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Comparison of Physicochemical Characteristics and Bioactivity of Olive Oil Mill Wastewaters from Traditional and Water-Saving ARA-Controlled Three-Phase Decanter

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Abstract: Olive mill wastewater (OMW) is one of the most environmentally concerning food processing effluents due to its phytotoxicity. Recently, several bioactive compounds with potential applications in food, pharmaceutical, and agricultural industries have been identified in OMW. This study aimed to compare, for the first time, the physico-chemical characteristics and biological activity of OMW obtained by two different types of three-phase decanters: a traditional one and a water-saving ARA decanter. DPPH, ABTS, FRAP, and β-carotene bleaching tests were used to investigate the antioxidant effects. The inhibition of key enzymes involved in hyperglycemia and hypolipidemia were also assessed. A high concentration of phenolic compounds was found in OMW obtained by the ARA-controlled system. Hydroxytyrosol resulted as the dominant compound, with a content of 502.3 mg/kg. OMW extract obtained by ARA decanter resulted as the most active in the FRAP test, with value of 67.23 μMFe (II)/g. A moderate inhibitory activity was found against α-amylase, α-glucosidase, and lipase enzymes. Data obtained by this study evidenced that the use of the ARA decanter allows for obtaining OMW extract characterized by a higher content of phytochemicals in comparison to those obtained by the traditional phase decanter, and a consequent higher biological activity. At the same time, the use of this equipment allows for the reduction of environment impact.

Keywords: olive mill wastewater; phenolic compounds; antioxidants activity; hypoglycemic; hypolipidemic

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1. Introduction

The annual production of table olives and olive oil reaches up to 10 and 2000 million tones, respectively. Olive mill wastewater (OMW) generation reached 30 million tons annually only in the Mediterranean basin [1]. The disposal and treatment of this waste are the main problems of the olive oil industry due their high organic load and content of phytotoxic and antibacterial phenolic substances, which avoid biological degradation [2]. The high polyphenol content of the wastewater is the major environmental problem caused by the olive mills [3]. Due to these characteristics, the disposal of OMWs in urban sewage treatment plants is not viable.

Several procedures are currently applied to manage OMWs. One of the most used methods is the application of OMWs as soil amendment in agricultural land to recover some organic materials useful in the field or industry. However, the direct use of OMWs in agriculture is limited by the antimicrobial and phytotoxic effects related to their polyphenolic content and low pH [4–6]. However, phenols are responsible of the high values of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) due their organic matter content. Hence, their recovery and reuse could have a consequent positive impact on COD and BOD reduction.

The recovery of phenolic compounds able to counteract several pathological conditions such as metabolic syndrome and obesity from the food by-products industry is a topic of

great research interest. In fact, in this way, the problem of wastes is treated with a circular economy perspective by transforming it from a cost into something still able to generate a profit; perhaps even helping to eliminate the environmental problems deriving from its management. Several research articles have evidenced that OMW is a rich source of phytochemical compounds [7,8].

In this context, our work analyzed, for the first time, the physico-chemical parameters and the bioactivity of OMW obtained by two different types of three phase decanters: traditional and water-saving ARA system controlled [9–13]. The analysis included BOD and COD. Moreover, OMW extracts were investigated for their total polyphenols and flavonoids content and for quantification of selected markers by HPLC analysis. The antioxidant potential and enzymes (lipase, α-glucosidase, and α-amylase) inhibitory activity were also assessed.

2. Materials and Methods

2.1. Reagents

All analytical grade solvents and reagents used in this research were purchased from Sigma-Aldrich S.p.a. (Milan, Italy).

2.2. OMW Sampling

OMW was sampled at the centrifugal separator, operating with traditional three-phase centrifugal decanters and three-phase ARA, in the province of Reggio Calabria (Italy). Decanters are a type of equipment made up of a cylindrical conical bowl. Inside this conical vase, there is a hollow component equipped with helical blades. A slight difference between the rotation speed of the drum and that of the internal auger determines the movement of the olive paste (oil and OMW) as they are pushed to the end.

The most important part of the three-phase decanter is the rotating section composed of a cylindrical–conical drum where the screw, rotating at differential speed, is housed.

