II Conference FoodWaStop
Cordoba, Spain
4-5 March, 2025

Funded by the European Union

VALORIZATION OF PHENOLIC COMPOUNDS RECOVERED FROM OLIVE OIL BYPRODUCTS AND THEIR POTENTIAL USE IN FOOD MODEL SYSTEMS

ARIOLA DEVOLLI, KATERINA PIKULI, EDLIRA SHAHINASI

Department of Chemistry, Faculty of Bi,otechnology and Food, Agricultural University of Tirana, Albania; adevolli@ubt.edu.al

Abstract

Olive oil production using three-phase and two-phase decanter systems generates different by-products, such as olive mill wastewater (OMWW) and olive pomace (OP), which have environmental, social, and economic significance in Albania, where olive oil production is one of the major agricultural sectors. By-products of the olive oil industry are considered low-cost sources containing high-added value compounds, and bioactive compounds including polyphenols that show remarkable antioxidant properties, carbohydrates, fibers, and pigments. These compounds are known to be associated with beneficial effects on human health and play an important role in food formulation, increasingly valued features by consumers who in the last decades are more aware of the role that the diet plays in health which has led to an increase in the demand for natural and sustainable functional ingredients and products.
This study is focused on evaluating the environmental impact of OMWW and on valorization approaches of bioactive compounds recovered from OMWW and OP.
Recovery of valuable ingredients from olive oil by-products by applying different innovative extraction techniques is a great challenge and an excellent economic opportunity for the olive oil sector which also lowers the environmental charge of their wastewater discharges.

The main goal of the current study is to evaluate the total phenolic content (TPC) and antioxidant activity of OP and OMWW extracts recovered through liquid-liquid solvent extraction using different extraction techniques. OP samples were obtained from both olive oil production systems (two and three-phase decanter) while OMWW samples were collected from different three-phase olive mills operating in Albania.
The results of the physicochemical analysis showed that samples of OMWW had an acid pH, high levels of organic load, and higher levels of total nitrogen, oils, and grease compared to allowed effluent discharge limits according to Albanian standards. The biodegradability index of OMWW samples exceeds the threshold of 3, confirming that our samples are partially or not biodegradable and that the total phenolic content was 5.5 -8.42 g/l.
The total phenolic content and antioxidant activity of OP samples generated from the manufacturing of olive oil using two and three-phase centrifugation systems varied in the range of 0.7-0.8 g gallic acid equivalent/L extract, antioxidant activity was 80-105% antiradical activity, and total antioxidant capacity ranged from 90-109% TAC. Based on the obtained results OP and OWWW have antioxidant qualities and may be useful in food formulation.

Materials and Methods

Sample collection and treatment

Samples of OMWW and OP were collected from olive mills operating in the southern and central parts of the Albania country from November 2023 to January 2024. Fresh OMWW samples were characterized by deep dark brown up to black color, with a strong specific olive oil smell. Samples of OP were dried at 45°C to 50°C for 48h in a try dryer to prevent the degradation of phenolic compounds, then finely grounded by using a mill flour with an average particle diameter of about 1mm.

Fig 1. Location of OMWW and OP sampling stations

Recovery of phenolic compounds from OMWW and OP samples

The extraction procedure of phenolic compounds from OMWW was performed as shown in Figure 2.

