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Çanakkale İli Zeytin Karasu ve Pirina Atıklarının Endüstriyel Valorizasyon Potansiyelleri

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Öne Çıkanlar:
- Biyoaktif bileşenler,
- Zeytin atıkları,
- Fenolik maddeler

Anahtar Kelimeler:
- Antioksidan aktivite,
- Hidroksitirazol,
- Sürdürülebilirlik,
- Zeytinyağı,

ÖZET:

Zeytinyağı üretim sürecinde ortaya çıkan karasu ve pirina, çevresel açıdan önemli atıklar olmasının yanı sıra, biyoaktif bileşenler bakımından zengin bir içeriğe sahiptir. Bu çalışmada, Çanakkale ilindeki on farklı zeytinyağı üretim tesisinden temin edilen karasu ve pirina örneklerinin fizikokimyasal bazı parametreleri ile antioksidan aktivite kapasiteleri belirlenmiştir. Analizler sonucunda toplam fenolik bileşen oranlarının özellikle Z5>Z1>Z10>Z9 numaralı istasyonlarda diğer bölgelere kıyasla oldukça yüksek olduğu tespit edilmiştir. Ayrıca, hidroksitirozol içeriğinin Z1>Z9>Z8>Z10 istasyonlarında belirgin seviyede yüksek olduğu, tirazol oranlarının ise özellikle Z1>Z7 numaralı istasyonlarda dikkate değer düzeyde olduğu saptanmıştır. Antioksidan kapasitenin değerlendirilmesi kapsamında, DPPH ve %ABTS katyon radikali giderme aktivitelerinin en yüksek Z8 numaralı istasyonda gözlendiği belirlenmiştir. İndirgeme gücü aktivitesinin ise en yüksek Z1 numaralı istasyonda olduğu tespit edilmiştir. Elde edilen bulgular, zeytin işleme atıklarının yüksek antioksidan kapasitesinin, içerdiği zengin biyoaktif bileşenlerle güçlü bir korelasyon gösterdiğini ortaya koymaktadır. Sonuç olarak, zeytin atıklarının biyoaktif bileşenler açısından değerli bir kaynak olduğu ve bu bileşenlerin gıda, tarım ve sağlık gibi çeşitli endüstriyel alanlarda değerlendirilme potansiyeline sahip olduğu düşünülmektedir.

Industrial Valorization Potential of Olive Mill Wastewater and Olive Pomace Wastes in Çanakkale Province

Highlights:
- Bioactive components
- Olive waste
- Phenolic substances

Keywords:
- Antioxidant activity,
- Hydroxytyrosol,
- Sustainability,
- Olive oil

ABSTRACT:

The olive mill wastewater (OMW) and olive pomace, which are significant environmental wastes generated during the olive oil production process, are also rich in bioactive compounds. In this study, the physicochemical parameters and antioxidant activity capacities of OMW and olive pomace samples obtained from ten different olive oil production facilities in Çanakkale province were determined. The analyses revealed that the total phenolic compound content was particularly high in stations Z5>Z1>Z10>Z9 compared to other regions. Moreover, hydroxytyrosol content was found to be significantly high in stations Z1>Z9>Z8>Z10, while tyrosol levels were notably high in stations Z1>Z7. In the evaluation of antioxidant capacity, the highest DPPH and %ABTS cation radical scavenging activities were observed in station Z8. Additionally, the highest reducing power activity was detected in station Z1. The findings indicate a strong correlation between the high antioxidant capacity of olive processing waste and its rich bioactive compound content. In conclusion, olive by-products are considered a valuable source of bioactive compounds, with potential applications in various industrial fields such as food, agriculture, and healthcare.

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INTRODUCTION

The olive (*Olea europaea* L.) is a perennial fruit species that exhibits genetic alternation and is primarily cultivated in the Mediterranean basin due to its specific climatic requirements. Due to its nutritional value and economic significance, olives must be processed either into table olives for direct consumption or into olive oil. Olive processing methods are categorized into two main technologies: olive oil production and table olive processing. However, both processes generate various waste products that contribute to environmental pollution (Khdair and Abu-Rumman, 2020).

Since a large proportion of olives are processed for oil extraction, the most significant environmental concerns arise following the olive oil production process. Although different processing technologies are employed in olive oil production, regardless of the method used, two primary by-products, known as olive pomace and olive mill wastewater (OMW), are generated at the end of the production process (Tunalıoğlu and Bektaş, 2010). Olive pomace can be utilized as raw material in various industries through secondary processing, including biofuel production, animal feed, and pomace oil extraction. In contrast, OMW poses serious environmental challenges due to its high organic matter content and phenolic compounds and has not yet been adequately exploited for economic purposes (Tunalıoğlu and Armağan, 2008).