Separation using centrifugal force takes place in the drum. In a three-phase centrifugal decanter, the product is separated into a light liquid phase (oil), a heavy liquid phase (water), and a solid phase (fragments of stone and olive pulp). An evolution of the three-phase decanter is the water-saving decanter, "ARA". This equipment, thanks to a special design based on a longer cylindrical part of the drum and shorter beach sections complemented by a pressure cone drum, allows for obtaining a lower wastewater volume and an oil richer in polyphenolic substances. As mentioned above, the so-called 'third generation' decanter centrifuge has an innovative geometry, which guarantees greater performance thanks to a longer cylindrical part of the drum and shorter beach sections, and a special drum with cone system (Figure 1). Samples investigated in this work were obtained from olives harvested in October 2021 and immediately processed. The resultant OMWs were stored at −20 °C until further analysis.

2.3. Analytical Determinations

The pH-meter Crison basic 20, with a 50 cm electrode, was used to evaluate pH. The dry matter content was evaluated at 105 °C up to a constant weight. Ash content was determined in a muffle furnace at 550 °C for 24 h. Mineral matter of Ca, Mg, K, Na, Fe, Zn, and Cu was determined by atomic absorption spectrophotometry (AAS) (Analyst 100 Atomic Perkin Elmer, Milan, Italy) after nitric acid digestion at 150 °C for 2 h.

2.4. Chemical Oxygen Demand (COD)

To determine COD, an ECO 6 high-temperature incubator with an automatic program was used (Velp Scientifica s.r.l., Milan, Italy) together with the following reagents: potassium dichromate solution (0.25 N); mercury sulphate; sulfuric acid concentrated to 98%, containing silver sulphate (0.07%); standard solution of ammonium iron(II) sulphate, or Mohr's salt (0.1 N); *o*-phenanthroline/ferrous sulphate indicator. To 10 mL of the bichromate solution was added 1 spatula tip of Mercury Sulphate, 30 mL of concentrated sulfuric

acid, and 20 mL of sample. This was subsequently boiled for 2 h in test tubes equipped with a reflux condenser at a temperature of 190 °C. At the end of incubation, it was allowed to cool and 80 mL of distilled water and 3–4 drops of indicator were added. The excess of bichromate was retro-titrated (color changing from green to orange) with a 0.1 N solution of Ammonium iron (III) sulphate. Titration was carried out on all samples, including the sample. Chemical Oxygen Demand (COD) was expressed in mg/L and the following formula was obtained:

$$\text{COD (mg/L)} = (V_1 - V_2)\ N\ 8000\ d/V$$

where:

V_1 = standard volume used for titration of control;
V_2 = volume used for titration of sample;
V = volume of analyzed sample;
N = normality of the titrant solution;
8000 = equivalent weight of oxygen in mg;
D = dilution of sample.

