LWT - Food Science and Technology 93 (2018) 323–328

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Phenolic composition and antimicrobial activity of Algerian olive products and by-products

Wahiba Yakhlef[a], Rabah Arhab[b], Concepción Romero[c], Manuel Brenes[c], Antonio de Castro[c], Eduardo Medina[c,*]

[a] Department of Molecular and Cellular Biology, Abbes Laghrour University, Khenchela, Algeria
[b] Department of Nature and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria
[c] Food Biotechnology Department, Instituto de la Grasa (IG-CSIC), Campus Universitario, Building 46, Ctra. Utrera, Km 1, 41013, Seville, Spain

ARTICLE INFO

Keywords:
Olive cultivars
Olive oil
Olive mill wastewaters
Polyphenols
Antimicrobials

ABSTRACT

This work characterized the phenolic profile of virgin olive oil, pomace, and olive mill wastewater (OMWW) of the three Algerian cultivars Blanquette, Rouguette and Sigoise, which were generated by traditional discontinuous press and 3-phase centrifugal extraction processes. Results revealed the cv. Blanquette as the cultivar with the highest phenolic content and no conclusive differences between the oil extraction processes were found. The antimicrobial activity exerted by OMWW and olive oils was attributed to their phenolic content, especially glutaraldehyde-like compounds. The most bactericidal OMWW, which presented the highest phenolic concentration (10,828 mg/L), showed a minimal bactericidal concentration (MBC) of 2.5% for all target microorganisms. In addition, a concentration of 452 mg/kg of total polyphenols in the olive oil reduced more than 3 log units from the initial inoculum in 30 min. Olive derived products showed a remarkable antimicrobial activity that makes them a good source of natural antimicrobials.

1. Introduction

Olea europaea L. represents one of the most important crops in Mediterranean countries. According to the International Olive Oil Council, Algeria is the ninth largest producer country of olive oil in the world, with around 87.5 tons in the 2015/16 season (IOC, 2017). For olive oil production, fruits are harvested from the tree from November to February, crushed in a grinder and malaxed to increase the released oil yield. The oil extraction from the olive paste can be conducted according to different processes: (i) traditional discontinuous press, (ii) 3-phase centrifugal, and (iii) 2-phase centrifugal extraction systems (Klen & Vodopivec, 2012). Three different products are generated with the traditional discontinuous press and the 3-phase system, (olive oil, pomace, and olive mill wastewater (OMWW)), and only two (olive oil and pomace) with the 2-phase system.

Virgin olive oil, unlike other vegetable oils, is consumed directly without refining, preserving its characteristic aroma and organoleptic properties. Olive oil is appreciated worldwide for its nutritional value, flavor and for its healthy properties. The increasing popularity of olive oil is due to its high concentration in monounsaturated fatty acid (oleic acid), but the presence of minor compounds such as phenolic compounds also contributes to the nutritional valorization of this fat; in particular, these substances possess antioxidant, antimicrobial and antitumoral activities, among others (Brenes, Medina, Romero, & de Castro, 2007; D'Angelo et al., 2005; Visioli et al., 2005). The phenolic profile and the quality of olive oils depends on several factors such as cultivar (Laincer et al., 2016), maturation of the fruits, time of harvesting, and the oil extraction process used (Bengana et al., 2013; Laribi et al., 2009).

Even though less eco-friendly, traditional press process and 3-phase centrifugal processes are still in use in many olive mills in Algeria that generate large amounts of OMWW. The seasonal production of OMWW receives insufficient treatment in the plant with a final destination in large ponds for evaporation or dumping in the soil or rivers, with the concomitant environmental and management problems due to their high polluting load and toxicity (Chatzistathis & Koutsos, 2017; McNamara, Anastasiou, O'Flaherty, & Mitchell, 2008). The chemical characteristics of these OMWW can vary from different origins, but they present a high concentration of organic substances such as sugars, proteins, carotenoids, tocopherols (Dermeche, Nadour, Larroche, Moulti-Mati, & Michaud, 2013) and phenolic compounds (El-Abbassi, Kiai, & Hafidi, 2012), and could be considered as a potential source of valuable natural biocompounds with a feasible recovery (Rubio-Senent, Martos, Lama-Muñoz et al, 2015). Precisely, olive products and by-

* Corresponding author.
E-mail address: emedina@ig.csic.es (E. Medina).

https://doi.org/10.1016/j.lwt.2018.03.044
Received 18 October 2017; Received in revised form 22 February 2018; Accepted 18 March 2018
Available online 20 March 2018

products possess an antimicrobial activity against phytopathogenic bacteria and fungi which have been associated with their phenolic compounds concentration (Brenes et al., 2011). The presence of polyphenols in this waste product open up the possibility of using it in agriculture as fertilizer or for restoration of degraded soils making this by-product more valuable (Chatzistathis & Koutsos, 2017; Medina et al., 2011).

The objective of the present work was i) to study the polyphenols composition present in virgin olive oil, pomace and OMWW from Algerian olive oil mills, considering extraction processes (traditional discontinuous press and 3-phase centrifugal system) and olive cultivars (Sigoise, Blanquette and Rougette); ii) to determine *in vitro* the antimicrobial activity of Algerian virgin olive oil and OMWW and their bioactive phenolic compounds against bacteria, in order to highlight their bactericidal potential.