Turkey is one of the world's leading olive and olive oil producers, with production concentrated mainly in the Mediterranean, Aegean and Marmara regions. Türkiye's total olive production in 2023 was 1,520,000 tons. According to the data, 74.7% of the total olive trees in Turkey are oil varieties (FAO, 2023). In Türkiye, olive oil production is mainly carried out in provinces with Mediterranean climates such as Balıkesir, Manisa, Çanakkale, İzmir, Aydın, Hatay and Gaziantep, and there has been a 146.7% increase in olive production compared to previous years (TÜİK, 2024; Hocaoğlu et al., 2018). Approximately 1,050 olive oil factories are operating throughout Turkey, half of which use continuous (uninterrupted flow) processing systems (Hocaoğlu et al., 2018). Çanakkale is one of the significant provinces in terms of olive production, with olive-growing areas covering 60% of the province's total fruit-growing land and 10% of its agricultural land. Olive production in the province is predominantly concentrated in the Ezine and Ayvacık districts, accounting for 39% and 30% of total production, respectively, followed by Bayramiç with 11%. In contrast, no olive production is carried out in the Çan and Yenice districts. On average, 94,788 tons of olives were processed annually in olive oil mills across Çanakkale between 2011 and 2015. The by-product quantities generated in these mills are also noteworthy, with the annual average olive pomace production ranging between 79 and 22.590 tons, while OMW production varies between 88 and 25.100 tons, depending on the district (Hocaoğlu et al., 2018).

The by-products generated during olive oil production are significant not only due to their environmental impacts but also because of their potential applications in the energy and agricultural sectors. Depending on the processing technology, olive pomace contains 2–12% residual oil, which, once extracted, is typically pelletized or compacted and used as a direct combustion fuel source. This method is particularly favored by dairies and small-scale industrial enterprises and is also widely applied in olive oil mills to meet hot water demands. The annual thermal capacity of the olive pomace utilized through these methods in Çanakkale has been calculated as 703.090 GJ/year. Additionally, olive pomace serves as an important biomass source for biogas production and can also be converted into biochar, a soil conditioner that has gained attention in recent years (Tunalıoğlu and Bektaş, 2010; Hocaoğlu et al., 2018).

On the other hand, OMW, a waste product of three-phase olive processing plants, is well-suited for methane production due to its composition, making it a viable biomass source for biogas generation. In the total biogas conversion of olive oil production residues in Çanakkale, olive pomace accounts for 58% of methane production. However, OMW remains a major environmental issue in Turkey, as it does in other leading olive-producing countries. This issue has become more pronounced, particularly after the year 2000, with the expansion of olive cultivation areas and the increase in production driven by agricultural policies. In line with Turkey's European Union harmonization process, regulatory developments concerning environmental legislation have further emphasized the importance of OMW management. Within this framework, the Ministry of Environment, Urbanization, and Climate Change of the Republic of Turkey issued a directive on January 23, 2023, mandating that all three-phase olive oil processing plants transition to two-phase production by September 2023. Compared to the three-phase system, the two-phase production method offers a more environmentally sustainable alternative, as it requires less water consumption and generates significantly lower amounts of OMW (Tunalıoğlu and Bektaş, 2010; Hocaoğlu et al., 2018; Benaddi et al., 2023).

OMW primarily contains compounds inherent to olives. However, its high concentrations of Biochemical Oxygen Demand (BOD), suspended solids, Chemical Oxygen Demand (COD), oil and grease, as well as various phenolic and polyphenolic compounds with phytotoxic properties, indicate that wastewater from the olive oil production sector has a significant pollution potential. OMW generally consists of polysaccharides, lipids, proteins, and various polycyclic and monocyclic aromatic molecules, which are known to enhance microbial anaerobic activities. It is characterized by an acidic pH and a high organic matter content, containing substantial amounts of suspended solids, pectins, sugars, phenolic compounds, and vegetable oils. Additionally, due to its aromatic compounds and a mixture of simple and complex sugars, OMW is recognized as a high-energy source. The organic compounds present in OMW primarily include sugars, nitrogenous compounds, volatile acids, polyalcohols, pectins, oils, polyphenols, and tannins, the latter of which contribute to its dark coloration (Çelik et al., 2008).