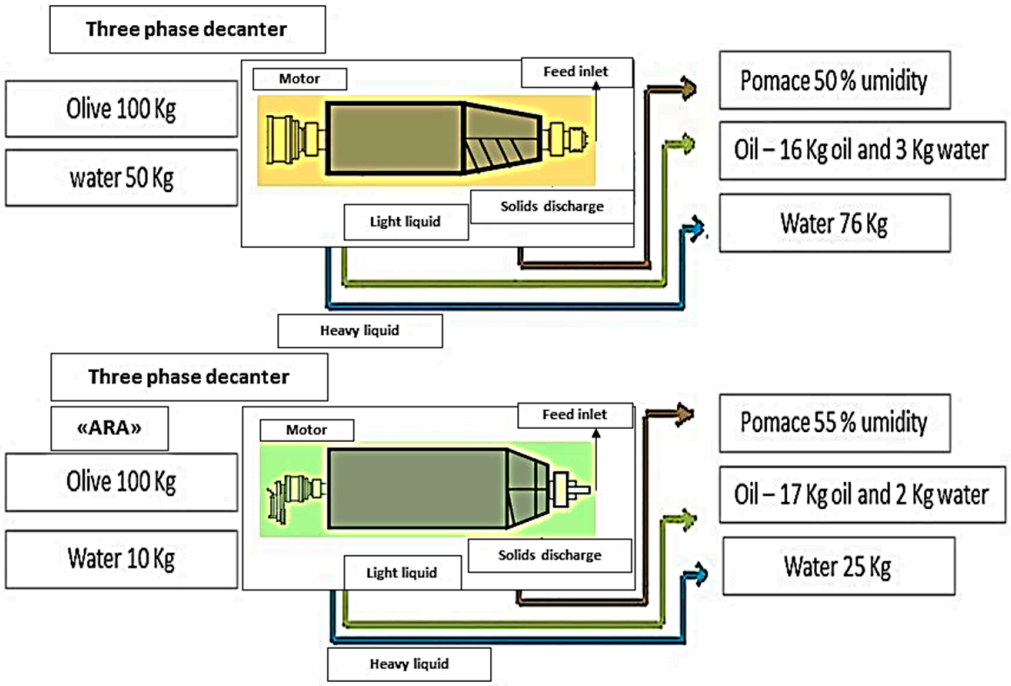

Figure 1. Traditional decanter with a long cone and innovative decanter "ARA" with a short cone.

2.5. Biological Oxygen Demand (BOD)

Oxygen consumption was measured nanometrically using the "Velp Scientifica BMS 6 BOD Analysis" (Velp Scientifica S.r.l., Milan, Italy). The microorganisms present in the sample of water containing biodegradable organic substances consume oxygen and produce a corresponding quantity of carbon dioxide. The sample was continually agitated during incubation to avoid the formation of concentration gradients. Incubation was carried out using a refrigerated thermostat set to 20 °C. Biological oxygen demand (BOD) was expressed as mg $O_2 \cdot L^{-1}$ (weight of oxygen consumed) in 5 days a 20 °C.

2.6. Total Sugar

To determine total sugars, the Dubois method was used, which is based on the hydrolysis of glycosidic linkages by concentrated sulfuric acid and the successive rehydration on of the monosaccharides [14]. The rehydrated products react with the phenol to give a spectrophotometrically measurable orange color (490 nm). Fifty g/L of phenol crystals and

98% concentrated sulfuric acid were used. A cuvette was used to mix 200 µL of sample, 200 µL of phenol solution, and 1 mL of sulfuric acid. It was left to react for 30 min and then absorbance was measured at 490 nm. The calibration curve was obtained using glucose in the range of 0–200 µg/L as a standard.

2.7. Residual Fat in OMW

The determination of oil in the olive mill wastewater was carried out by extraction in n-hexane. Fifty milliliters of n-hexane and 25 mL of OMW, previously centrifuged and filtered, were placed in a separation funnel. They were vigorously agitated and a saturated solution of NaCl was added to break the emulsion. The final organic phase, obtained after the four extractions (50 mL of n-hexane added to the aqueous phase), was re-placed in the separation funnel with a saturated solution of NaCl (ratio 1:2) and the organic phase was transferred to a conical flask, to which sodium bisulfite was added to eliminate any water present. Finally, it was filtered and weighed. The anhydrous organic phase was transferred to a 250 mL round-bottom flask, and it was dried using a rotavapor. The extract was recovered using 2 mL of methanol.

2.8. Phenolic Extraction Procedure

OMW (10 mL) was acidified with HCl (pH 2), then 15 mL of *n*-hexane was added, and the mixture was centrifuged (3000 rpm for 5 min). After that, the OMW was mixed with ethyl acetate (10 mL) and centrifuged again in the same condition. Once the phase dissolved in ethyl acetate was separated, the solvent was evaporated under vacuum at 40 °C. The residue was re-dissolved in a methanol: water solution (1:1 *v:v*) and subjected to analysis.

2.9. Total Phenols Content (TPC) and Total Flavonoids Content (TFC)

The total phenols content (TPC) was determined by using the Folin–Ciocalteu procedure [15]. The absorbance was measured at 725 nm using a UV-Vis Jenway 6003 spectrophotometer (Carlo Erba, Milan, Italy).

For the Total Flavonoid Content (TFC) determination, the method based on the formation of a flavonoid–aluminum complex was applied [15]. The absorbance was read at 510 nm.

2.10. HPLC Phenolic Profile

Selected phenolic compounds have been qualitatively and quantitatively analyzed following the procedure reported by Mateos et al. [16]. For this purpose, an HPLC Knauer (Asi, Advanced Scientific Instruments, Berlin, Germany) smart line pump 1000, coupled with a UV Waters 486 detector and a reversed phase C18 column (120 Å, 4 µm, 4.6 mm ID3250 mm, 5 µm particle size) (Polymer Laboratories), was used.