2. Material and methods

2.1. Olive samples and extraction process

A total of 54 samples of olive oil (18), pomace (18) and olive mills waste waters (OMWW) (18) were collected in December from various mills located in Roknia, Bouati Mahmoud, Guelma, Bouchegouf and Medjez Sfa, all of them in the region of eastern Algeria (36° 28′ 00″ N, 7° 27′ 00″ E) during the season 2016/2017. Samples were from three different olive cultivars (cv. Rouguette (R), cv. Blanquette (B) and cv. Sigoise(S)) and were obtained by two different extraction methods, traditional by pressure (P) and 3-phase continuous by centrifugation (C). Three lots of each combination were studied. Samples of olive oil were filtrated through cellulose paper and kept at ambient temperature until analyses. Pomace samples were extracted immediately with dimethyl sulfoxide (DMSO), and OMWW were acidified with phosphoric acid at pH 2 in order to avoid the oxidation of the phenolic compounds. Both were kept frozen at −30 °C for further analysis after two months of storage.

2.2. Phenolic compounds analysis by HPLC

The phenolic composition of olive oils was studied according to Medina, de Castro, Romero, and Brenes (2006). Briefly, an extraction of 0.6 mL of olive oil was obtained using 3 × 0.6 mL of *N, N*- dimethylformamide (DMF). The extract was washed with hexane, and N_2 was bubbled into the DMF extract to eliminate the residual hexane. Then, the extract was filtered (0.2 μm pore size) and 20 μL were injected into the chromatograph. The extraction of phenolic compounds from pomace was based on methodology reported elsewhere (Romero, Medina, Mateo, & Brenes, 2017). Around 10 g of pomace was mixed in an Ultra-Turrax homogenizer (Ika, Breisgau, Germany) with 30 mL DMSO. After 30 min of resting contact, the mixture was centrifuged at 6000 × *g* for 5 min (22 °C). Then, an aliquot of 250 μL of DMSO extract was mixed with 250 μL of internal standard (0.2 mM syringic acid in DMSO) and 500 μL of DMSO. For the case of OMWW, 250 μL of samples were centrifuged and mixed with 250 μL of internal standard (2 mM syringic acid in distilled water) and 500 μL of distilled water. Finally, all samples were filtered through a 0.22 μm pore size nylon filter and an aliquot (20 μL) was injected into the chromatograph. The chromatographic system consisted of a Waters 717 plus autosampler, a Waters 600 E pump, a Waters column heater module and a Waters 996 photodiode array detector (PDA). For olive oils samples was also used a Jasco FP-920 fluorescence detector (Jasco, Tokyo, Japan). They were connected in series and operated with Empower 2.0 software (Waters Inc.). A 25 cm × 4.6 mm i.d., 5 μm Spherisorb ODS-2 (Waters) column at a flow rate of 1 mL min − 1 and a temperature of 35 °C was used in all experiments. Separation was achieved by gradient elution using (A) water (pH 2.5 adjusted with 0.15% phosphoric acid) and (B) methanol described by Ramírez, Medina, Brenes, and Romero (2014). Phenolic compounds were monitored at 240, 280 and 320 nm with the PDA detector and the fluorescence detector only for olive oil samples. The evaluation of each compound was performed using a regression curve with the corresponding standard. Hydroxytyrosol (Hy), luteolin, luteolin 7-glucoside, rutin and apigenin were purchased from Extrasynthese SA (Genay, France). Hydroxytyrosol glycol, tyrosol (Ty), 4-ethylphenol, caffeic, *p*-coumaric and vanillic acids were purchased from Sigma Chemical Co. (St Louis, MO, USA). Hydroxytyrosol 1-O-glucoside, salidroside, ester of cafeic acid and comselogoside were quantified using the response factors of Hy, Ty, caffeic acid, and *p*-coumaric acid, respectively. Hydroxytyrosol 4-O-glucoside, pinoresinol, 1-acetoxypinoresinol, the elenolic acid (EA), the oleuropein aglycon (HyEA) and ligustroside (TyEA), and the dialdehydic form of decarboxymethyl elenolic acid free (EDA) or linked to hydroxytyrosol (HyEDA) or tyrosol (TyEDA) were obtained by semi-preparative HPLC (Medina, Brenes, Romero, García, & de Castro, 2007; Medina, Romero, Brenes, de Castro, & García, 2008). The analyses were performed in duplicate.

The identification of the isolated phenolic compounds from the extract was made using a HPLC-MS system described by Susamci, Romero, Tuncay, and Brenes (2017). The chromatographic system consisted of a Waters 2695 Alliance with a pump, column heater and autosampler modules, and the detection was carried out with a Waters 2998 photodiode array detector and a mass single-quadrupole detector (QDa, Waters, USA). The QDa mass detector was operated in the negative mode (ESI-), the capillary voltage was set at 0.8 kV, the cone voltage to 15 V, and nitrogen was used as nebulizer gas with de-solvation temperature set at 600 °C. The flow rate was 1 mL/min, and the column, solvent and gradient conditions were the same as mentioned above.

Total phenolic compounds in olive oils is the sum of Hy, Hy glycol, Tyr, 4-ethylphenol, pinoresinol, 1-acetoxypinoresinol, apigenin, vanillin, luteolin, *p*-coumaric acid, EDA, HyEDA, TyEDA, EA, HyEA and TyEA. Total phenolic compounds in pomace is the sum of Hy, Hy 1-O-glucoside, Hy 4-O-glucoside, Ty, salidroside, HyEDA, rutin, luteolin, luteolin 7-glucoside, *p*-coumaric acid, vanillic acid, cafeic acid, ester of cafeic acid, and comselogoside. Total phenolic compounds in OMWW is the sum of Hy, Hy glycol, Hy 1-glucoside, Hy 4-glucoside Ty, salidroside, EDA-like, HyEDA, EA, cafeic acid, ester of cafeic acid and comselogoside.

