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Phenolic Profile and Antioxidant Properties of Sarıulak Olive Fermented with Red Beetroot

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Abstract

BACKGROUND/AIMS: Phenolic compounds are essential for enhancing the quality of table olives, contributing to their taste, aroma, color, and shelf life. Their antioxidant properties also provide health benefits, including protection against low-density lipoprotein oxidation and prevention of oxidative damage. The purpose of this study is to analyze the phenolic composition and antioxidant characteristics of the Sarıulak olive variety, which is fermented alongside red beetroot (*Beta vulgaris* L.).

MATERIALS AND METHODS: In this study, geographically certified Tarsus Sarıulak olives sourced from Mersin, Türkiye, and red beets obtained from Bursa, Türkiye, were utilized. Preliminary trials demonstrated that the optimal outcome was achieved with a 20% red beetroot ratio. The fermentation process, which lasted 128 days, was conducted using 10 kg of green olives in 9 liters of 7% brine solution. Fermentation was carried out in 12 L PVC-reinforced polyethylene airtight containers. Phenolic compounds were identified through high-performance liquid chromatography, while total phenolic content and antioxidant activity were evaluated using a spectrophotometer.

RESULTS: Olives contain around 30 phenolic compounds, with total phenolic content ranging from 100 to 800 mg/kg. Fresh olives, rich in oleuropein and hydroxytyrosol, have higher phenolic content than processed ones. In this study: oleuropein content in fresh olives ranged between 667.16 and 721.18 mg/kg, but dropped to 17.35-20.91 mg/kg during fermentation. Red beetroot addition further reduced oleuropein levels to 14.25-22.41 mg/kg, indicating enhanced hydrolysis. Hydroxytyrosol levels increased by 38% in red beet-added olives, while they decreased by 54% in the control group. Antioxidant activity improved significantly, reaching 32.43% with red beet addition, compared to 12.67% in the control.

CONCLUSION: The results demonstrate that incorporating red beetroot during olive fermentation enhances the hydrolysis of oleuropein, increases hydroxytyrosol levels, and boosts antioxidant activity. These effects contribute to producing a healthier olive product with higher functional value. Future studies are recommended to further explore the underlying mechanisms and long-term stability of these benefits.

Keywords: Sarıulak olive, red beetroot, fermentation, phenolic compounds, antioxidants

INTRODUCTION

Olive cultivation is primarily practiced in Mediterranean countries like Spain, Türkiye, Greece, Italy, and others, and Italy, due to the specific climate requirements of olive trees. Currently, 93% of the world's olive trees are in these regions. In the 2022/2023 season, Türkiye ranked second in global olive oil production with 380,000 tons after Spain's 665,800 tons, while Greece produced 345,000 tons, and Italy, 288,900 tons. These countries are key players in global olive oil production.[1] The Sarıulak olive variety, cultivated in Mersin and Adana, accounts for approximately 6% of the olive trees in the Mediterranean region. This variety yields medium-sized, cylindrical fruits with an oil content ranging from 18% to

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22% and can be utilized for both green and black table olives.[2] Sensitive to low temperatures, the Tarsus Sarıulak variety originates from Tarsus in Mersin.[3,4] The primary objective of table olive processing is to remove[5] phenolic acids, flavonoids, sugars (approximately 95% sucrose), vitamins (A, B1, B2, B3, C, folate), and essential minerals (calcium, potassium, magnesium, iron). Betalains in red beet prevent oxidation, scavenge free radicals, and offer health benefits, including probiotic effects and enhanced antioxidant activity. They also improve nutritional and sensory quality by increasing microbial diversity during fermentation.[6-14] Red beetroot is rich in betalains, pigments that give it red and yellow hues. These include red betacyanins and yellow betaxanthins, with betanin being the dominant betacyanin, making up 75-95% of betalains. Betanin is widely used as a food colorant (E162). Interest in betalains is growing due to their stability in mildly acidic conditions and better water solubility compared to anthocyanins, making them a preferred natural colorant in the food industry.[15-17] Phenolic compounds are key elements that improve the quality of table olives by enhancing their taste, aroma, color, and shelf life, alongside providing their antioxidant benefits. Additionally, these compounds are recognized for their positive effects on human health, including anti-inflammatory and antimicrobial properties, which contribute to overall well-being.[18,19] Phenolic compounds in olives are essential for health benefits, owing to their antioxidant properties, protective effects against low-density lipoprotein oxidation, and potential to prevent oxidative damage.[5] These compounds, including flavonoids and phenolic acids, significantly contribute to the sensory qualities and health effects of olive products. Oleuropein, a major secoiridoid in olives, provides strong antioxidant properties and acts as a natural defense against pests.[18] Oleuropein consists of three key components: an apolar elenolic acid, a secoiridoid water-soluble glucose molecule, and hydroxytyrosol (HTrz), a polyphenol with antioxidant properties. While elenolic acid has no direct function, it helps form oleuropein, a strong antioxidant, when glucose and hydroxytyrosol are added.[5,20,21] This study aims to identify the phenolic profile and antioxidant properties of the Sarıulak olive variety fermented with red beetroot (*Beta vulgaris* L.) while also evaluating the feasibility of producing beetroot-enriched olives.