2.11. Antioxidant Activity

The radical scavenging activity was assessed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tests as previously reported [17]. The Ferric Reducing Ability Power (FRAP) was performed following the method previously described [17]. The ability of OMW extracts to counteract the peroxidation process was assessed using β-carotene bleaching test [17].

2.12. Evaluation of α-Amylase and α-Glucosidase Inhibitory Activity

To investigate the potential α-amylase and α-glucosidase inhibitory activities, the procedures previously reported were adopted [17].

2.13. Pancreatic Lipase Inhibitory Activity

The evaluation of pancreatic lipase inhibitory activity was assessed as previously described by Loizzo et al. [15].

2.14. Statistical Analysis

Results were expressed as means ± standard deviation (S.D) (n = 3). The inhibitory concentration 50% (IC_{50}) was calculated by using Prism GraphPad Prism version 4.0 software (GraphPad Software, San Diego, CA, USA). Tukey's multiple range test and Dunnett's test were applied to the data set using one-way analysis of variance (ANOVA) using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Analytical Parameters

Table 1 reports the results of analytical data obtained from OMW derived from three-phase traditional and three-phase ARA extraction mills.

Table 1. OMW analytical determinations of samples obtained from press extraction and centrifugation technology oil mills.

Analytical Parameters	Units	Three Phase Decanter "ARA"	Three Phase Decanter	Sign.
pH	–	5.3 ± 0.3 [a]	5.8 ± 0.9 [a]	ns
Dry extract	%	16.5 ± 0.9 [a]	9.6 ± 0.8 [b]	**
TPC	g/L	5.6 ± 0.6 [a]	1.7 ± 0.2 [b]	**
TFC	g/L	0.5 ± 0.04 [a]	0.3 ± 0.03 [b]	**
COD	mg/L	115,000 ± 23.9 [a]	48,000 ± 8.9 [b]	**
BOD	mg/L	14,725 ± 53.9 [a]	5595 ± 12.5 [b]	**
Sugar	%	3.7 ± 0.8 [a]	2.1 ± 0.2 [b]	**
Fat	%	1.0 ± 0.3 [a]	0.6 ± 0.1 [b]	**
Ash	%	1.8 ± 0.1 [a]	0.7 ± 0.1 [b]	**
Na	mg/kg	126 ± 8.9 [a]	73.5 ± 6.5 [b]	**
Ca	mg/kg	350 ± 12.7 [a]	173 ± 5.8 [b]	**
Mg	mg/kg	62.5 ± 8.9 [a]	45 ± 7.5 [b]	**
K	mg/kg	6652 ± 21.9 [a]	2003 ± 23.3 [b]	**
Fe	mg/kg	37.6 ± 5.9 [a]	17.5 ± 3.8 [b]	**
Cu	mg/kg	2.4 ± 0.8 [a]	0.9 ± 0.6 [b]	**
Zn	mg/kg	3.1 ± 0.4 [a]	1.7 ± 0.3 [b]	**

Data are expressed as mean ± S.D.; Differences were evaluated by one-way analysis of variance (ANOVA) completed with Tukey's test. Means in the same row with different small letters differ significantly ($p < 0.05$). ns: not significant; ** significant.

Water addition used in three-phase traditional extraction was found to have little or no effect on pH value. This result is in accordance with El-Abbassi et al. [13] and Aggoun et al. [14], while it significantly affected the other parameters. COD is used for quantification of pollution in water after wastewater treatment.

Means values of COD were 115,000 mg/L and 48,000 mg/L for three-phase traditional and three-phase ARA obtained OMW, respectively, whereas BOD values showed a similar trend and averaged at 14,725 mg/L for three-phase traditional and 5595 mg/L for three-phase ARA extraction technology, respectively. The average reducing sugar content was 3.7% in three-phase traditional-obtained OMW and 2% in three-phase ARA-obtained OMW. Mineral analysis showed high potassium content in vegetation water as one the most interesting elements for OMW disposal on agricultural soil [13].