The composition of OMW generally consists of 83–96% water, 3.5–15% organic matter, and 0.2–2.0% mineral salts. Furthermore, the plant sap of the olive tree contains valuable trace elements, as well as high levels of potassium, phosphorus, organic components, pectins, sugars, phenolic compounds, and vegetable oils. Due to its aromatic compounds and diverse sugar content, this type of wastewater possesses substantial energy potential. The composition of OMW, which is rich in organic matter and derived from oil seepage and water-washed oil residues, varies depending on the applied processing technology and production method (Babadostu, 2014). Particularly, OMW is considered a critical wastewater requiring treatment due to its high BOD: 50–100 g/L, COD: 80–220 g/L, and total organic carbon (TOC: 10–110 g/L) levels. The tannins, polyphenols, and polyalcohols it contains exhibit antimicrobial activity, leading to high toxicity. Given its complex physicochemical structure and significant environmental impact, determining the composition and physicochemical parameters of OMW is crucial for both pollution control and assessing its potential for industrial applications (Paraskeva and Diamadopoulos, 2006).

Currently, various sources are being utilized for the production of phenolic compounds and the extraction of naturally occurring antioxidant molecules. The disposal of olive oil production by-products, such as OMW and olive pomace, which are generated in large quantities worldwide, is considered a major environmental challenge. However, the phenolic compounds and natural antioxidants present in these by-products offer significant potential for environmental, social, economic, and health-related applications. The aim of this study is to determine the characteristic properties and pollution parameters of OMW and olive pomace wastes obtained from olive oil production facilities operating within the Çanakkale province, to analyze their phenolic compound content, and to evaluate their antioxidant activity capacities. The findings obtained from this study will contribute to the assessment of the environmental impacts of these wastes and to the identification of their biotechnological potential.

MATERIALS AND METHODS

Fieldwork

In the Çanakkale province, 10 different olive oil production facilities utilizing 2-phase and 3-phase processing systems were identified, and visits to these factories were conducted on November 23, 2023. Since OMW and olive pomace were obtained from commercial companies, the names of the firms have not been disclosed. Information regarding the firms from which OMW and olive pomace samples were collected, as well as the olive oil production phases, is presented in Table 1.

Certain physicochemical parameters examined in the project, including pH, dissolved oxygen and, electrical conductivity, were measured on-site using an HQ40d18 ecological kit from the Hach–Lange brand and recorded accordingly. Samples collected from each station were stored in two 5-liter bottles for the analysis of oil and grease, TOC, total nitrogen, total phenolic compounds, hydroxytyrosol, oleuropein, tyrosol, and vanillic acid. The samples were transported to the laboratory under cold chain conditions. Until the commencement of laboratory analyses, all samples were stored at -80°C.

Table 1. Information on the businesses from which olive mill waste and olive pomace samples were obtained

Station Number/District	Olive Variety	Growing Area	Collection Time	Sample Type/ Pomace	Sample Type/ OMW	Production Phase	Storage Time	Harvest Method/ Mechanical
Z1 /Gökçebayır	Oil/Edremit/Gemlik/ Arbequina (little)	Çanakkale surroundings, Lapseki, Çardak	Oct-Dec	+	-	2 phase	If there are too many olives, immediately; Waiting for maximum 2 days	Beater and machine
Z2/Gökçebayır	Edremit	Gökçebayır	Oct-Nov	+	+	3 phase	Waiting for 1-2 days	Machine
Z3/Gökçebayır	Edremit/Gemlik	Gökçebayır	Oct-Nov-Dec	+	+	3 phase	Waiting for 1 day	Machine
Z4/Gökçebayır	Edremit	Ayvacık	Nov- Dec	-	+	3 phase	Immediately	Machine
Z5/Geyikli	Oil/Edremit/Gemlik/ Arbequina	Geyikli	Nov- Dec	+	-	2 phase	Maximum 1 day	Machine
Z6/Geyikli	Oil/Edremit/Gemlik/ Arbequina/Domat	Lapseki, Geyikli, Yapıldak	Nov- Dec	+	+	2 phase	Immediately	Machine and wooden stick
Z7/Kemallı	Edremit/Gemlik (little)	Kemalli and surrounding villages	Nov- Dec	+	+	3 phase	Waiting for 2-3 days	Machine and wooden stick
Z8/Ayvacık	Edremit	Ayvacık	Nov- Dec	OMW-pomace mixed		3 phase	Waiting for 1 day	Machine
Z9/Ayvacık	Edremit (mixed sample of 15 companies)	ND	Nov- Dec	-	+	3 phase	Immediately	ND
Z10/Ezine	ND	ND	Nov- Dec	-	+	3 phase	ND	ND

*ND: Not Data, Oct: October, Nov: November, Dec: December

Physicochemical Analysis

pH analysis

pH measurement in the field was made with the Hatch – Lange brand ecological kit pH meter with catalog number HQ40d18.