2.3. Isolation of phenolic compounds

OMWW SP (generated from Sigoise cultivar by a traditional extraction by pressure) was selected because it contained less solids in suspension and was easier to handle. A 1:1 (v:v) mixture of OMWW SP and ethyl acetate was vortexed for 1 min and then centrifuged at 9.000 g for 5 min. This step was repeated five times. Ethyl acetate extract was evaporated under vacuum in a rotavapor at 35 °C and suspended in distilled water (pH 5) using the same initial volume. A blank of ethyl acetate extract with water was also performed. Furthermore, the OMWW (SP) was centrifuged at 9.000 g for 5 min and ultrafiltered through a membrane of 3000 Da (Whatman, Sigma-Aldrich).

Phenolic compounds (Hy 1-O-glucoside, EDA-like and EA) were isolated from the ethyl acetate extract by analytical HPLC. The column, gradient and equipment were the same as those used for the phenolic compound analysis. Aqueous mobile phase was acidified with 2 N HCl (522 μL/L). Fractions were collected peak-by-peak manually. The pooled extract for each peak was evaporated under vacuum to dryness, and the residue was dissolved in acetic acid/sodium acetate buffer (pH 5.0). Finally, the concentration of each compound was measured by HPLC. A control run of HPLC was also performed injecting deionized water and collecting the corresponding fractions.

2.4. Bacterial strains and culture conditions

Pseudomonas fluorescens (CECT 378), Staphylococcus aureus (CECT

239), Escherichia coli (CECT 434), and *Enterococcus faecalis (CECT 481)* were obtained from the Spanish Type Culture Collection (Burjasot, Valencia, Spain). *P. fluorescens*, *S. aureus*, and *E. coli* were cultured in nutrient broth prepared with (g/L) "Lab-Lemco" powder (Oxoid) 5, Neutralized bacteriological peptone (Oxoid) 10, NaCl 5 and agar 15 for solid medium (pH 7.2). *E. faecalis* was cultured in Brain Heart Infusion (Oxoid Ltd., Basingstoke, Hampshire, England) with or without 1.5% agar. *P. fluorescens* was cultured at 29 °C, and other strains were cultured at 37 °C. These strains were chosen as they are commonly used in antimicrobial testing (European Standard EN 1276, 1997). All strains were kept at - 80 °C in the adequate culture broths with glycerol (15–20%) until use. Every target strain was cultured twice in its corresponding broth, from the frozen stock, before testing, and overnight cultures were routinely used for inoculum preparation.

2.5. Antimicrobial assays

Three olive oils and OMWW (RC, SP and BC) were selected according to the concentration of total polyphenols: low, intermediate and high phenolic concentration, respectively. RC was obtained from Rougette cultivar by 3-phase continuous extraction, BC from Blanquette cultivar by 3-phase continuous extraction, and SP from Sigoise cultivar by traditional pressure extraction.

An antimicrobial test was performed with all strains against the selected OMWW. The pH of the OMWW were adjusted to 5.5, filtered through 0.22 µm of diameter, and diluted with sterile water at pH 5.5 before use. One mL of OMWW (at concentrations of 0.25, 0.50, 1.25, 2.5, 5, 10, 20, 40 and 60%) was inoculated and mixed with 50 µL of an overnight culture of the target microorganism diluted with saline solution (0.85 g/100 mL NaCl) to get an initial population between 5.51 and 6.07 log CFU/mL. The mixture was incubated at room temperature for 30 min and vortexed occasionally. After treatment, culturable survivors were determined by plating these mixtures or the corresponding decimal dilutions (0.1% peptone water) on the appropriate solid media plating with a Spiral Plater (Don Whitley Sci. Ltd., model WASP 2, Shipley, U.K.). Colonies were enumerated with an automated counter (Countermat, IUL Instruments, Barcelona, Spain). A control with water was performed and the antimicrobial test was performed in duplicate. The minimal bactericidal concentrations (MBC) of OMWW under these experimental conditions were determined as the lowest concentrations at which 3-log drops in CFU per milliliter were obtained (National Committee for Clinical Laboratory Standards, 1999).

The bactericidal activity assay of olive oil was based on the method reported by Medina et al. (2006). A 2 mL mixture (1:1) of olive oil with sterilized phosphate-buffered saline with Tween 20 (Sigma-Aldrich) was inoculated with 50 µL of the refreshed target strain previously diluted with saline solution to obtain an initial inoculum between 5.90 and 5.70 log CFU/mL. The mixture was incubated at room temperature for 30 min with occasional shaking and then plated to count survivors on appropriate media. Controls with no oil were also performed. Trials were carried out in duplicate. This test was performed with the oils RC, BC and SP against all strains.

The strain *S. aureus* CECT 239 was chosen for testing the effect of the ethyl acetate extract of OMWW SP, the OMWW extracted, ultrafiltered and isolated compounds. A mixture of 200 µL of isolated compounds at different concentrations was inoculated with a 50 µL of an overnight culture of the microorganism diluted with saline solution (0.85 g/100 mL NaCl) to get an initial population of 5.86 log CFU/mL. The mixture was incubated at room temperature for 30 min and viable count was determined by plating as mentioned before. Isolated compounds were tested at a concentrations range of 8–69 mg/L for Hy, 19–150 mg/L for EDA, and 40–320 mg/L for EA. They corresponded to concentrations found in the OMWW SP diluted at 2.5–20%. Isolated compound solutions were prepared diluting with sterile acetic acid/sodium acetate buffer at pH 5.5. The experiments were carried out in duplicate and a blank with mobile phases from HPLC was performed as a control.