3.2. OMW Phytochemicals Content

Phenolic compounds are the most investigated natural compounds for their health positive effects, mainly antioxidant, anti-inflammatory, anticancer, cardioprotective, and antimicrobial activities [18]. The use of an ARA decanter increases the concentration of TPC by about three times (5.6 mg gallic acid equivalents (GAE)/L vs. 1.7 mg GAE/L) for three-phase ARA and three-phase traditional decanter, respectively [19].

A similar trend was observed with TFC that, with the use of three-phase ARA extraction, reaches a value of 0.5 mg quercetin equivalents (QE)/L.

The ARA decanter is structured to ensure a lower use of water than other equipment, thus producing a small volume of OMW and, at the same time, an oil rich in polyphenols.

An oil richer in polyphenols can also be obtained by using a third-generation variable dynamic pressure cone decanter, as previously demonstrated by Catalano et al. [10] and Amirante et al. [11].

Table 2 reports the chemical profile of OMW obtained by the two different extraction procedures. Four compounds (4-hydroxyphenylacetate, hydroxythyrosol, oleuropein, and tyrosol) were identified as the most abundant (Figure 2). Concentrations of 502.3 and 373.3 mg/kg were found for hydroxytyrosol by using three-phase ARA decanter and three-phase decanter, respectively. This result is in accordance with Aggoun et al. [20].

Table 2. HPLC analysis of phenolic compounds (mg/L).

Phenolic Compounds	Three Phase Decanter ARA	Three Phase Decanter	Sign.
Caffeic acid	15.2 ± 1.2 ^{a}	8.1 ± 0.2 ^{b}	**
p-Coumaric acid	12.3 ± 0.9 ^{a}	6.4 ± 0.1 ^{b}	**
Ferulic acid	9.3 ± 0.7 ^{a}	6.9 ± 0.2 ^{b}	**
Luteolin	24.7 ± 2.6 ^{a}	15.2 ± 1.2 ^{b}	**
4-Hydroxyphenylacetate	112.8 ± 9.7 ^{a}	72.6 ± 2.6 ^{b}	**
Hydroxytyrosol	502.3 ± 9.7 ^{a}	373.3 ± 6.8 ^{b}	**
Oleuropein	198.2 ± 8.9 ^{a}	106.8 ± 5.4 ^{b}	**
Tyrosol	156.8 ± 3.6 ^{a}	89.7 ± 6.1 ^{b}	**
Vanillic acid	57.8 ± 2.9 ^{a}	29.4 ± 3.2 ^{b}	**
Verbascoside	42.1 ± 5.8 ^{a}	26.7 ± 4.1 ^{b}	**
Apigenin	31.6 ± 2.6 ^{a}	18.4 ± 1.6 ^{b}	**
o-Cumaric acid	11.34 ± 0.7 ^{a}	5.3 ± 0.3 ^{b}	**
Floretic acid	21.1 ± 1.02 ^{a}	12.8 ± 0.8 ^{b}	**

Data are expressed as mean ± S.D.; Differences were evaluated by one-way analysis of variance (ANOVA) completed with Tukey's test. Means in the same row with different small letters differ significantly ($p < 0.05$). ** significant.

Using the three-phase ARA decanter, a double concentration of tyrosol was obtained (156.6 mg/kg vs. 89.7 mg/kg for three-phase and three-phase ARA, respectively). Values of 198.2 mg/kg and 106.8 mg/kg of oleuropein were found for the three-phase ARA and three-phase decanter, respectively. Caffeic acid, *p*-coumaric acid, ferulic acid, luteolin, 4-hydroxyphenylacetate, verbascoside, and vanillic acid are the other identified compounds.

Previously, Gueboudji et al. [21] investigated the phenolic profile of OMW derived from the cold extraction of Algerian olive oil and reported the presence of caffeic, quinic, and 4,5-di-*O*-caffeoyquinic acids, and kaempferol as the main abundant compounds.

Apigenin, caffeic acid, catecol, *p*-cumaric acid, diosmetin, and hydroxytyrosol were the major compounds in OMW obtained by the processing of "Dolce di Rossano" and "Carolea" olive varieties using a three-phase decanter and, successively, a spray dryer system at low temperatures [22]. Polyphenol OMW-enriched fractions were obtained by Tundis et al. [23] using microfiltration, nanofiltration, and reverse osmosis. Reverse osmosis retentate showed a high hydroxytyrosol, tyrosol, oleuropein, verbascoside, vanillic acid, and luteolin content. Successively, the same research group investigated the phytochemical content of microfiltered OMW treated by direct contact membrane distillation (MD). Results

evidenced that MD retentate was five times richer in hydroxytyrosol and verbascoside and about seven times richer in oleuropein than the feed [24].