Electrical conductivity analysis

Electrical conductivity was made with the Hatch – Lange brand ecological kit with catalog number HQ40d18.

Oil and grease determination

Oil and grease determination of the OMW and/or olive pomace samples taken from each factory was made as a service purchase through ÇOMÜ Science and Technology Application and Research Center (ÇOBİLTUM).

Total organic carbon determination

It was made as a service purchase for TS 8336 test method through TÜBİTAK Bursa Test and Analysis Laboratory (TÜBİTAK BUTAL).

Total phenolic substance and total nitrogen determination

It was made as a service purchase through ÇOBİLTUM.

HPLC analysis

Samples prepared for the qualitative analysis of phenolic compounds (hydroxytyrosol, oleuropein, tyrazole and vanillic acid) found in OMW and olive pomace were sent to TÜBİTAK Bursa Test and Analysis Laboratory Directorate and were obtained through service procurement.

Antioxidant Activity Determination

DPPH radical scavenging activity

The ability to neutralize free radicals was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Blois (1958). The test samples underwent incubation at room temperature in darkness for 30 minutes, followed by measurement of absorbance at 517 nm relative to the blank. The inhibition percentage of the samples was derived using Formula 1. Ascorbic acid (AA) was employed as the standard reference.

$$\%\ \mathbf{DPPH}: \left[\frac{\mathbf{A0 - A1}}{\mathbf{A0}}\right] \mathbf{X100} \qquad (1)$$

A0: DPPH radical absorbance without sample or standard
A1: DPPH radical absorbance with sample

ABTS cation radical scavengin activity

The evaluation of ABTS cation radical scavenging potential was conducted following the microplate technique described by Archarya (2017). A solution containing 7 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2.45 mM potassium persulfate ($K_2O_8S_2$) was prepared at a 2:3 ratio and left to incubate for 16 to 20 hours. The obtained blue-green ABTS solution was diluted with ethanol until its absorbance reached 0.7±0.1 at 750 nm. Subsequently, 10 µL of either the test sample or reference standard, along with 190 µL of the ABTS reagent, was dispensed into 96-well microplates and incubated in darkness at 25ºC for 5 minutes. The inhibition percentage was determined by recording absorbance at 750 nm and applying Formula 2.

$$\%\ \text{Inhibition} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100 \qquad (2)$$

Ac: Absorbance value of the control, As: Absorbance value of the sample

Reducing power capacity

The determination of reducing power capacity was carried out using the microplate technique outlined by Oyaizu (1986). In a 96-well microplate, 10 µL of either the test sample or reference compound (ascorbic acid), 25 µL of phosphate buffer (0.2 M, pH 6.6), and 25 µL of 1% potassium ferricyanide ($K_3(Fe(CN)_6)$) were combined and allowed to incubate at room temperature for 20 minutes. After incubation, 25 µL of 10% trichloroacetic acid (TCA), 85 µL of distilled water, and 8.5 µL of 0.1%

ferric chloride ($FeCl_3$) were sequentially added to each well and mixed thoroughly. Absorbance measurements were performed at 700 nm following an additional 15-minute incubation.

RESULTS AND DISCUSSION

Physicochemical Parameter Findings of Stations

Physicochemical parameter findings of samples taken from all stations are given in Table 2.