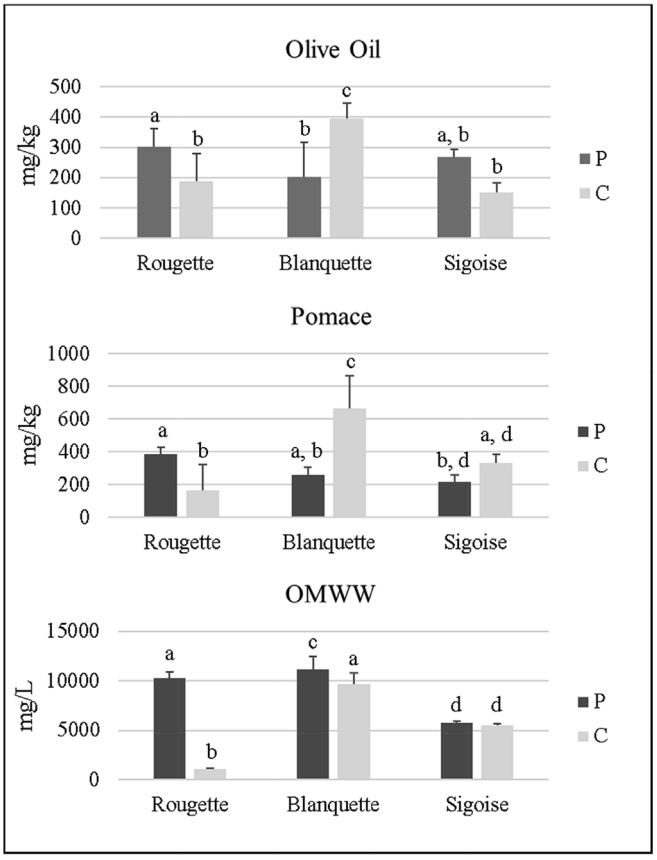

Fig. 1. Total phenolic compounds in olive products from cv. Rougette, cv. Blanquette and cv. Sigoise. See section 2.2 for the sum of single phenolic compounds. Three samples of each cultivar were run in duplicates. P corresponds to traditional discontinuous by pressure, and C corresponds to 3-phase continuous by centrifugation. Bars show standard deviation. Same letters within the same columns designates no statistically significant differences ($p < 0.05$) according to the Duncan's New Multiple Range Test.

2.6. Statistical analysis

Data were expressed as mean values and standard deviations (SD) were calculated. Comparison among mean variables was carried out by the Duncan's multiple range tests and the differences considered significant when $p < 0.05$. Statistica software version 7.0 was used for data processing (Statistica for Windows, Tulsa, OK, USA).

3. Results and discussion

3.1. Phenolic compounds in olive products

The phenolic composition in olive products obtained by traditional discontinuous and 3-phase centrifugal systems for the three Algerian cultivars studied is shown in Fig. 1, Figs. S1 and S2. Virgin olive oil obtained from cv. Blanquette by 3-phase centrifugal system showed the highest concentration of total phenols (395 mg/kg of oil), significantly higher than for traditional pressure extraction (202 mg/kg of oil). By contrast, Rougette and Sigoise cultivars presented higher phenolic concentrations extracted by traditional system (300 and 267 mg/kg of oil, respectively) than 3-phase (187 and 150 mg/kg of oil, respectively), although this difference was only significant for the cv. Rougette. The same tendency was observed for the phenolic composition in olive pomace (Fig. 1). The highest phenolic concentration was found for the cv. Blanquette by the 3-phase extraction (661 mg/kg of pomace) and the lowest for the cv. Rougette with the same system (165 mg/kg of

pomace). There were no significant differences between the extraction systems for the cv. Sigoise. The virgin olive oils analyzed presented a phenolic content in line with those obtained by Douzane et al. (2013). However, other authors have quantified higher concentrations of phenolic compounds for the Blanquette cultivar (Laincer et al., 2016). This variability of concentration among the same cultivars depends on many factors, including geography, agronomy, seasons and environmental factors. According to Klen and Vodopivec (2012), the traditional press system provides a higher transfer rate of phenols to oil than the 3-phase centrifuge. This corresponds to the Rougette and Sigoise cultivars studied, whereas it is not fulfilled for the Blanquette cultivar. Regarding the oil extraction system in this study, there were no conclusive differences between the different oil extraction processes.

The majority of phenols of the olive fruits were flushed away with the wastewater generated in olive oil production, only 0.3–1.5% from total polyphenols are found in the olive oil (Klen & Vodopivec, 2012). The highest phenolic concentration was found in the OMWW, which ranged from 10,030 to 1184 mg/L for the Rougette cultivar, 11,155 and 9690 mg/L for the Blanquette cultivar, and 5760 and 5469 mg/L for the Sigoise cultivar by traditional pressure system and 3-phase respectively. OMWW from Rougette and Blanquette cultivars exhibited much higher polyphenols by traditional than 3-phase extraction systems, unlike that observed for other Algerian olive cultivars (Aggoun et al., 2016). Otherwise, there were no significant differences for the Sigoise cultivar.

