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Extraction and Quantification of Polyphenols of Olive Oil Mill Wastewater from the Cold Extraction of Olive Oil in the Region of Khenchela-Algeria

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Abstract
In the olive industry, the extraction operation requires large quantities of water; therefore, this industry generates large quantities of olive oil mill wastewater (OMW), which is heavily loaded with polyphenols. The objective of this work is to extract and quantify main phenolic compounds from OMW after the evaluation of its physicochemical properties. The extraction of phenolic compounds was done by the liquid-liquid method with ethyl acetate, acetone and methanol to find the most suitable solvent. The amount of total polyphenols was evaluated by the Folin-Ciocalteu method using gallic acid as standard phenolic acid. The amount of flavonoids was determined by the aluminum trichloride method. The results of physicochemical analysis have shown that the OMW is an acidic liquid (pH = 5.05 ± 0.03) and it has a high electrical conductivity (EC= 14.40 ± 0.25 mS/cm), high humidity (H = 86 ± 3.1%). It was too loaded with organic matter (OM = 8.3 ± 0.05%), dry matter (DM = 16.2 ± 0.81%), mineral matter (MM% = 1.25 ± 0.07), total suspended solids (TSS = 0.9 ± 0.05%) and fatty matter (FM = 2.02 ± 0.04%). The results of the extraction show that the content of total phenolic and flavonoids in the methanolic extract of OMW are respectively 950 ± 14.2 µg GAE/ mg of extract and 80.6 ± 17.27 µg QE/mg of extract. The LC-MS results revealed the presence of 10 phenolic compounds. The major identified was quinic acid with a concentration of 23.940 ppm. According to the results obtained, methanol is the suitable solvent for the extraction of polyphenols and flavonoids from OMW that have great importance in the pharmaceutical and food industries.
Keywords: OMW; Polyphenols; Total phenolic content; Total flavonoid content; LC-MS.

Introduction

By-products represent a major disposal problem for the food industry, but they are also promising sources of bioactive compounds (El-Abbassi, 2013). Because it is well known that olives and their derivatives are high in phenol substances with significant antioxidant and nutraceutical properties (De Leonardis et al., 2007a), they may find use in the pharmaceutical industry. Olive oil mill wastewater (OMW), one of the main by-products of olive oil production, is a significant pollutant in the Mediterranean region (El-Hajjouji, 2007). OMW chemical composition is highly variable both qualitatively and quantitatively (Khatib et al., 2009). This liquid is characterized by a purple-dark brown to black color because of the presence of tannins and phenolic compounds of low molecular weight (Hocaoglu et al., 2018), with a pH range of 3-6 and strong olive oil odor. In addition, it has high organic pollution, high electrical conductivity and high nutrient content (El-Hajjouji, 2007). The high concentration of polyphenols is a major contributor to the environmental problems caused by OMW.

These compounds are difficult to decompose and present phytotoxicity, toxicity against aquatic organisms or suppression of soil microorganisms (El-Abbassi, 2013). The treatment of OMW is extremely difficult due to its large volume and the high concentration of organic matter. Nowadays, OMW is valorized industrially to recover polyphenols (Rahmanian et al., 2014)This study is devoted to the physicochemical, quantitative and qualitative analysis of the content of phenolic compounds in OMW obtained from the cold extraction of olive oil from Khenchela in eastern Algeria.

Materials and methods

Sampling

The samples of OMW were taken from a modern olive oil cold extraction unit (temperature does not exceed 25 °C) located at the level of the commune of Baghai in the wilaya of Khenchela in eastern Algeria. It is an Italian-made oil mill, created in 2016. It has a capacity of 4 quintals per hour (q/h) and a daily capacity of 120 quintals and the by-products of olive crushing are OMW (60 L/h) and olive pomace (60 q/h). The samples of OMW were obtained during the harvest season in February 2019. Before extracting polyphenols, we conduct physicochemical analyses on the samples to determine their properties. All analysis was performed in triplicate.