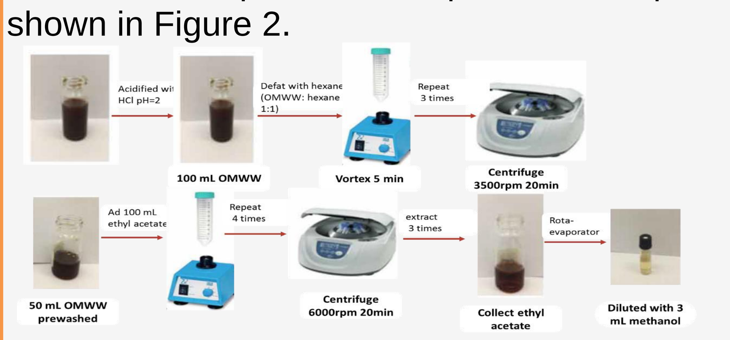


Fig 2.extraction procedure of phenolic compounds from OMWW

The recovery of phenolic compounds from OP has been performed by UAE technique using 10 g of OP and 50 mL of each extraction solvents: water, methanol:water (80:20), ethanol:water (50:50), ethanol:water (80:20), and n-hexane). After the extraction of phenolic compounds from OP, the supernatants were filtered using 0.45 μm Millipore syringe filters and stored at - 20^{0}C for further analysis.

Statistical analysis: All collected data were subject to statistical analysis (ANOVA and PCA) using the SPSS software ver.29. Descriptive statistics, mean, standard deviation (SD), and confidence interval (CI) were used to analyze related OMWW and OP properties.

Analytical methods

- The characterization of OMWW effluents was based on the study of various physicochemical parameters.
- The pH and EC were measured in situ using a multi-parameter water analyzer.
- The TSS was determined gravimetrically. BOD content was determined by the Winkler method, COD concentration was also determined according to standard method 5220D (APHA, 2017).
- TP measurement and TN expressed as the sum of nitrite nitrogen, nitrate nitrogen, and Kjeldahl nitrogen were deterrmined according to standard methods for the examination of water and wastewater, while Na, Ca, and K were determined using Flame Atomic Absorption Spectrometer.
- The total antioxidant activity of phenolic extracts was performed using the DPPH radical scavenging activity assay. The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method.

Results and Discussion

The study is focused on the environmental impact of OMWW generated from the three-phase extraction process. The results obtained from physicochemical analyses were evaluated according to discharge limits allowed by the Albanian authorities. As can be seen from the results presented in Table 1, all OMWW samples are considered acidic (pH 4.4-5.3) compared to the allowed limits (6-9) for wastewater discharge.

Table 1. Statistical results of physicochemical parameters of OMWW samples.

Parameter	Unit	Vlora region			Elbasan region			Tirana region		
		Mean	SD	CI	Mean	SD	CI	Mean	SD	CI
pH	-	4.83	0.26	0.30	4.83	0.37	0.42	4.83	0.37	0.42
EC	mS/cm	9.68	2.12	2.40	9.41	1.45	1.64	9.41	1.45	1.64
TSS	g/L	23.97	4.54	5.14	30.93	6.21	7.02	30.93	6.21	7.02
TN	mg/L	621.67	13.60	15.39	460.33	32.40	36.66	460.33	32.40	36.66
TP	mg/L	263.00	77.16	87.32	195.33	39.84	45.08	195.33	39.84	45.08
COD	g/L	201.70	4.58	5.18	172.47	34.00	38.47	172.47	34.00	38.47
BOD	g/L	45.23	6.81	7.71	34.27	3.56	4.02	34.27	3.56	4.02
Oil & grease	g/L	6.11	0.49	0.56	7.50	0.73	0.82	7.50	0.73	0.82
TPC	g/L	8.11	0.23	0.26	6.20	0.57	0.64	6.20	0.57	0.64
Na	g/L	0.77	0.03	0.04	1.03	0.05	0.06	1.03	0.05	0.06
K	g/L	6.27	0.26	0.30	3.75	0.41	0.47	3.75	0.41	0.47
Ca	g/L	0.72	0.12	0.14	0.95	0.06	0.07	0.95	0.06	0.07

Table 2. The biodegradability index (Ib), COD/BOD ratio

Ib index	OMWW-Vlora	OMWW-Elbasan	OMWW-Tirane
COD/BOD	5.13	5.03	5.30

OMWW effluents in the three studied regions contain considerably higher quantities of organic load expressed by COD and BOD, higher levels of EC, TSS, TN, and TP than the permitted level according to Albanian discharge standards. The values of K, Na, and Ca in the current study were higher than those reported from different studies. Based on this, we can conclude that the high K, Na, and TN levels could be attributed to fertilizer and organic matter use in the study areas. The TPC of OMWW effluents ranged from 5.5 g/L to 8.42 g/L, which is significantly higher than the results reported by other studies.

