C, Harizanis C, Tsimidou M, Boskou DG (2002) Biophenols in table olives. J Agri Food Chem 50(13):3688–3692

Caponio F, Difonzo G, Calasso M, Cosmai L, De Angelis M (2019) Effects of olive leaf extract addition on fermentative and oxidative processes of table olives and their nutritional properties. Food Res Int 116:1306–1317

Cemeroglu B (2010) Gıda Analizleri Genişletilmiş 2. Baskı. Gıda Teknolojisi Derneği Yayınları. Ankara

Durante M, Tufariello M, Tommasi L, Lenucci MS, Bleve G, Mita G (2018) Evaluation of bioactive compounds in black table olives fermented with selected microbial starters. J Sci Food Agri 98(1):96–10

Erbay B, Kucuksayan S, Kucukoner E (2010) Renklendirilmiş fermente "Memecik" çeşidi zeytinlerin fiziksel, kimyasal ve duyusal özellikleri. Akademik Gıda 8(6):13–18

Gallardo-Guerrero L, Gandul-Rojas B, Moreno-Baquero JM, López-López A, Bautista-Gallego J, Garrido-Fernández A (2013) Pigment, physicochemical, and microbiological changes related to the freshness of cracked table olives. J Agri Food Chem 61(15):3737–3747

Gandul-Rojas B, Gallardo-Guerrero L (2018) Pigment changes during preservation of green table olive specialities treated with alkali and without fermentation: effect of thermal treatments and storage conditions. Food Res Int 108:57–67

García-Serrano P, de Los SB, Sánchez AH, Romero C, Aguado A, García-García P, Brenes M (2020) Progress on green table olive processing with KOH and wastewaters reuse for agricultural purposes. Sci Total Environ 746:141150

Golomb BL, Morales V, Jung A, Yau B, Boundy-Mills KL, Marco ML (2013) Effects of pectinolytic yeast on the microbial composition and spoilage of olive fermentations. Food Microbiol 33(1):97–106

Halkman AK (2005) Merck gıda mikrobiyolojisi uygulamaları. Başak Matbaacılık, Ankara

IOOC (2004) Trade standards applying to table olives. Document COI/OT/NC No. 1. https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OT-NC1-2004-Eng.pdf. Accesed 10 May 2022

Karaoglan HA, Keklik NM, Isikli ND (2019) Degradation kinetics of anthocyanin and physicochemical changes in fermented turnip juice exposed to pulsed UV light. J Food Sci Technol 56(1):30–39

Khosravi H, Saedi SI, Rezaei M (2021) Real-time recognition of on-branch olive ripening stages by a deep convolutional neural network. Sci Hortic 287:110252

Kiai H, Hafidi A (2014) Chemical composition changes in four green olive cultivars during spontaneous fermentation. LWT-Food Sci Technol 57(2):663–670

Kiai H, Raiti J, El Abbassi A, Hafidi A (2020) Chemical profiles of Moroccan Picholine olives and its brines during spontaneous fermentation. Int J Fruit Sci 20(3):S1297–S1312

Malheiro R, Sousa A, Casal S, Bento A, Pereira JA (2011) Cultivar effect on the phenolic composition and antioxidant potential of stoned table olives. Food Chem Toxicol 49(2):450–457

Nielsen SS (2017) Food analysis laboratory manual. Springer, Berlin

Othman NB, Roblain D, Chammen N, Thonart P, Hamdi M (2009) Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. Food Chem 116(3):662–669

Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A (2006) Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. J Agri Food Chem 54(22):8425–8431

Perpetuini G, Prete R, Garcia-Gonzalez N, Khairul Alam M, Corsetti A (2020) Table olives more than a fermented food. Foods 9(2):178

Pino A, Vaccalluzzo A, Solieri L, Romeo FV, Todaro A, Caggia C, Arroyo-Lopez FN, Bautista-Gallego J, Randazzo C (2019) Effect of sequential inoculum of beta-glucosidase positive and probiotic strains on brine fermentation to obtain low salt Sicilian table olives. Front Microbiol 10:174

Ramírez E, Gandul-Rojas B, Romero C, Brenes M, Gallardo-Guerrero L (2015) Composition of pigments and colour changes in green table olives related to processing type. Food Chem 166:115–124

Ramírez E, Brenes M, de Castro A, Romero C, Medina E (2017) Oleuropein hydrolysis by lactic acid bacteria in natural green olives. LWT-Food Sci Technol 78:165–171

Rocha J, Borges N, Pinho O (2020) Table olives and health: a review. J Nutr Sci 9:e57

Rodrigues N, Marx IM, Dias LG, Veloso AC, Pereira JA, Peres AM (2019) Monitoring the debittering of traditional stoned green table olives during the aqueous washing process using an electronic tongue. LWT-Food Sci Technol 109:327–335

Rodríguez-Gómez F, Ruiz-Bellido MA, Romero-Gil V, Benítez-Cabello A, Garrido-Fernández A, Arroyo-López FN (2017) Microbiological and physicochemical changes in natural green heat-shocked Aloreña de Málaga table olives. Front Microbiol 8:2209

Talhaoui N, Taamalli A, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A (2015) Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits. Food Res Int 77:92–108

Tofalo R, Schirone M, Perpetuini G, Angelozzi G, Suzzi G, Corsetti A (2012) Microbiological and chemical profiles of naturally fermented table olives and brines from different Italian cultivars. Antonie Van Leeuwenhoek 102(1):121–131