Physicochemical Characteristics

The pH and electrical conductivity (EC) were directly measured in the sample using a pH meter and a conductivity meter. The pH value was determined with a pH meter (AdwaAD1000). Electrical Conductivity (EC) was determined using a conductivity meter type (inoLab WTW). Fatty matter (FM) is determined by the chloroform / methanol method described by Aissam (2003). Dry matter (DM), humidity (H %), total suspended solids (TSS), organic matter (OM), mineral matter (MM) were performed according to the Standard Methods (APHA, 2005). Dry matter content (DM) and humidity (H%) were measured by drying at 105 °C for 24 h. Organic matter (OM) was calculated by the difference between the dry weight of the OMW and its weight after calcination. Mineral matter (MM) was determined by weighing after ignition in a muffle furnace type (Nabertherm) at 550 °C, for 24 hours. Analyzes were carried out in triplicate.

Polyphenol Extraction Method

It was done by liquid-liquid extraction according to the method described by De Marco et al. (2007) with slight modifications. Before extraction, acidification is carried out to promote the elevation of the solubility of phenolic compounds in organic solvents (Obied et al., 2005a), each time used as a solvent to extract acetone, ethyl acetate and methanol. Then delipidation with hexane to remove the lipid fraction. The dry residue is stored in 6 mL of methanol and kept cold.

Total Phenolic Content (TPC)

The total phenolic content of each extract was determined following the Folin–Ciocalteu method described by Kahkonen et al. (1999) with slight modifications. At a fraction of 0.1 mL of the methanolic extract, 0.5 mL of the Folin-Ciocalteu reagent and 4 mL of sodium carbonate 1M (Na_2CO_3) are added after an incubation of 90 minutes at room temperature. The absorption was measured at 760 nm at the visible UV spectrophotometer against a white without extract. In order to quantify the total polyphenols, a standard range based on gallic acid is prepared in parallel under the same conditions. To determine the linearity zone, a mother solution (1 g/L) of the pure gallic acid standard is prepared and diluted at different concentrations (100 to 500 mg/L). The results were expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE / mg of extract). Analyzes were carried out in triplicate.

Total Flavonoid Content (TFC)

The quantification of total flavonoid content of each extract was performed by Kim et al. (2003)

method with slight modifications. 1 mL of the extract and standard (dissolved in methanol) with the proper dilutions are added to 1 mL of a solution of sodium nitrite 0.5 M ($NaNO_2$) and 150 µL of an aluminum trichloride solution 0.3 M ($AlCl_3$). The mixture is vigorously stirred. Then let stand for 15 minutes. The absorption is measured at 430 nm using a visible UV spectrophotometer. The flavonoids were quantified using a linear calibration curve achieved by a pure quercetin standard prepared at different concentrations (2.5 to 40 mg/L) under the same conditions as the sample. The results were expressed as micrograms quercetin equivalents per milligram of extract (µg QE / mg of extract). Analyzes were carried out in triplicate.

LC-MS Separation and Identification of Phenolic Compounds

The analysis for phenolic compounds was performed on a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadripole 2020 detector system. This instrument was equipped with an Inertsil ODS-4 C18 3 µm column (L150×3.0 mm i. d). The column temperature was set at 40°C and dissolving line temperature was 275 °C. Phenolic compounds were identified by comparison with retention time of the standards of phenolic compounds. The lab standards were LGC and Sigma Aldrich.

Results and discussion

Physicochemical Characteristics

The physicochemical properties of OMW studied are presented in (Table 1).

Table 1. Physicochemical properties of olive oil mill wastewater studied.