3.2. Antimicrobial activity of olive products

Three OMWW (RC, BC and SP) were selected for antimicrobial tests according to their concentration in total polyphenols (Table 1). The OMWW generated from cv. Blanquette BC showed the highest phenolic content (10,828 mg/L), ten times more than OMWW RC from cv. Rougette (1096 mg/L). Both were obtained by a 3-phase continuous olive oil extraction system. The OMWW from cv. Sigoise generated by traditional pressure system (SP) showed an intermediate concentration in total polyphenols (5762 mg/L). Among all the phenolic compounds, Hy, Hy 4-O-glucoside, EDA-like and EA composed around 90% of the total phenols and derivatives. To a lesser extent, hydroxytyrosol glycol, Hy 1-O-glucoside, tyrosol and salidroside showed lower concentrations, being very low or null for caffeic acid and caffeic acid ester (Table 1). No oleuropein concentration was found in any samples.

Several dilutions of these OMWWs were inoculated with selected microbial strains to get the MBC shown in Table 2. After 30 min of incubation at room temperature, the highest antimicrobial activity corresponded to OMWW BC, which also presented the highest phenolic content. This OMWW reduced 3 log units of the 4 strains tested when the concentration of total polyphenols was 2.5%. The OMWW SP showed a MBC of 10% for *E. coli* and 5% for the rest of the microorganisms. The lowest activity was observed for OMWW RC that showed a MBC of 60% for *S. aureus* and *E. coli* and more than 60% for *E. faecalis* and *P. fluorescens*.

Table 2
MBCs of OMWWs against target bacteria.

OMWW	MBC (%)			
	S. aureus	*E. faecalis*	*E. coli*	*P. fluorescens*
RC	60	> 60	60	> 60
SP	5	5	10	5
BC	2.5	2.5	2.5	2.5

As mentioned, OMWWs are sources of phenolic compounds which are known to exhibit antimicrobial effects (Leouifoudi, Harnafi, & Zyad, 2015; Thielmann, Kohnen, & Hauser, 2017), but this activity cannot be assigned to a particular substance or to the polymeric fraction of this liquid. To elucidate the compounds responsible for this antimicrobial activity, OMWW SP was subjected to a phenolic extraction with ethyl acetate. The extract was enriched in Hy, Tyr, EDA-like and EA (Table 1), and the extracted OMWW held Hy 4-O-glucoside and salidroside. Only the Hy glycol was found in both phases at similar concentration. In parallel, the OMWW SP was ultrafiltered through a membrane of 3000 Da to remove the polymers so that simple phenols remained in the filtered OMWW at the same concentration than the original (Table 1). The SP extract of phenolic compounds, the extracted OMWW SP and the ultrafiltered OMWW SP were inoculated with *S. aureus* and the antimicrobial activity is shown in Fig. 2A. After 30 min of contact, the ultrafiltered OMWW SP exerted the same MBC (5%) as the non-filtered. The extract of OMWW SP at 5% reduced around 2 log units and reached the MBC at 10% presenting good bactericidal properties. Nevertheless, the extracted OMWW SP reached the MBC at a concentration of 40%. During the extraction, certain phenolic compounds such as Hy, EDA-like and EA diffused to the ethyl acetate extract and, consequently, the antimicrobial activity in the extracted OMWW decreased. Some of these compounds were isolated from the extract by HPLC, identified by MS-HPLC. The MS spectra of isolated compounds exhibited a signal at m/z 153 and 241 for hydroxytyrosol and EA respectively, as previously described by Medina et al. (2007). EDA-like exhibited a signal at m/z 199 (Fig. S3) as previously described for the first time by Medina et al. (2011), and subsequently characterized as (E)-3-(1-oxobut-2-en-2-yl) glutaric acid (Rubio-Senen, Martos, García et al., 2015).

Isolated compounds were tested individually against *S. aureus* (Fig. 2B). Neither hydroxytyrosol nor EA showed antimicrobial activity at concentrations studied. The same results were obtained for hydroxytyrosol, its glucosides, and tyrosol against lactic acid bacteria (Medina et al., 2007). Only the EDA-like compound showed antimicrobial activity at a concentration of 150 mg/L, which corresponded

Table 1
Phenolic composition (mg/L) of OMWWs and their extracts used for the antimicrobial assays.

Compound	RC	BC	SP	SP extract	Extracted SP	SP ultrafiltered
Hy Glycol	150[a] (21)	230 (4)	258 (0)	132 (2)	159 (8)	325 (22)
Hy 1-O-Glucoside	27 (5)	23.8 (0)	23 (4)	–	–	–
Hydroxytyrosol	108 (5)	1409 (63)	427 (11)	348 (2)	102 (3)	465 (10)
Hy 4-O-Glucoside	211 (11)	3150 (73)	1748 (92)	54 (3)	1897 (142)	1927 (91)
Salidroside	33 (3)	265 (2)	125 (5)	–	119 (9)	135 (1)
Tyrosol	14 (3)	76 (7)	425 (4)	53 (2)	–	54 (0)
Caffeic acid	–	3 (1)	1 (0)	–	–	–
Ester of caffeic	–	1 (0)	1 (0)	–	–	–
Comselogoside	–	1 (1)	2 (1)	1 (0)	–	–
EDA-like	465 (31)	3828 (359)	1252 (46)	1118 (23)	0 (0)	1237 (13)
EA	87 (2)	1840 (131)	1884 (4)	1582 (15)	0 (0)	1805 (6)
Total phenols	1096 (60)	10828 (344)	5762 (118)	3287 (16)	2277 (162)	5949 (129)

[a] Data are the mean of duplicates. Standard deviation shown in parentheses. Abbreviation: cv. Rouguette (R), cv. Blanquette (B), cv. Sigoise(S), traditional by pressure (P) and 3-phase continuous by centrifugation (C). See section 2.2 for phenolic compounds abbreviations.