Table 2. Physicochemical parameter changes of OMW samples

Parameter	Stations									
	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9	Z10
pH	2.87	2.72	2.30	3.27	NM	3.08	2.92	2.94	3.30	2.45
Electrical conductivity (µS/cm)	7.72	3.12	0.42	11.32	NM	8.98	2.29	6.04	11.06	4.79
Dissolved oxygen (mg/L)	0.89	0.78	1.14	1.03	NM	1.22	0.76	2.38	0.25	0.65
Total organic carbon (g/L)	10.9 ± 1.4	11.1 ± 0.6	1.3 ± 0.5	< 0.1	86.5 ± 0.1	8.8 ± 0.3	9.7 ± 0.8	71.3 ± 0.4	102 ± 1	22.6 ± 3.8
Total nitrogen (mg/L) (%)	0.078	0.03	0.024	0.014	0.13	0.015	0.008	0.050	0.09	0.06
Total phenolic (Polyphenol) (mg/kg GAE)*	733.74	272.51	360.95	76.79	934.54	95.47	158.22	271.15	491.04	499.04
Oil and grease (mg/L)	1921	8136.8	1370.4	13.6	NM	1523.2	49.6	1723	NM	6070
Oleroupein (mg/L)	15.5 ± 0.5	4.0 ± 0.4	1.9 ± 0.1	0.2 ± 0.1	7.6 ± 0.2	2.9 ± 0.4	2.0 ± 0.2	18 ± 1	5.9 ± 0.4	1.9 ± 0.1
Hydroxytyrasol (mg/L)	289 ± 4	3.7 ± 0.3	-	0.8 ± 0.1	90 ± 1	-	-	118 ± 11	271 ± 7	116 ± 8
Tyrazole (mg/L)	84 ± 1	15 ± 1	-	1.1 ± 0.1	50 ± 1	-	-	72 ± 3	63 ± 5	62 ± 5
Vanillic acid (mg/L)	8.9 ± 0.1	2.0 ± 0.2	-	0.2 ± 0.1	4.5 ± 0.1	-	-	12 ±1	12 ±1	2.7 ± 0.1

*Z1: Station 1; NM: Not Measured

It was determined that the pH values in the OMW samples taken from all stations were in the range of 2.30- 3.30 and showed high acidity. Electrical conductivity values vary between 0.42- 11.06 µS/cm. The lowest conductivity value was obtained from station Z3, and it is thought that factors such as rainfall, infiltration of the OMW sample into the soil or evaporation are effective in the low conductivity measurement. It was determined that the dissolved oxygen value in all stations was in the range of 0.25 mg/L- 2.38 mg/L and was at very low values, thus presenting unsuitable conditions for aerobic life. Since station Z5 consists of solid pomace sample, pH, electrical conductivity and dissolved oxygen measurements could not be made. It is seen that the total organic carbon amount varies between <0.1- 102 ± 1 g/L and the highest organic carbon amount was taken from station Z9. The total nitrogen amount varies between 0.008 – 0.13 mg/L and the highest nitrogen amount was obtained from station Z5. Total phenolic content varied between 95.47-934.54 mg/kg at all stations, and the highest rate was obtained at station Z5, similar to the total nitrogen amount (Table 2).

The oil and grease ratios ranged from 13.6–8136.8 mg/L; the lowest oil and grease ratio was found at station Z4, and the highest at station Z2. It is thought that the low oil and grease ratio may be due to olive processing processes. It was determined that the ratios of oleroupein, tyrazole, and vanillic acid obtained from the samples varied between 0.2 ± 0.1 - 18 ± 1 mg/L; 0.8 ± 0.1 - 289 ± 4 mg/L; 1.1 ± 0.1 - 84 ± 1 mg/L; 0.2 ± 0.1- 12 ±1 mg/L, respectively. Hydroxytyrosol, tyrazole and vanillic acid could not be determined from stations Z3, Z6 and Z7. While the station with the lowest content in terms of four parameters was Z4; oleroupein was determined at the highest level in Z8, hydroxytyrosol and tyrazole at Z1, and vanillic acid at Z8 and Z9. It is observed that the highest rate in terms of phenolic components is obtained in hydroxytyrosol (Table 2). Olive oil processors report that the high amount of phenolic substances in olive oil and olive pomace, while it should pass from processed olives to olive oil, is due to the fact that olive oil is obtained from olives that are not ripe enough and to human error in the processing.

In our study, correlations were also calculated to explain the relationship between all parameters examined at the stations. According to the Pearson correlation coefficient, significant ($p<0.05$) positive correlations obtained in all parameters are shown with an asterisk (Table 3).