The value of the Ib index exceeds the limit threshold of biodegradability (Ib=3) therefore OMWW effluents present a high, polluting power with toxic effects and are considered among the "strongest" industrial effluents which indicates that OMWW effluents are not suitable for biological treatment and therefore must be considered a physicochemical treatment before discharge to the environment or sewer system.

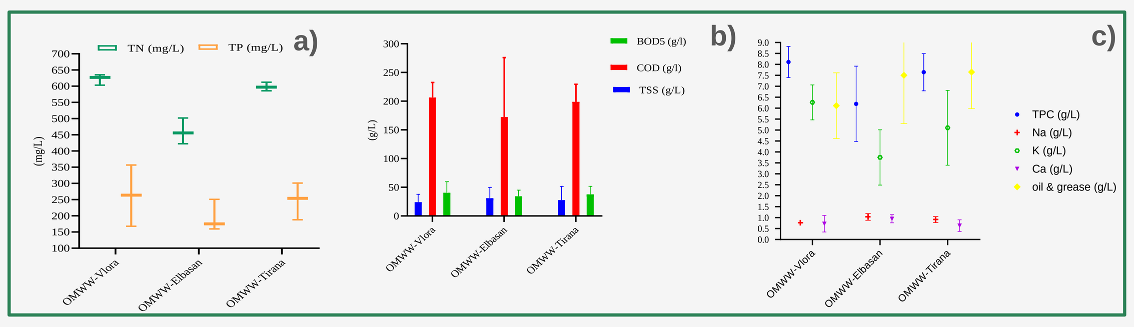


Figure 3. Spatial changes of physicochemical parameters in three studied regions: *a) Vlora region; b) Elbasan region and c) Tirana region.*

Compared to other studied areas, OMWW samples collected in the Vlora region, contain a higher level of TN, TP, organic load, TPC, and K.

Results of the Pearson correlation indicated a moderate to strong correlation between the physicochemical parameters of OMWW. A significant positive correlation (r ranged from 0.71 to 0.98) between TPC and COD of OMWW effluents in the three studied areas was noticed.

Table 3. Principal component analysis of physicochemical parameters of OMWW

Principal Component	Extraction Sums of Squared Loadings		
	Total	% Variance	% Cumulative
1	5.392	44.933	44.933
2	2.121	17.677	62.61
3	1.77	14.753	77.364
4	1.145	9.542	86.906
5	0.675	5.622	92.528

Principal component analysis (PCA) was conducted to find loadings between physicochemical parameters of OMWWs in studied areas.

Table 4. Component matrix of all measured parameters.

Parameter	Component 1	2	3	4
pH	-0.064	0.827	-0.214	0.354
EC	-0.105	-0.06	0.615	0.76
TSS	-0.577	0.438	-0.572	-0.031
TN	0.955	0.186	0.044	0.032
TP	0.399	-0.442	-0.538	0.187
COD	0.679	0.572	0.217	-0.072
BOD	0.646	-0.419	0.444	-0.296
Oil&Grease	-0.478	0.45	0.628	-0.087
TPC	0.896	0.38	-0.206	0.03
Na	-0.867	0.12	0.149	-0.206
K	0.87	-0.13	-0.041	0.314
Ca	-0.746	-0.363	-0.147	0.403