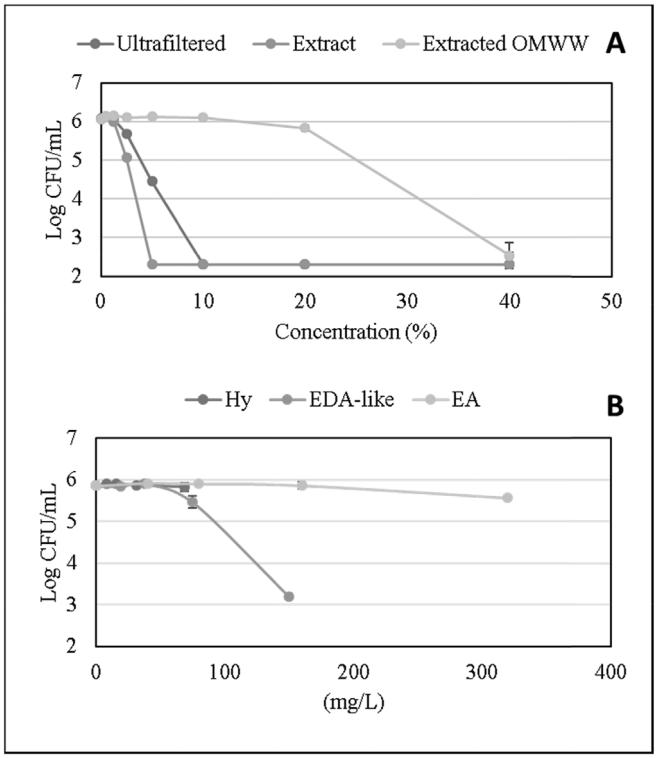

Fig. 2. Antimicrobial activity of OMWW SP extract (panel A) and isolated phenolic compounds (panel B) against *S. aureus* after 30 min of contact. Bars means standard deviations. See section 2.1 for abbreviations.

to the content found in the OMWW extract when it was diluted to around 13%. Hence, this antimicrobial activity agrees with the MBC found for the extract.

The antimicrobial activity of OMWW has widely been studied and several compounds have been attributed as responsible (Capasso et al., 1995; Obied, Bedgood, Prenzler, & Robards, 2007) but any individual compound explained this bioactivity by itself. However, it has been demonstrated that the most bactericidal compounds in olive products are glutaraldehyde-like compounds such as HyEDA and EDA (Medina, Brenes, García, Romero, & de Castro, 2009). EDA is an oleuropein derivative well studied in table olive elaborations (Medina et al., 2008). The presence of a dialdehydic structure in the molecule of EDA confers a strong antimicrobial activity, which is similar to that of the commercial biocide glutaraldehyde (Medina et al., 2009). In alperujo, the compound EDA-like is detected in high concentrations (Medina et al., 2011) and its origin has been suggested to be the antimicrobial EDA by a hydrolytic reaction during the storage and/or processing. The MS spectra differ in an oxygen atom from *m/z* 183 and 199 for EDA and EDA-like respectively. Although EDA-like had less antimicrobial activity against *Pseudomonas savastonoi* (Medina et al., 2011), the high amount of EDA-like compound present in the OMWW explains their bactericidal properties against bacteria and other phytopathogenic microorganisms (Medina et al., 2011; Rubio-Senent et al., 2015).

3.3. Antimicrobial activity of virgin olive oil

In the same way as OMWW, three virgin olive oils were selected for antimicrobial activity testing against target microorganisms. BC oil showed the highest concentration in total polyphenols (452 mg/kg of oil), followed by SP (370 mg/kg of oil) and RC (126 mg/kg of oil) (Table 3). Among the phenolic compounds, Hydroxytyrosol and tyrosol derivatives are the most abundant in the olive oil, where HyEA, HyEDA, TyEA and TyEDA are the majority. Also, the lignans pinoresinol and 1-acetoxypinoresinol were found at high concentrations in all the olive oils (Table 3). After inoculation and 30 min of contact with the oils (50%), only BC showed antimicrobial activity against *S. aureus* and *E. faecalis* (Fig. 3). This oil reduced the initial inoculum by more than 3 log units and no effect was observed for the rest of the oils and strains. Laincer et al. (2014) found a high antimicrobial activity in Algerian virgin olive oils against *S. aureus* and *Bacillus subtilis*. This bactericidal activity was attributed to the phenolic compounds of olive oil as being mainly responsible (Brenes et al., 2007). The effect of each individual phenolic compound was studied *in vitro* and showed that TyEDA and HyEDA presented a potent antimicrobial activity (Romero, Medina, Vargas, Brenes, & de Castro, 2007) due to the presence of the dialdehydic structure in their molecules (Medina et al., 2009). Both compounds possess the EDA structure linked to the phenols tyrosol and hydroxytyrosol respectively, that increases their lipophilic character and antibacterial efficacy. The results obtained in this work are in agreement with the previous studies. The BC olive oil presented higher concentrations of the oleuropein and ligustroside derivatives than RC and SP oils (Table 3) which is related to a higher antimicrobial activity.

Table 3
Phenolic compounds (mg/kg of oil) of olive oils used in the antimicrobial assays.