Parameters	Values
pH	5.05 ± 0.03
EC (mS/cm)	14.10 ± 0.25
H%	86 ± 3.1
OM%	8.3 ± 0.05
DM%	16.2 ± 0.81
MM%	1.25 ± 0.07
TSS%	0.9 ± 0.05
FM%	2.02 ± 0.04

Based on obtained results, OMW is an acidic liquid effluent (pH = 5.05 ± 0.03), too loaded with mineral and organic matter expressed in terms of a high value of electrical conductivity (EC = 14.40 ± 0.25 mS/cm), (H = 86 ± 3.1%), (DM = 16.2 ± 0.81 %), (OM = 8.3 ± 0.05 %), and (TSS = 0.9 ± 0.05 %), (MM = 1.25 ± 0.07 %), (FM = 2.02 ± 0.04 %).

OMW was distinguished by a foul odor and a brown to reddish brown coloration, as well as a cloudy appearance and a strong odor reminiscent of olive oil.

The composition of OMWs is widely discussed in the literature. Our results are in the same range as those obtained by Kadi et al. (2020) and Bombino et al. (2021). OMW is an acidic liquid, with pH values from 3 to 5, due to the presence of phenolic acids and fatty acids with an electrical conductivity value of 16.79 mS / cm (El-Abbassi, 2013). This high conductivity could be explained by the high content of organic compounds in the form of salts. Indeed, dissolved mineral salts are good conductors. Generally, it is composed of dry matter (6% to 17%), and of organic matter (4 to 16%) (El-Abbassi, 2013; Degirmenbasi and Takac, 2018).

OMW exhibits a highly polluted release in the form of residual liquid whose composition is variable due to different factors, such as degree of maturation, cultivars of olives, growing systems, salting practice for olive preservation, climatic conditions and the process used for olive oil extraction and use of pesticides and fertilizers (De Felice et al., 1997; El-Abbassi et al., 2017).

Total Phenolic and Flavonoids Contents

The total phenolic content (TPC) of extracts was estimated according to the calibration curve prepared from gallic acid *($y = 0.0048\ x + 0.0111$, $R^2 = 0.9778$).* According to the results obtained in Figure 1, the content of OMW in phenolic contents differs between the extracts of the three solvents. The methanol extracts contain the highest concentration (950 ± 14.2 µg GAE / mg of extract) followed by the ethyl acetate extract (902.7 ± 10.5 µg GAE / mg of extract), While the low concentration is recorded in the acetone extract (778.1 ± 12.92 µg GAE /mg of extract).

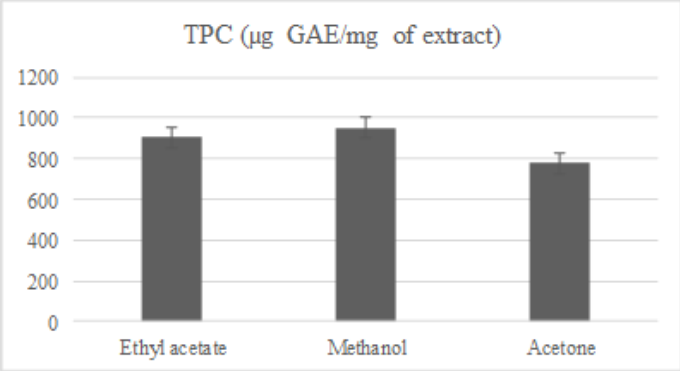

Figure 1. Total polyphenol contents of the three extracts of olive oil mill wastewater.

Numerous researchers have determined the level of polyphenols: values of (0.8 ± 0.02 mg GAE/mL) quantified by Kadi et al. (2020) and (0.65 ± 0.36 g/L) found by Bombino et al. (2021).

The total flavonoid contents (TFC) was calculated following the calibration curve prepared from quercetin *($y = 0.0109\ x + 0.0073$, $R^2 = 0.9684$).* The results of total flavonoid content are presented in (Figure 2).