Table 3. Correlation values of all parameters

Parameters	pH	Electrical conductivity	Dissolved oxygen	Total organic carbon	Total nitrogen	Total phenolic	Oil and grease	Oleroupein	Hydroxytyrasol	Tyrazole	Vanillic acid
pH	1	**0.937*** 0.001	**0.985*** 0.001	0.394 0.231	**0.947*** 0.001	**0.945*** 0.001	**0.527*** 0.096	**0.972*** 0.001	**0.975*** 0.001	**0.953*** 0.001	**0.843*** 0.001
Electrical conductivity		1	**0.974*** 0,001	0.087 0,003	**0.996*** 0,001	**0.999*** 0,001	0.233 0,490	**0.991*** 0,001	**0.862*** 0,001	**0.999*** 0,001	**0.743*** 0,006
Dissolved oxygen			1	**0.947*** 0.001	0.077 0.899	**0.994*** 0.001	0.331 0.390	**0.997*** 0.001	**0.864*** 0.001	**0.947*** 0.001	0.309 0.001
Total organic carbon				1	0.309 0.355	**0.994*** 0.001	**0.98*** 0.001	0.448 0.167	**0.99*** 0.001	**0.854*** 0.002	**0.983*** 0.001
Total nitrogen					1	**0.994*** 0.001	0.115 0.735	**0.977*** 0.001	0.189 0.577	**0.832*** 0.003	0.132 0.700
Total phenolic						1	**0.994*** 0.001	**0.995*** 0.001	**0.937*** 0.002	**0.9*** 0.006	**0.986*** 0.001
Oil and grease							1	0.261 0.439	**0.994*** 0.001	**0.843*** 0.002	**0.9*** 0.001
Oleroupein								1	0.336 0,312	**0.855*** 0,002	0.277 0,409
Hydroxytyrasol									1	**0.982*** 0.001	**0.996*** 0.001
Tyrazole										1	**0.947*** 0.001
Vanillic acid											1

Significant positive correlations were obtained for all parameters except pH - total organic carbon; electrical conductivity - total organic carbon; dissolved oxygen - total nitrogen; total organic carbon - total nitrogen; electrical conductivity - oil grease; dissolved oxygen - oil grease; dissolved oxygen - vanillic acid; total organic carbon -oleroupein; total nitrogen - oil grease; total nitrogen - hydroxytyrosol; total nitrogen - vanillic acid; oil grease -oleroupein and oleroupein and hydroxytyrosol - vanillic acid parameters (Table 3).

Leouifoudi et al. (2015) conducted a comprehensive HPLC analysis of both olive pomace and OMW, identifying various bioactive phenolic compounds, including simple phenols, phenolic acids, secoiridoids, flavonoids, and lignans. They observed that the phenolic composition was influenced by environmental factors. In olive pomace waste, compounds such as hydroxytyrosol, tyrosol, vanillic acid, sinapic acid, oleuropein, verbascoside, rutin, luteolin, quercetin, and secoiridoid derivatives were identified. In OMW, compounds like hydroxytyrosol, tyrosol, vanillic acid, sinapic acid, caffeic acid, p-coumaric acid, verbascoside, luteolin, apigenin, oleuropein, and secoiridoid derivatives were found.

Silvan et al. (2019) applied an advanced hybrid separation technique to isolate phenolic and secoiridoid compounds from crude olive mill wastewater. This method integrated pressure-driven cross-flow membrane microfiltration, ultrafiltration, and solid-phase extraction. The researchers fractionated the extracts into five groups based on composition and evaluated their antibacterial potential against different *Campylobacter* strains. The findings showed that the fraction with the highest levels of phenolics and secoiridoids exhibited significant antibacterial activity, with varying effects depending on the bacterial strain.

Sar and Akbaş (2023) investigated the potential of OMW as a source of bioactive phenolic compounds, considering its use as an eco-friendly alternative to synthetic chemicals. Three different solvents, ethanol, ethyl acetate, and methanol, were used for phenolic extraction. Among them, the methanol extract had the highest total phenolic content (4.03 g GAE/L) and exhibited the strongest antibacterial activity. However, none of the extracts showed antifungal activity against *Candida* *albicans*. Their findings suggest that olive mill wastewater, particularly methanol-based extracts, holds promise as a source of bioactive compounds for applications in nutraceuticals and the food industry, offering a sustainable alternative to synthetic additives.

Several studies have highlighted the extensive diversity of phenolic compounds present in OMW and pomace by various researchers (Rodríguez-López et al., 2020; Delgado et al., 2023). Investigations into the biological activities of phenolic constituents in olive oil wastewater and pomace have demonstrated that hydroxytyrosol exhibits antioxidant (Rietjens et al., 2007; Bertelli et al., 2020), cardioprotective (Bogani et al., 2007; Visioli, 2012), anticancer (Bernini et al., 2017), anti-inflammatory (Martínez et al., 2019), neuroprotective (Bertelli et al., 2020), antimicrobial, and antiviral effects (Lee et al., 2016), as well as antidiabetic potential (Hamden et al., 2009). Numerous studies have also documented the antioxidant (Mancebo-Campos et al., 2014), cardioprotective (Andreadou et al., 2007), anticancer (Han et al., 2009), anti-inflammatory (Domitrović et al., 2012), and neuroprotective (Diomede et al., 2013) properties of oleuropein, along with its antimicrobial effects (Leouifoudi et al., 2015; Sar and Akbaş, 2023). Additionally, tyrosol has been recognized for its antioxidant (Giovannini et al., 1999), cardioprotective (Samuel et al., 2008), anti-inflammatory (Chandramohan and Pari, 2016), and neuroprotective (Bu et al., 2007) activities.