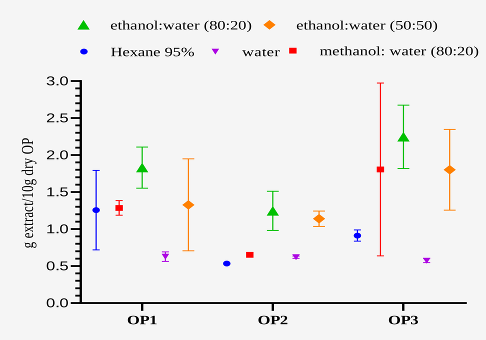


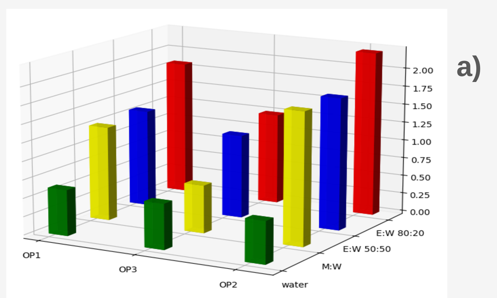
Figure 4. Yield extract of OP provided by UAE

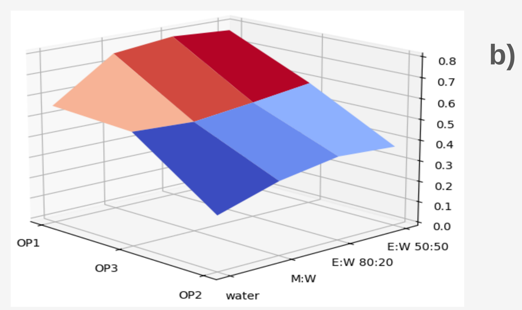
The study also focused on evaluating TPC, antioxidant activity, and antioxidant capacity of OP extracts. Samples of OP had a brown dark color and pH values ranged from 4.6 to 5.4. The moisture content of OP samples is ranged 45-72%.
Figure 4 displays the amount of extract (in grams) obtained from 10 grams of dry OP (%w/w) using various solvents in solid-liquid ultrasound-assisted extraction (UAE).

Table 5. TPC, TAA, and TAC of OP samples.

Solvents-UAE	OP1	OP2	OP3
TPC (g GAE/L extract)			
water	0.5521 ± 0.0145	0.2446 ±0.0013	0.5245 ±0.0788
methanol:water (80:20)	0.7505 ±0.0105	0.3341 ±0.0066	0.5113 ±0.0657
ethanol:water (80:20)	0.7832 ±0.0460	0.3841 ±0.0250	0.5457 ±0.0039
ethanol:water (50:50)	0.7652 ±0.0512	0.3681 ±0.0560	0.5842 ±0.01707
TAA (%RAC)			
water	67.2911 ±5.2556	60.2112 ±1.8586	70.5365 ±2.1262
methanol:water (80:20)	79.1557 ±8.2821	85.6641 ±0.8125	101.6614 ±0.5534
ethanol:water (80:20)	104.242 ±5.5341	84.6522 ±4.9167	91.1491 ±7.8173
ethanol:water (50:50)	90.3727 ±2.2457	83.2298 ±1.8750	88.8198 ±2.2998
%TAC			
water	67.165 ±3.3524	41.6651 ±5.6148	70.9350 ±1.2689
methanol:water (80:20)	79.3032 ±6.3572	43.7053 ±1.6522	47.6354 ±8.7512
ethanol:water (80:20)	109.604 ±0.9864	52.2223 ±2.6422	89.9117 ±4.8113
ethanol:water (50:50)	99.7032 ±3.4680	37.942 1±5.6443	73.7440 ±6.1254

The highest amount of TPC, TAA, and TAC compounds was recorded in the samples of olive pomace (OP1 and OP3) obtained from three-phase production systems. The highest TPC values in OP samples were obtained using ethanol:water (80:20) as extraction solvent. OP samples obtained from the *Kalinjoti* cultivar (OP1) showed higher levels of TPC, TAA, and TTAC compared with OP samples obtained from other cultivars.