MATERIALS AND METHODS

Olive Samples and Experimental Design

In this study, geographically registered Tarsus Sarıulak olives from Mersin, Türkiye, and red beets from Bursa, Türkiye, were used. The fermentation lasted 128 days, involving 10 kg of green olives in 9 liters of 7% brine. The olives were fermented in two salt solutions: 7% NaCl (Sample Y) and 7% NaCl + 20% red beetroot (sample K). They were placed in 25 kg polyethylene crates, stacked up to three high in a closed truck, and transported to the Food Engineering Department of Çukurova University on the same day. The peels of the red beets were removed, and chopped into small pieces before being added to the olive fermentation process (day 0). Preliminary studies showed that the optimal red beetroot supplement was 20% (2 kg beetroot). The fermentation was conducted in airtight vessels kept in a cool, dark environment under controlled conditions, with salt concentrations maintained at 7%. After fermentation, the olives were stored at -20 ºC for further analysis.

Chemicals

Methanol (CAS No 67-56-1), acetic acid (CAS No 64-19-7), and hexane (CAS NO 110-54-3) of high-performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany). Standards for phenolic compounds, including hydroxytyrosol (3,4-Dihydroxyphenyl Ethanol, CAS NO 10597-60-1), Tyrosol (CAS NO 501-94-0), verbascoside (CAS NO 61276-17-3), p-coumaric acid (CAS NO 501-98-4), vanillic acid (CAS NO 121-34-6), oleuropein (CAS NO 32619-42-4), rutin (CAS NO 153-18-4), and apigenin-7-glucoside (CAS NO 578-74-5), were supplied by Sigma-Aldrich [St. Louis, MO, United States of America (USA)] and Fluka Chemie GmbH (Buchs, Switzerland). The chemicals Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid CAS NO 53188-07-1) and the Folin-Ciocalteu (CAS NO 12111-13-6) phenol reagent were sourced from Merck, while 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CAS NO 1898-66-4) was purchased from Sigma-Aldrich.

Preperation of Sample Extracts

One point five grams of olive with the pit removed, ground into a paste using a grinder, was weighed and 20 mL of 80% methanol was added. After being broken down using an ultraturrax, the sample was washed twice with n-hexane (CAS NO 110-54-3) to remove the oil, and homogenized by vortexing. The extract was obtained by centrifuging at 6000 rpm for 10 minutes (min). After passing through a Durapore 0.45 µm, 47 mm PTFE (Polytetrafluoroethylene) membrane filter, the sample was directly injected into the HPLC system. For the phenolic compound analysis, 20 mL of methanol (80:20) was added to the 1.5 g olive sample, and the extract was obtained by centrifugation. The sample was blended and washed twice with 10 mL of n-hexane to remove the oil.

Determination of Total Phenolic Content

The method proposed by[22,23] was carried out with slight modifications. In the proposed method, phenolic compounds reduce phosphomolybdic-phosphotungstic agents in the Folin-Ciocalteu solution to form a blue-colored complex; this color change is measured spectrophotometrically at 765 nm.

Determination of Antioxidant Activity

In the analysis of olive extracts, the spectrophotometric method proposed by[24] was adapted to be suitable for this purpose. For the analysis, 100 µL of extract was combined with 3000 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH*; 0.025 g/L in 80% methanol). For the control sample, 100 µL of pure water was used instead. The samples were subsequently blended and kept in the dark for one hour to allow the reaction to stabilize. After this incubation period, the absorbance of the samples was recorded at 515 nm using a spectrophotometer (Perkin Elmer Lambda 25 UV/VIS, Massachusetts, USA, 2005), with an 80% methanol solution serving as the reference.