Antioxidant Activity Results of Stations

DPPH radical scavenging activity

When the DPPH free radical scavenging activity at a concentration of 20 µg/mL in samples taken from different stations was examined, it was found that ascorbic acid (control standard)>Z8>Z3>Z1>Z10>Z7>Z9>Z5>Z6>Z2>Z4. The highest antioxidant activity was determined at station Z8 (79.76±0.51%), and the lowest activity was determined at station Z4 (64.65±4.80%) (Figure 1).

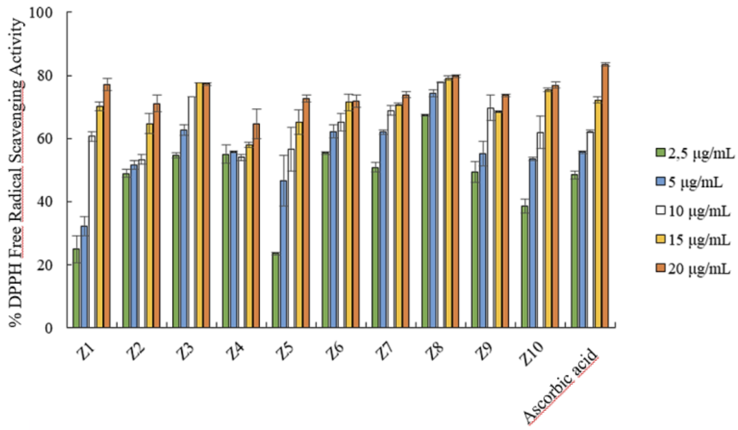

Figure 1. % DPPH free radical scavenging activity of OMW and olive pomace samples

DPPH free radical scavenging activity, in terms of effectiveness, the oleroupein level (18±1mg/L) was higher than the other stations in Z8 station, which follows the standard antioxidant, while oleroupein (0.2±0.1mg/L), hydroxytyrosol (0.8±0.1mg/L), and tyrosol (1.1±0.1 mg/L) were at the lowest levels in Z4. At the same time, the total phenolic content was found to be 76.79 mg/kg GAE, which was the lowest value among the other stations in Z4.

%ABTS cation radical scavenging activity

%ABTS cation radical scavenging activity was found to be between 91.91±0.44% and 71.29±1.14%. The highest activity was determined at station Z8 and the lowest activity was determined at station Z9. The cation radical scavenging activity in samples taken from four different stations (Z8, Z3, Z7 and Z2) was higher than the ascorbic acid used as control (Figure 2).

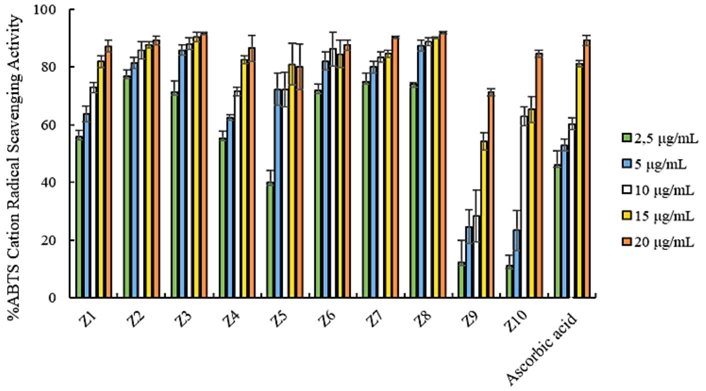

Figure 2. %ABTS cation radical scavenging activity of OMW and olive pomace samples

ABTS cation radical scavenging activity, as well as DPPH free radical scavenging activity, showed better activity at Z8 station than others. The two antioxidant studies conducted also showed parallelism in this respect. At the same time, it was determined that four stations (Z8, Z3, Z7 and Z2) were more effective than the standard antioxidant used as the control group.

Reducing power activity

The reducing power activity was found to be between 3.43±0.11 and 0.14±0.005. The highest activity was determined at station Z1 and the lowest at station Z4. In addition, the activity at station Z1 was higher than the ascorbic acid used as control (Figure 3).