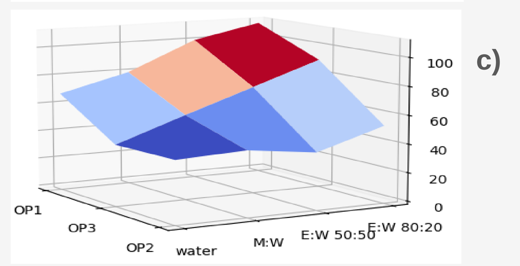


Figure 5. Three-dimensional plots showing the effect of extraction solvent and olive oil production processes on extraction yield (a); on TPC (b); and on TAC (c) of olive OP samples.

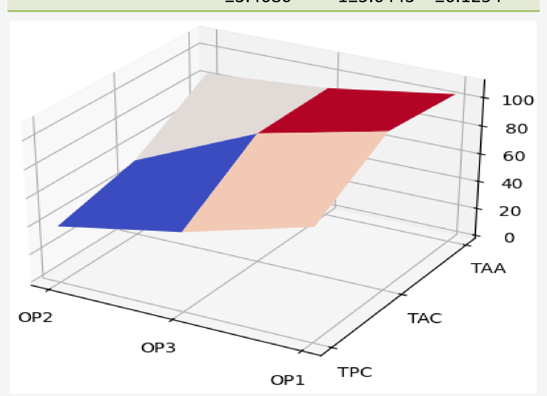


Figure 6. Three-dimensional plots showing the influence of olive oil production processes on the recovery rate of TPC, TAA, and TAC from OP using ethanol:water (80:20) as extraction solvent.

The optimum extraction efficiency of antioxidant compounds was obtained using a combination of ethanol-water (80:20) as extraction solvent. The highest levels of TPC, TAA, and TAC were obtained from OP samples of 3-phase centrifugation systems compared to 2-phase centrifugation systems (OP2).

Conclusions

The results indicated that Olive Mill Wastewater effluents in all studied areas exceeded the national standards for wastewater discharged to surface waters and municipal sewage treatment plants. OMWW effluents were characterized by higher levels of TPC, COD, BOD, TN, TP, and TSS compared to other studied regions.
The biodegradability index of OMWW effluents exceeded the limit threshold of biodegradability (Ib >5) due to the high organic load. Application of cleaner production options and proper environmental waste management systems in olive oil industries are needed to reduce their environmental impact.
The results showed that the obtained extracts contained a significant amount of phenolic compounds and antioxidant activity. However, they significantly depended on olive cultivars, olive oil production processes, and the polarity of the extraction solvent.
The study highlighted the usage of solid by-products generated from the olive oil industry (OP) as a valuable source of bioactive compounds in food formulations, which can extend the shelf-life.

References

- Abbattista, R., Ventura, G., Calvano, C.D., Cataldi, T.R.I., & Losito, I. (2021). Bioactive Compounds in Waste By-Products from Olive Oil Production: Applications and Structural Characterization by Mass Spectrometry Techniques. Foods 2021, 10(6), 1236.
- APHA. 2017. Standard Methods for the Examination of Water and Waste Water, 23th ed. (Clesceri, L. S., Greenberg, A. E. & Eaton, A. D. eds.). America Public Health Association, Washington, DC.
- Benincasa, C., Pellegrino, M., Romano, E., Claps, S., Fallara, C., Perri, E. 2022. Qualitative and Quantitative Analysis of Phenolic Compounds in Spray-Dried Olive Mill Wastewater. Front. Nutr. 8:782693. doi: 10.3389/fnut.2021.782693
- Pikuli, K and Devolli, A. 2024. Total phenolic content and antioxidant activity evaluation of olive mill pomace extract. Scientific Bulletin. Series F. Biotechnologies, Vol. XXVIII, No. 1, 2024 ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364
- Yangui, A., Abderraba, M. 2018. Towards a high yield recovery of polyphenols from olive mill wastewater on activated carbon coated with milk proteins: Experimental design and antioxidant activity. Food Chem. 262: 102-109.