High-Performance Liquid Chromatography Analysis of Phenolic Compounds

The phenolic compounds in the extracts were analyzed using HPLC on a Shimadzu LC-20AT system (Kyoto, Japan, 2006) with a diode array detector and a 4.6 mm × 250 mm i.d., 5 µm particle size reversed-phase C18 column (Waters, USA). The mobile phase consisted of water with 5% formic acid (solvent A) and acetonitrile (solvent B), with a gradient elution starting at 5% formic acid, progressing to 10% B at 15 min, 15% B at 20 min, 25% B at 30 min, 35% B at 50 min, 55% B at 75 min, 40% B at 53 min, 75% B at 90 min, and finally 100% B at 93 min. The column was maintained at 30 °C with a flow rate of 1 mL/minimum and an injection volume of 40 µL. Detection occurred at wavelengths of 280 nm and 320 nm. The phenolic compounds were identified and quantified by comparing their retention times with those of standard compounds, and the results were expressed as mg/kg on a wet weight basis.

Statistical Analysis

The results of the analysis of raw olives and those obtained during fermentation were subjected to analysis of variance. Duncan's multiple range test was used to assess significant differences, with IBM SPSS Statistics 22 statistical software employed for the analysis.

RESULTS

Total Phenolic Content and Antioxidant Activity of Table Olives

The total phenolic content and antioxidant activity of the studied olives are presented in Figures 1, 2. Table olives and olive oils are noted to be valuable sources of "functional foods" due to the phenolic antioxidant compounds they contain.[25] It has been reported that the total phenolic content in olives varies depending on the variety.[26] The total phenolic content of olives at the start of fermentation ranged from 4601.17 to 4879.49 mg gallic acid/kg, decreasing to 1451.76 to 1827.74 mg/kg by the end. The highest phenolic content was observed at day 0, with a slight decrease by day 60 and more noticeable reduction in the control sample compared to the red beet-enriched olives. In a study on green olive fermentation with 10% and 20% red beet,[27] reported an initial total phenolic content of 3584.70 mg gallic acid/kg. As fermentation progressed, total phenolic content decreased, with a slight increase at the end. Antioxidant activity was higher at the start (52.49%-54.50%) than at day 60 and day 128, showing a significant difference between day 60 and day 128 ($p<0.05$). Previous studies have shown that unfermented green olives have the highest antioxidant capacity (89%), with a decline observed during fermentation.[28,29] In this study, the lowest antioxidant activity at the end of fermentation (12.67%) was recorded in the control sample, while the highest value (32.43%) was observed in the olives with red beet addition. The increase in antioxidant activity was attributed to the red beet supplementation. Antioxidant activity values decreased during the middle of the fermentation process. It has been suggested that the reduction in antioxidant capacity during processing is linked to the decrease in total phenolic compounds, with a linear relationship between total phenolics and antioxidant capacity.[26,30] Several studies have reported that betalains exhibit strong antioxidant activity, indicating that the addition of red beet enhances the antioxidant activity of olives. This is due to the positive correlation between betalain content and antioxidant properties. Plants rich in betalains are recognized for their high antioxidant capabilities.[31] A study showed that olives with red beet had higher antioxidant capacity at the start, which decreased during fermentation, peaking in the middle. Increased red beet content also raised antioxidant activity.[27]

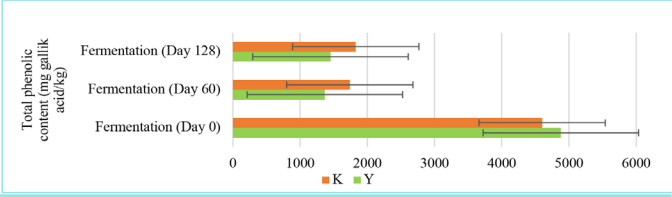

Figure 1. Total phenolic content of table olives (on a wet basis). Bars with different letters indicate significant differences ($p<0.05$). Y represents 7% NaCl and K represents 7% NaCl + 20% red beetroot.