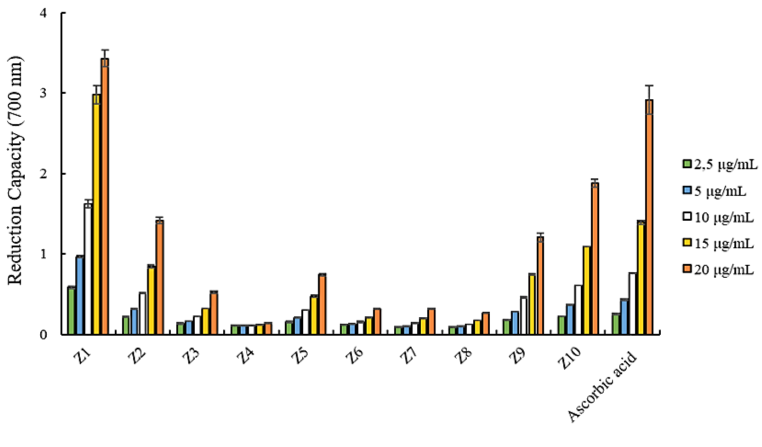

Figure 3. Reducing power activity of OMW and olive pomace samples

The reducing power capacity analysis revealed that Station Z1 exhibited a greater reducing capacity than ascorbic acid across all tested concentrations. This station also demonstrated higher levels of hydroxytyrosol (289±4 mg/L) and tyrosol (84±1 mg/L) compared to samples from other stations. Additionally, the total phenolic content was measured at 733.74 mg/kg GAE, while the oleropein concentration reached 15.5±0.5 mg/L, ranking as the second highest among the analyzed stations.

The most important phenolic compounds contained in olive mill wastewater are hydroxytyrosol and oleuropein in olive leaves. It has been determined that these two compounds are more effective against oxidation than standard antioxidants (Butyl hydroxy toluene (BHT) and Vitamin E) (Aruoma et al., 1998). The obtained data indicate an inverse relationship between oleuropein and hydroxytyrosol levels, with a decline in oleuropein concentration accompanied by an increase in hydroxytyrosol levels.

De Leonardis et al. (2007) reported that while oleuropein decreases during the ripening and processing of olives, the level of hydroxytyrasol increases. The antioxidant capacities of mill wastewater and pomace wastes are consistent with the antioxidant capacity data given in the literature, and it has been proven once again within the scope of the study that the variability depends on climatic data. The high antioxidant capacity of these wastes reveals their high potential in the synthesis of active substances that will play a role in the prevention of degenerative diseases caused by oxidative stress such as cancer, Parkinson's, and Alzheimer's.

CONCLUSION

In this study, the physicochemical properties, phenolic compound contents, and antioxidant activity capacities of OMW and olive pomace wastes obtained from olive oil production facilities operating in the Çanakkale province were investigated. The analyses revealed that the total phenolic content was high at certain stations, with bioactive compounds such as hydroxytyrosol and tyrosol being particularly concentrated at specific locations. The DPPH and ABTS free radical scavenging activities, along with the reducing power assays, demonstrated that these wastes possess strong antioxidant properties. These findings indicate that olive processing wastes have significant potential for biotechnological applications.

The results suggest that OMW and olive pomace can be evaluated for industrial applications. The high content of phenolic compounds implies that these wastes could serve as natural antioxidant sources in the food, pharmaceutical, and cosmetic industries. The localized accumulation of hydroxytyrosol-rich samples at specific stations highlights the necessity for further studies focusing on the purification and industrial utilization of these compounds. The study also found that the low pH values and high organic matter content of OMW pose a potential environmental risk, necessitating the development of biotechnological treatment methods. Advanced treatment techniques such as bioremediation or membrane filtration systems could mitigate the environmental impact of OMW. In this context, future research should focus on developing sustainable and economically viable solutions for OMW management.

Another significant finding of this study is the potential of olive pomace waste for biomass utilization. Olive pomace has been identified as a valuable resource for biogas production and energy recovery. Therefore, the establishment of biogas plants in local olive oil production facilities could provide substantial benefits in terms of waste management and energy generation. Additionally, the use of OMW and olive pomace as agricultural fertilizers or soil conditioners could be considered a sustainable waste management strategy. However, due to their phytotoxic effects, these wastes must undergo pre-treatment before being directly applied to agricultural fields. Future research should explore appropriate pre-treatment methods to ensure the safe and effective use of these components in agriculture.

In conclusion, olive processing by-products should not merely be regarded as waste but rather as valuable resources containing biotechnologically and industrially significant compounds. Therefore, future studies should focus on developing new application strategies to enhance the efficient utilization and sustainable management of these wastes.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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