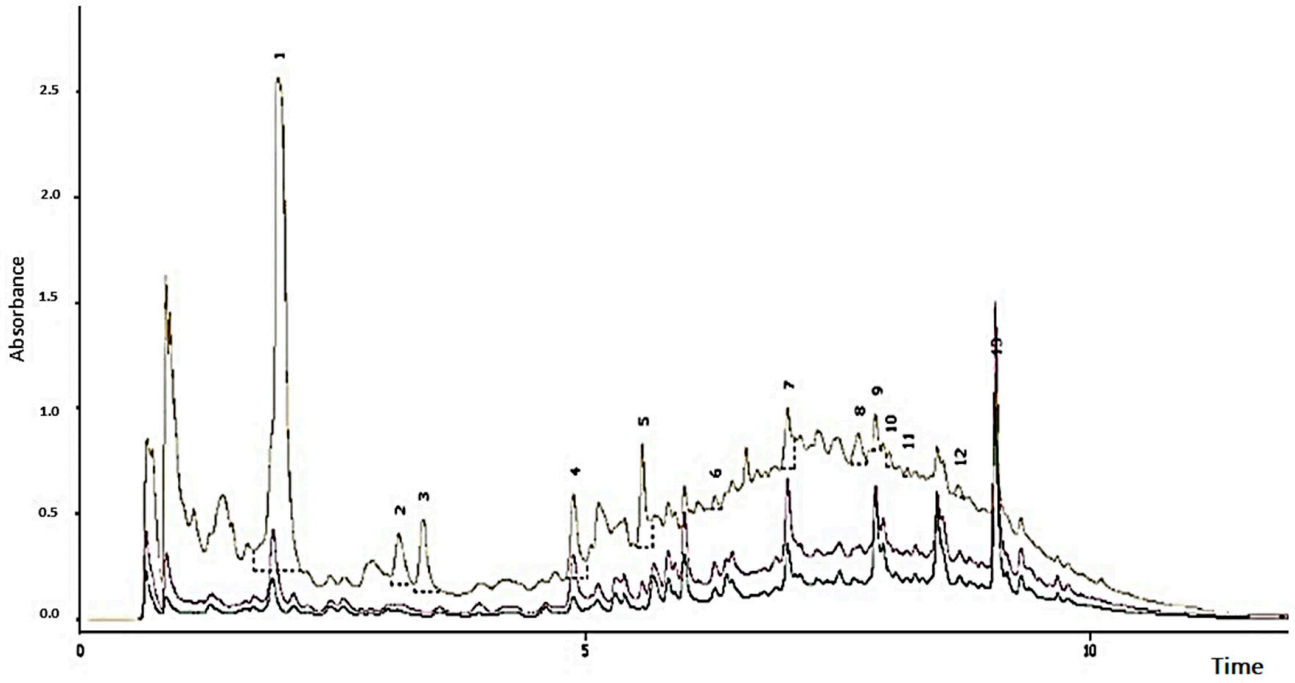

Figure 2. HPLC-DAD identified compounds in OMW extract 1: hydroxytyrosol; 2: 4-Hydroxyphenylacetate; 3: Tyrosol; 4: Hydroxytyrosol; 5: caffeic acid; 6: floretic acid 7: *p*-cumaric acid; 8: ferulic acid; 9: verbascoside; 10: luteolin; 11: *o*-cumaric acid; 12: apigenin; 13: oleuropein.

A similar approach was applied by Russo et al. [25], which used a complex process of microfiltration, reverse osmosis, and then vacuum membrane distillation using hydrophobic hollow fiber membranes. The application of this process allows for the obtainment of a purified OMW with a concentration of valuable polyphenols in a smaller volume (TPC values in the range 1.5–15 g GAE/L).