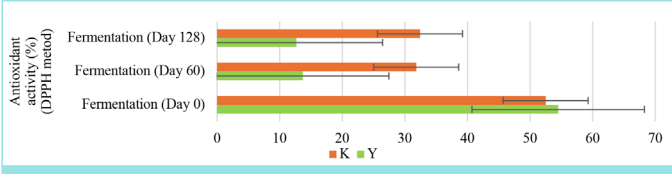

Figure 2. Antioxidant activity of table olives (on a wet basis). Bars with different letters indicate significant differences ($p<0.05$). Y represents 7% NaCL and K represent 7% NaCL + 20% red beetroot.

DPPH: 2,2-diphenyl-1-picrylhydrazyl.

Phenolic Compounds Profile of Table Olives

The amounts of phenolic compounds in the table olives are presented in Table 1. Monounsaturated fats and phenolic compounds are key contributors to the nutritional value of olive products, with phenolic compounds comprising 1-2% of fresh olives.[19] Olive fruit contains various phenolic compounds, including glycosides like oleuropein, ligstroside, verbascoside, rutin, anthocyanins, as well as simple phenols and phenolic acids.[32] Olives contain about 30 simple phenolic compounds, with total phenolic content ranging from 100 to 800 mg/kg.[33] In their study on Tunisian olives,[19] identified 13 simple phenolic compounds in the olive fruit flesh, noting that the main components were hydroxytyrosol, tyrosol, and vanillic acid. Studies by[34,35] have shown that the concentration of phenolic compounds in olives and olive oil typically ranges from 50 to 800 mg/kg. Significant variations in phenolic content were observed among different table olive samples. The phenolic compounds identified across all samples included hydroxytyrosol, tyrosol, verbascoside, p-coumaric acid, vanillic acid, oleuropein, rutin, and apigenin-7-glucoside. At the start of fermentation, oleuropein levels ranged from 624.36 mg/kg to 997.33 mg/kg, decreasing substantially by the end to 21.65 mg/kg (sample K) and 45.79 mg/kg (sample Y). These phenolic compounds in olives are typically in complex forms, which break down into simpler compounds like hydroxytyrosol and tyrosol. Oleuropein and hydroxytyrosol are the predominant phenolic compounds in fresh olives, with hydroxytyrosol being formed because of oleuropein hydrolysis. Hydroxytyrosol is a critical marker for monitoring the reduction in bitterness in olives.[36] At the start of fermentation, hydroxytyrosol levels were similar across samples (221.20 mg/kg to 221.81 mg/kg). However, hydroxytyrosol increased in the red beet-enriched samples, reaching 357.72 mg/kg by the end, a 38% increase, while the control sample saw a 54% decrease. Tyrosol levels decreased during fermentation, with final values of 11.89 mg/kg (sample Y) and 13.64 mg/kg (sample K), showing significant differences ($p<0.05$). Rutin levels also decreased, from 58.48 mg/kg (sample Y) and 65.46 mg/kg (sample K) to 1.76 mg/kg (sample K) and 3.27 mg/kg (sample Y) by the end. Statistically significant differences in rutin levels were observed among the samples, consistent with previous research that reported higher rutin contents in raw Sarıulak variety olives.[37] The initial vanillic acid levels in the table olives ranged from 19.16 mg/kg in sample Y to 33.66 mg/kg in sample K, decreasing over fermentation. By day 128, the content dropped to 3.24 mg/kg in sample Y and 4.04 mg/kg in sample K. However, red beet-enriched olives showed an increase in vanillic acid, with a significant difference between samples ($p<0.05$). Verbascoside levels started at 719.72 mg/kg in sample Y and 835.32 mg/kg in sample K, declining during fermentation to 19.56 mg/kg and 650.22 mg/kg, respectively, with a significant difference ($p<0.05$). The variation in verbascoside was attributed to the addition of red beet. Apigenin levels increased from 6.92 mg/kg (sample Y) and 9.60 mg/kg (sample K) to 27.54 mg/kg and 28.80 mg/kg, respectively, with no significant difference ($p>0.05$). p-Coumaric acid levels decreased, with a slight increase in sample K (red beet-enriched) at the end, but no significant difference was observed between samples ($p>0.05$).