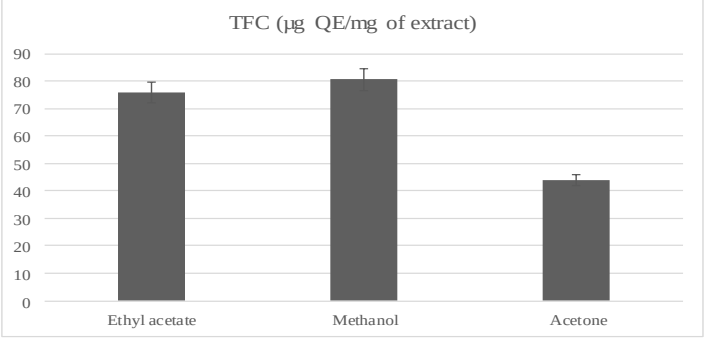

Figure 2. Total flavonoid contents of the three extracts of olive oil mill wastewater.

According to the results obtained, the content of flavonoid from OMW differs between the extracts of the three solvents. The methanol extracts contain the highest concentration (80.6 ± 17.27 µg QE / mg of extract) followed by the ethyl acetate extract (75.8 ± 16.61 µg QE / mg of extract), While the low concentration is recorded in the acetone extract (43.9 ± 9.77 µg QE / mg of extract).

The level of flavonoids is largely determined by many researchers; Kadi et al. (2020) found a value of (0.065 ± 0.001 mg QE/mL) and (0.056 ± 0.005 QE/mL).

Moussaoui (2007) showed that the phenolic composition of OMW depends on the cultivar, the degree of maturity of the olive, the climatic conditions and the technological processes used for the separation of the two oily and aqueous phases. This is what made the phenolic fraction of OMW characterized by great complexity (Bianco et al., 2003).

Identification and Quantification of Phenolic Compounds by LC-MS Analysis

The quantitative analysis results of major phenolic compounds identified in the extract of OMW are summarized in Table 2.

Thirty-one compounds were screened by liquid chromatography mass spectrometry LC-MS. Only ten compounds were identified and quantified in the methanolic extract. They were quinic acid (23.940 ppm), p-coumaric acid (1.427 ppm), rutin (0.255 ppm), luteolin-7-o-glucoside (0.197 ppm), naringin (0.154 ppm), quercetrin (quercetin-3-o-rhamonosid) (0.084 ppm), kampherol (3.635 ppm), quercetin (0.470 ppm), apegenin (0.843 ppm) and cirsiliol (2.352 ppm). The major identified phenolic compound was quinic acid with a concentration (23.940 ppm). Its chemical structure was presented in (Figure 3).

Table 2. LC-MS analysis of methanolic extract of olive oil mill wastewater.

Nº	Identified Molecules	Retention time	M/h	Concentration ppm
1	Quinic acid	2.031	191	23.940
2	Gallic acid	-	169	N.D.(Peak)
3	Protocatechuic acid	-	153	N.D.(Peak)
4	Catechin (+)	-	289	N.D.(Peak)
5	Caffeic acid	-	179	N.D.(Peak)
6	Syringic acid	-	197	N.D.(Peak)
7	1,3-di-O-caffeoyquinic acid	-	515	N.D.(Peak)
8	Epicatechin	-	289	N.D.(Peak)
9	p-coumaric acid	22.417	163	1.427
10	Rutin	25.350	609	0.255
11	Transfrolic acid	-	193	N.D.(Peak)
12	Hyperoside (quercetin-3-o-galactoside	-	463	N.D.(Peak)
13	Luteolin-7-o-glucoside	25.958	447	0.197
14	3,4-di-O-caffeoyquinic acid	-	515	N.D.(Peak)
15	Naringin	27.465	579	0.154
16	Rosmarinic acid	-	359	N.D.(Peak)
17	4,5-di-O-caffeoyquinic acid	-	515	N.D.(Peak)
18	Quercetrin (quercetin-3-o-rhamonosid	28.350	447	0.084
19	Apegenin-7-o-glucoside	-	431	N.D.(Peak)
20	o-coumaric acid	-	163	N.D.(Peak)
21	Salviolinic acid	-	717	N.D.(Peak)
22	Kampherol	33.310	285	3.635
23	Quercetin	33.317	301	0.470
24	Trans cinnamic acid	-	147	N.D.(Peak)
25	Silymarin	-	481	N.D.(Peak)
26	Narangenin	-	271	N.D.(Peak)
27	Apegenin	35.894	269	0.843
28	Luteolin	-	285	N.D.(Peak)
29	Cirsiliol	36.983	329	2.352
30	Cirsilineol	-	343	N.D.(Peak)
31	Acacetin	-	283	N.D.(Peak)