Compounds	Olive Oil		
	RC	BC	SP
Hydroxytyrosol	11[a] (1)	5 (0)	9 (0)
Hy Glicol	–	1 (0)	2 (0)
Hy- EDA	–	27 (0)	16 (3)
Hy- EA	11 (1)	99 (11)	66 (2)
Tyrosol	1 (0)	11 (1)	19 (2)
Ty - EDA	18 (4)	38 (1)	28 (0)
Ty- EA	7 (0)	185 (21)	142 (4)
4-ethylphenol	8 (0)	–	–
Pinoresinol	52 (3)	62 (1)	62 (6)
1- Acetoxypinoresinol	16 (1)	22 (2)	24 (2)
Luteolin	2 (0)	2 (0)	2 (0)
Apigenin	1 (0)	1 (0)	1 (0)
Total phenols	126 (8)	452 (35)	371 (13)

[a] Data are the mean of duplicates. Standard deviation shown in parentheses. See section 2.1 and 2.2 for abbreviations.

4. Conclusions

The results obtained in the present work have characterized the phenolic profile of products generated during olive oil extraction of three Algerian olive cultivars. Regarding the phenolic composition of olive products, no significant differences were found between the traditional discontinuous press and 3-phase centrifugal olive oil extraction processes. The antimicrobial test with isolated phenolic compounds revealed that EDA-like possesses a high bactericidal activity *in vitro*, and the high concentrations in the OMWW can explain the antimicrobial properties of this by-product. Moreover, the presence of glutaraldehyde-like compounds such as TyEDA and HyEDA in the virgin olive oils confer them with a strong antimicrobial activity. These results support that the high amount of antimicrobial compounds in the products derived from olive oil extraction such as virgin olive oil, pomace and OMWW, are a good source of natural antimicrobials with potential in healthcare, animal feed, the food industry or as pesticides in agriculture.

Acknowledgements

This research was supported by the Algerian Ministry of Higher Education and Scientific Research and the Spanish Government (Project AGL2016-76820-R, AEI / FEDER, UE).

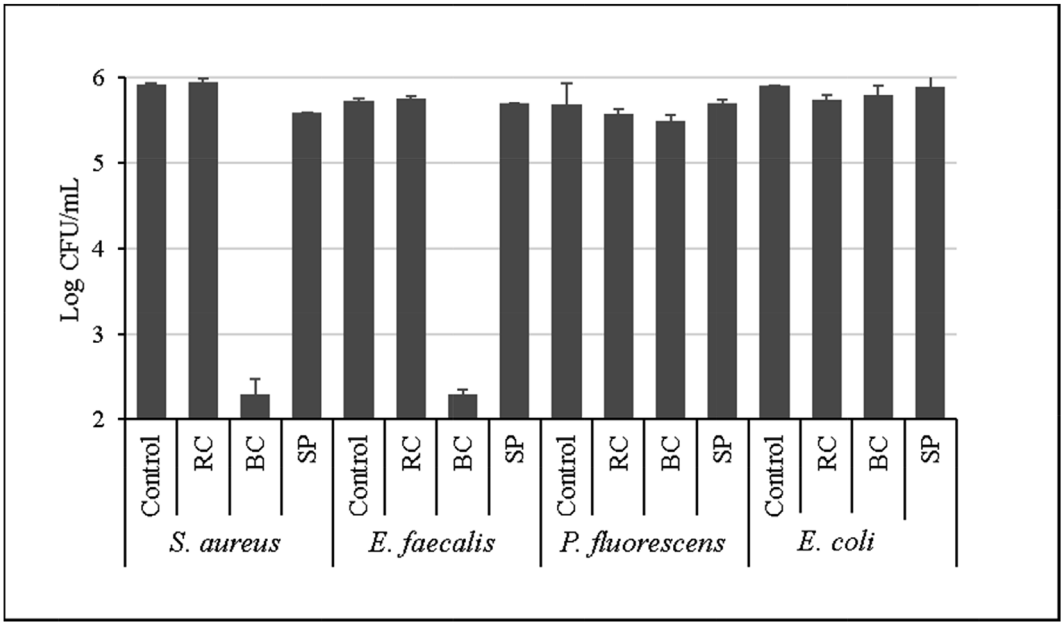

Fig. 3. Antimicrobial activity of olive oils against targeted microorganisms after 30 min of contact. Test was run in duplicates. Bars means standard deviations.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2018.03.044.

References

Aggoun, M., Arhab, R., Cornu, A., Portelli, J., Barkat, M., & Graulet, B. (2016). Olive mill wastewater microconstituents composition according to olive variety and extraction process. *Food Chemistry, 209*, 72–80.

Bengana, M., Bakhouche, A., Lozano-Sánchez, J., Amir, Y., Youyou, A., Segura-Carretero, A., et al. (2013). Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil. *Food Research International, 54*, 1868–1875.

Brenes, M., García, A., de los Santos, B., Medina, E., Romero, C., de Castro, A., et al. (2011). Olive glutaraldehyde-like compounds against plant pathogenic bacteria and fungi. *Food Chemistry, 125*, 1262–1266.

Brenes, M., Medina, E., Romero, C., & de Castro, A. (2007). Antimicrobial activity of olive oil. *Agro Food Industry Hi-tech, 18*, 6–8.

Capasso, R., Evidente, A., Schivo, L., Orru, G., Marcialis, M. A., & Cristinzio, G. (1995). Antibacterial polyphenols from olive oil mill wastewaters. *Journal of Applied Bacteriology, 79*, 393–398.