Table 1. Phenolic compounds of table olives in mg/kg on wet basis

Phenolic compound		Fermentation (day 0)	Fermentation (day 60)	Fermentation (day 128)
Verbascosid	Y	769.95±6.52^{d}	20.07±0.69^{e}	19.56±0.80^{f}
	K	835.32±11.90^{c}	441.15±12.27^{b}	650.22±18.13^{b}
Vanillic acid	Y	19.16±2.26^{b}	3.71±0.63^{ab}	3.24±0.01
	K	33.66±1.33^{a}	0.98±0.26^{c}	4.04±0.38
Oleuropein	Y	714.17±9.91^{e}	46.31±0.13^{a}	45.79±2.51^{a}
	K	956.48±8.90^{b}	24.30±0.36^{e}	21.65±1.06^{a}
Rutin	Y	58.48±0.66^{c}	1.85±0.13^{b}	3.27±3.75
	K	65.46±0.53^{a}	2.33±0.97^{b}	1.76±0.24
Apigenin-7-glukozid	Y	9.60±3.73	29.52±1.19	28.80±5.22^{ab}
	K	6.92±0.59	26.27±0.78	27.54±0.13^{b}
P-coumaric acid	Y	3.05±0.05^{ab}	0.40±0.06^{b}	0.49±0.10
	K	4.34±0.98^{a}	0.32±0.00^{b}	0.56±0.01
Hydroxytyrosol	Y	221.81±6.92^{b}	200.70±6.94^{d}	101.10±9.90^{d}
	K	221.20±9.21^{b}	265.32±17.42^{b}	357.72±11.71^{a}
Tyrosol	Y	167.54±12.39^{ab}	15.65±2.72^{c}	11.89±1.10^{c}
	K	166.68±0.61^{ab}	10.03±11.07^{c}	13.64±2.25^{c}

Means with different superscript letters in the same row are significantly different ($p<0.05$). Y: 7% NaCL and K: 7% NaCL + 20% red beetroot.

DISCUSSION

Table olives and olive oils are recognized as functional foods due to their rich phenolic composition, which contributes to health-promoting antioxidant properties.[5,29] The results indicate a general decline in total phenolic content and antioxidant activity over fermentation, consistent with previous findings.[26,30] However, the addition of red beet significantly reduced the extent of these losses, indicating that betalains from red beet enhanced antioxidant properties.[20,21,31] The increased hydroxytyrosol content in the red beet-supplemented samples supports the hypothesis that oleuropein hydrolysis is more pronounced in these conditions.[36] This compound is also linked to bitterness reduction during olive fermentation. While rutin, vanillic acid, and verbascoside typically degrade over time, the enriched samples retained higher levels, suggesting a protective or synergistic role from red beet components.[19,27,31-33] These findings align with Ardıç,[27] who reported increased phenolic retention and antioxidant activity in beet-supplemented green olives.[27] Overall, this study confirms the positive effect of red beet addition on preserving the bioactive properties of table olives. The findings contribute to the growing body of knowledge supporting the use of natural plant-based fortification strategies in fermented vegetable products.[18,21,31]

CONCLUSION

The incorporation of red beet into table olive production improved the fermentation process. Although overall phenolic compound content decreased during fermentation, the addition of red beet reduced this decline, leading to higher antioxidant activity in the red beet-infused samples. The highest antioxidant activity recorded was 32.43%. Oleuropein levels declined rapidly during fermentation, but red beet promoted a faster breakdown compared to the control. Red beet-enriched olives also showed higher hydroxytyrosol levels, with a 38% increase in the enriched sample (K) compared to a 54% decrease in the control (Y). The study highlights the potential benefits of red beet in improving the quality and functional properties of table olives, emphasizing their importance in economy and health benefits. Further research is needed to explore future applications of this approach.

MAIN POINTS

- Red beet boosted the antioxidant activity in olives to 32.43% of their content, making it about 2.5 times higher than the control group.
- It slowed the decline of phenolic compounds during fermentation and increased hydroxytyrosol levels by 38%.
- It also accelerated the natural hydrolysis of oleuropein, improving phenolic transformation and health benefits.

ETHICS

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: H.E., Ş.G., Design: H.E., Ş.G., M.K., Data Collection and/or Processing: H.E., Ş.G., Analysis and/or Interpretation: H.E., Ş.G., Literature Search: Ş.G., Writing: H.E., Ş.G., M.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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