3.3. Antioxidant Activity

The antioxidant properties were assessed using a multi-target approach based on tests operating by different mechanisms of action (Table 3). Despite the different extraction technologies, no statistically significant differences were found between the OMW obtained with three-phase ARA and three-phase traditional extraction decanter methodology in both radical scavenging tests (DPPH and ABTS) (Table 3). OMW extract from ARA decanter technology showed a FRAP value comparable to the positive control BHT (67.2 μMFe (II)/g vs. 63.5 μMFe (II)/g). On the contrary, OMW extract obtained by the traditional three-phase decanter showed an IC_{50} value of 40.1 μg/mL. In the β-carotene bleaching test, the antioxidant activity is dependent on the substrate polarity. In fact, non-polar antioxidants can exert stronger antioxidant properties in emulsions systems due to their higher concentration in the lipid phase. This explains why extracts with higher content of TPC and TFC showed lower activity in this assay. The application of direct contact membrane distillation allows for obtaining better antioxidant properties than those observed by using the ARA decanter. In fact, values of 97.2 and 9.8 μg/mL were found for feed and MD retentate samples derived from OMW treatment against DPPH radicals by Tundis et al. [24]. The result obtained by feed, in FRAP, test is comparable to that obtained by ARA decanter (67.7 μMFe (II)/g vs. 67.2 μMFe (II)/g). Our results on DPPH assay are better that those found by El-Abbassi et al. [19] for OMW extracts obtained by three-phase process modern and semi-modern systems.

Table 3. Antioxidant activity.

Samples	DPPH Test §	ABTS Test §	β-Carotene Bleaching Test §	FRAP Test ^
Three-phase decanter	60.6 ± 3.1 ***	68.4 ± 3.1 ***	40.1 ± 2.2 ***	55.8 ± 1.3
Three-phase decanter ARA	55.9 ± 2.8 ***	55.4 ± 3.1 ***	69.9 ± 2.4 ***	67.2 ± 1.8
Positive controls				
Ascorbic acid	5.2 ± 0.8	1.6 ± 0.4		
BHT				63.5 ± 2.3
Propyl gallate			0.1 ± 0.0	

Data are expressed as means ± S.D. (n = 3). §: IC_{50} (μg/mL); ^: μM Fe(II)/g. Differences within and between groups were evaluated by one-way ANOVA followed by Dunnett's test (*** $p < 0.001$) where samples were compared with the positive control.

Moreover, our data are better than those reported for OMW permeate obtained by nanofiltration process (10.7 μM Fe(II)/g) [23]. Previously, Russo et al. [25] evidenced that the antioxidant activity is influenced by several factors (extraction, cultivar, area of collection, climatic conditions, stage of ripening). According to the literature, the antioxidant power is not only related to the TPC, since these compounds are not the only phytochemicals capable of exerting antioxidant activity present in OMW. In fact, El Yamani [26] compared the TPC and antioxidant activity (DPPH and FRAP test) of OMW derived from two three-phase decanters and a super-pressure system used to processes olives from Morocco. Despite the fact that OMW derived from the three-phase decanter showed the highest TPC value, this extract displayed the lowest DPPH radical scavenging potential (IC_{50} of 42.5 μg/mL), whereas IC_{50} values of 32.5 and 40.0 μg/mL were recorded for two-phase and super-pressure decanters, respectively.

Hydroxytyrosol, which is the dominant compound in OMW extract, exerted antioxidant activity by a different mechanism of action, including inhibition of cyclooxygenase, 5-lipoxygenase, the inducible form of nitric oxide synthase, etc. [27,28]. Similarly, the seco-iridoid oleuropein possesses antioxidant activity through a different mechanism of action. Moreover, oleuropein was able to exert ABTS radical scavenging activity and was more potent than vitamin C in the FRAP test [29].

3.4. Target Enzymes Linked to Metabolic Syndrome

Along with obesity, the metabolic syndrome is increasing worldwide. To date, the exact pathophysiology underlying the metabolic syndrome remains unknown. However, some factors including abdominal obesity and insulin resistance are more involved [30]. For this reason, counteracting the glycemic peak and reducing fat intake to avoid obesity appear to be useful. Herein, considering a circular economy approach, based on the reuse of OMWs as possible sources of bioactive compounds, we have tested OMW extracts against carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) and lipase. The strategy of reducing the digestibility of carbohydrates is considered a valid prophylactic approach to prevent diabetes mellitus type 2 (T2DM). Therefore, the consumption of foods rich in hydrolyzing enzyme inhibitors and/or nutraceuticals with the same activity is recommended for the prevention of T2DM.