Several researchers identified phenolic compounds by HPLC as Yakhlef (2019) that identified eleven (11) phenolic compounds at 280 nm. There were Hydroxytyrosol-glycol, hydroxytyrosol-1-o-glucoside, hydroxytyrosol, hydroxytyrosol-4-o glucoside, salidroside, tyrosol, caffeic acid,

comselogoside, EDA-like coffeic acid ester, and elenolic acid.

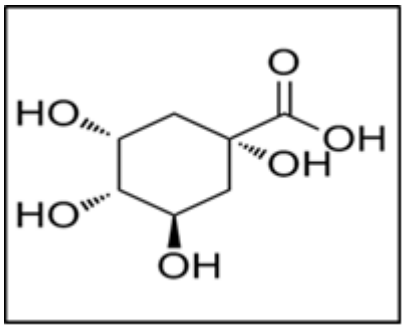

Figure 3. Chemical structure of identified quinic acid

Conclusion

This work allowed us to carry out a physicochemical and phytochemical study of OMW obtained from the cold extraction of olive oil. This study was devoted to the quantification and qualification of total phenolic and flavonoid contents using three different solvents. The results obtained indicate that OMW is highly polluted acidic liquid effluent and is rich in bioactive molecules. Extraction solvents have an influence on the content of polyphenols and flavonoids, which are due to their polarity. Methanol was the appropriate solvent for the extraction of bio-phenols from OMW. Ten phenolic compounds were identified. The major compound with a high concentration is quinic acid. Because OMW is a biological source of natural phenolic compounds and antioxidants, it is advisable to exploit them in the pharmaceutical and food industries.

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Author's Contributions

Zakia Gueboudji produced the experimental part and the manuscript under extensive supervision and revision by Pr. Kenza Kadi and Pr. Kamel Nagaz. All authors read and critically revised the manuscript.

References

Aissam H 2003. Etude de la biodégradation des effluents des huileries (margines) et leur valorisation par production de l'enzyme tannase. Thèse de doctorat national, spécialité microbiologie de l'environnement, Université Sidi Mohamed Ben Abdellah, Faculté des sciences, Dhar El Mehraz, FES, Maroc.

APHA 2005. Standard Methods for the Examination of Water and Wastewater. 21st Ed. American Public Health Association, Washington, DC, USA.

Bianco A. Buiarelli F. Cartoni GP. Coccioli F. Jasionowska R. Margherita P 2003. Analysis by liquid chromatography-tandem mass spectrometry of biophenolic compounds in olives and vegetation waters. Journal of Separation Science, 26(5): 409–416. doi: 10.1002/jssc.200390053.

Bombino G. Andiloro S. Folino A. Lucas-Borja ME. Zema DA 2021. Short-term effects of olive oil mill wastewater application on soil water repellency. Agricultural Water Management, 244: 1-10. doi: 10.1016/j.agwat.2020.106563.

De Felice B. Pontecorvo G. Carfagna M 1997. Degradation of wastewaters from olive oil mills by Yarrowia lipolytica ATCC 20 255 and pseudomonas pitida. Acta Biotechnologica, 17: 231-239. doi.org/10.1002/abio.370170306.