Acarbose, Voglibose, and Miglitol are the main prescribed drugs able to inhibit both carbohydrate-hydrolyzing enzymes [31]. However, these compounds are characterized by several side effects that make pharmacological approaches less attractive as therapeutic agents, which makes natural products viable alternatives [32].

Although all investigated OMW extracts showed an enzyme inhibitory activity lower than the positive controls, generally α-glucosidase enzyme was more sensible to the action of the extracts (IC_{50} values of 154.6 and 168.8 μg/mL for the three-phase ARA decanter and the traditional one, respectively).

At the same time, ARA decanter-derived extract exhibited a good α-amylase inhibitory activity (IC_{50} value of 181.5 μg/mL) (Table 4).

Table 4. Hypoglycemic and hypolipidemic activity.

Samples	α-Amylase §	α-Glucosidase §	Lipase §
Three-phase decanter	230.2 ± 4.9 ***	168.8 ± 3.9 ***	281.1 ± 4.48 ***
Three-phase decanter ARA	181.5 ± 3.7 ***	154.6 ± 3.5 ***	219.9 ± 4.05 ***
Positive controls			
Acarbose	50.2 ± 0.9	35.6 ± 1.1	
Orlistat			37.4 ± 1.3

Data are expressed as means ± S.D. (n = 3). §: IC_{50} (μg/mL); Differences within and between groups were evaluated by one-way ANOVA followed by Dunnett's test (*** $p < 0.001$) where samples were compared with the positive control.

IC_{50} values of 219.9 and 281.1 μg/mL were found for three-phase ARA decanter and three-phase decanter OMW extracts, respectively, against lipase. The ability of OMW extract to inhibit key enzymes linked to obesity and hyperglycemic condition was observed by Tundis et al. [23,24]. IC_{50} values of 65.3 and 66.2 μg/mL were found against α-amylase and α-glucosidase, respectively, by using retentate obtained by reverse osmosis. The same sample showed the highest lipase inhibitory activity (IC_{50} of 175.6 μg/mL) [23]. On the contrary, the application of direct contact membrane distillation did not show statistically significant differences in carbohydrate-hydrolyzing enzymes, whereas values of 181.0 and 400.8 μg/mL for MD retentate and feed, respectively, were recorded [24].

The high content of hydroxytyrosol contributes to the inhibitory activity of the OMW extract against α-amylase and α-glucosidase. In fact, this compound exhibited a strong α-glucosidase inhibitory activity (IC_{50} of 150 μM), whereas a moderate inhibition against α-amylase was found. An IC_{50} value of 400 μM was observed for oleuropein against α-glucosidase. Moreover, against α-glucosidase, hydroxytyrosol exhibited a non-competitive inhibition whereas uncompetitive inhibition was registered for oleuropein [33]. This phytochemical modulates adipogenic gene expression, adipocyte differentiation, and fat accumulation in humans [34].

4. Conclusions

Despite environmental pollution problems, numerous studies have shown that OMWs are a rich source of healthy constituents, mostly phenols, characterized by promising biological activities. From a circular economy point of view, the reuse of OMWs in industries could reduce the environmental impact derived from the disposal of this waste.

The present study compared, for the first time, the content of bioactive molecules and the biological properties of extracts obtained from OMWs, resulting from the use of two different types of three-phase decanters: traditional and water-saving (ARA).

The ARA system is designed to minimize the amount of wastewater. According to the literature, our results showed heterogeneous chemical–physical characteristics both in terms of organic and inorganic substances. Olive ripening stage, climatic conditions, variety of olive cultivation, geographical area, and storage time before olive pressing, as well as the decanter used, are responsible for this heterogeneity.

The results obtained with the ARA system show how this system allows for obtaining an extract not only rich in bioactive substances, but also more powerful, both as an antioxidant and in its ability to inhibit the enzymes used as targets to prevent or treat metabolic syndrome. Therefore, the application of this technology, with an innovative geometry, allows numerous advantages for the oil industry by minimizing the production of OMW, with a greater intrinsic value of the waste itself, thus reducing storage and disposal costs, as well as the impact on the environment in terms of pollution.

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