De Leonardis A. Macciola V. Lembo G. Aretini A. Nag A 2007a. Studies on oxidative stabilization of lard by natural antioxidants recovered from olive-oil mill wastewater. Food Chemistry, 100 (3):

998–1004. doi.org/10.1016/j.foodchem.2005.10.057.

De Marco E. Savarese M. Paduano A. Sacchi R 2007. Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chemistry, 104(2): 858-867. doi: 10.1016/j.foofchem.2006.10.005.

El-Abbassi A. Saadaoui N. Kiai H. Raiti J. Hafidi A 2017. Potential applications of olive mill wastewater as biopesticide for crops protection. Science of the Total Environment, 576: 10-21. doi: 10.1016/j.scitotenv.2016.10.032.

El-Abbassi A 2013. Valorisation des margines par isolement des polyphénols avec des procédés membranaires de séparation. Thèse de doctorat, Université Cadi Ayyad, Faculté des sciences, Semlalia, Marrakech, Maroc.

El-Hajjouji H 2007. Evolution des caractéristiques physico-chimiques, spectroscopiques et écotoxicologiques des effluents d'huileries d'olive au cours de traitements biologique et chimique. Thèse de doctorat, Institut national polytechnique de Toulouse, École doctorale : Sciences Écologiques, Vétérinaires, Agronomiques et Bioingénieries Spécialité, Ecologie et Agrosystèmes, France.

Hocaoglu SM. Gursoy Haksevenler BH. Basturk I. Talazan P. Aydoner C 2018. Hot wastewater recovery by using ceramic membrane ultrafiltration and its reusability in textile industry. Journal of Cleaner Production, 171, 220-233. doi.org/10.1016/jclepro.2017.10.015.

Kadi K. Mrah R. Hamli S. Lekmine S. Dib D. Addad D. Boukeria S. Gueboudji Z. Hafsaoui I 2020. Evaluation of the anticoagulant activity of margins from olives extraction in the Khenchela region. Journal of fundamental and applied sciences, 12 (2): 634-649. doi: 10.4314/jfas.v12i2.8.

Kahkonen MP. Hopia AI. Rauha JP. Pihlaja K. Kujala TS. Heinonen M 1999. Antioxidants Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry, 47(10): 3954-3962. doi.org/10.1021/jf990146l.

Khatib A. Aqra F. Yaghi N. Subuh Y. Hayeek B. Musa M. Basheer S. Sabbah I 2009. Reducing the environmental impact of olive mill wastewater, American Journal of Environmental Sciences, 5 (1): 1-6.

Kim DO. Chun OK. Kim YJ. Moon HY. Lee CY 2003. Quantification of Polyphenolics and Their Antioxidants Capacity in Fresh Plums. Journal of Agricultural and Food Chemistry, 51(22): 6509-6515. doi.org/10.1021/jf0343074.

Moussaoui R 2007. Valorisation des sous-produits de l'huilerie d'olive : grignons et margines. Thèse de doctorat, Université Mouloud Mammeri, Tizi Ouzou, Algérie, pp : 133.

Obied HK. Allen MS. Bedgood DR. Prenzler PD. Robards K. Stockmann R 2005a. Bioactivity and analysis of biophenols recovered from olive mill waste. Journal of Agricultural and Food Chemistry, 53(4): 823-837. doi: 10.1021/jf048569x.

Rahmanian N. Jafari SM. Galanakis CM 2014. Recovery and removal of phenolic compounds from olive mill wastewater. Journal of the American Oil Chemists' Society, 91: 1-18. doi: 10.1007/s11746-013-2350-9.

Yakhlef W 2019. Caractérisation des profils phénoliques et évaluation de l'activité antibactérienne du contenu phénolique des margines monovariétales. Thèse de doctorat en science, spécialité microbiologie appliquée, Université Larbi Ben M'Hidi, Faculté des sciences exactes et sciences de la nature et de la vie, Oum El-Bouaghi, Algérie.