Chatzistathis, T., & Koutsos, T. (2017). Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. *Agricultural Water Management, 190*, 55–64.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry, 48*, 1532–1552.

Douzane, M., Tamendjari, A., Abdi, A. K., Daas, M. S., Mendid, F., & Bellal, M. M. (2013). Phenolic compounds in mono-cultivar extra virgin olive oils from Algeria. *Grasas y Aceites, 64*, 285–294.

D'Angelo, S., Ingrosso, D., Migliardi, V., Sorretino, A., Donnarumma, G., Baroni, A., et al. (2005). Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biology & Medicine, 38*, 908–919.

El-Abbassi, A., Kiai, H., & Hafidi, A. (2012). Phenolic profile and antioxidant activities of olive mill wastewater. *Food Chemistry, 132*, 406–412.

European Standard EN 1276 (1997). *Chemical disinfectants and antiseptics—quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used*.

IOC, International Olive Council. World table olive figures. (2017). Available from http://www.internationaloliveoil.org/estaticos/view/132-world-table-olive-figures Accessed 2017 Sep.

Klen, T. J., & Vodopivec, B. M. (2012). The fate of olive fruit phenols during commercial olive oil processing: Traditional press versus continuous two- and three-phase centrifuge. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 49*, 267–274.

Laincer, F., Iaccarino, N., Amato, J., Pagano, B., Pagano, A., Tenore, G., et al. (2016). Characterization of monovarietal extra virgin olive oils from the province of Béjaïa (Algeria). *Food Research International, 89*, 1123–1133.

Laincer, A., Laribi, R., Tamendjari, A., Arrar, L., Rovellini, P., & Venturini, S. (2014). Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities. *Grasas y Aceites, 65* e001.

Laribi, R., Rovellini, P., Deflaoui, L., Aidli, A., Mettouchi, S., Arrar, L., et al. (2009). Evolution of biophenolic compounds in virgin olive oil during olive ripening of Algerian cultivars. *Rivista Italiana delle Sostanze Grasse, 86*, 151–161.

Leouifoudi, I., Harnafi, H., & Zyad, A. (2015). Olive mill waste extracts: Polyphenols content, antioxidant, and antimicrobial activities. *Advances in Pharmacological Sciences, 2015* 714138.

McNamara, C. J., Anastasiou, C. C., O'Flaherty, V., & Mitchell, R. (2008). Bioremediation of olive mill wastewater. *International Biodeterioration & Biodegradation, 61*, 127–134.

Medina, E., Brenes, M., Romero, C., García, A., & de Castro, A. (2007). Main antimicrobial compounds in table olives. *Journal of Agricultural and Food Chemistry, 55*, 9817–9823.

Medina, E., Brenes, M., García, A., Romero, C., & de Castro, A. (2009). Bactericidal activity of glutaraldehyde-like compounds from olive products. *Journal of Food Protection, 72*, 2611–2614.

Medina, E., de Castro, A., Romero, C., & Brenes, M. (2006). Comparison of the concentrations of phenolic compounds in olive oil and other plant oils: Correlation with antimicrobial activity. *Journal of Agricultural and Food Chemistry, 54*, 4954–4961.

Medina, E., Romero, C., Brenes, M., de Castro, A., & García, A. (2008). Profile of antilactic acid bacteria compounds during the storage of olives which are not treated with alkali. *European Food Research and Technology, 228*, 133–138.

Medina, E., Romero, C., de los Santos, B., de Castro, A., García, A., Romero, F., et al. (2011). Antimicrobial activity of olive solutions from stored alpeorujo against plant pathogenic microorganisms. *Journal of Agricultural and Food Chemistry, 59*, 6927–6932.

National Committee for Clinical Laboratory Standards (1999). *Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26-A*. Wayne, PA: National Committee for Clinical. Laboratory Standards.

Obied, H. K., Bedgood, D. R., Prenzler, P. D., & Robards, K. (2007). Bioscreening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscicidal activities. *Food and Chemical Toxicology, 47*, 1238–1248.

Ramírez, E., Medina, E., Brenes, M., & Romero, C. (2014). Endogenous enzymes involved in the transformation of oleuropein in Spanish table olive varieties. *Journal of Agricultural and Food Chemistry, 62*, 9569–9575.

Romero, C., Medina, E., Mateo, M. A., & Brenes, M. (2017). New by-products rich in bioactive substances from the olive oil mil processing. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.8460wileyonlinelibrary.com.

Romero, C., Medina, E., Vargas, J., Brenes, M., & de Castro, A. (2007). In vitro activity of olive oil polyphenols against Helicobacter pylori. *Journal of Agricultural and Food Chemistry, 55*, 680–686.

Rubio-Senent, F., Martos, S., García, A., Fernández-Bolaños, J. G., Rodríguez-Gutiérrez, G., & Fernández-Bolaños, J. (2015b). Isolation and characterization of a secoiridoid derivative from two-phase olive waste (alperujo). *Journal of Agricultural and Food Chemistry, 63*, 1151–1159.

Rubio-Senent, F., Martos, S., Lama-Muñoz, A., Fernández-Bolaños, J. G., Rodríguez-Gutiérrez, G., & Fernández-Bolaños, J. (2015a). Isolation and identification of minor secoiridoids and phenolic components from thermally treated olive oil by-products. *Food Chemistry, 187*, 166–173.

Susamci, E., Romero, C., Tuncay, O., & Brenes, M. (2017). An explanation for the natural de-bittering of Hurma olives during ripening on the tree. *Grasas y Aceites, 68* e182.

Thielmann, J., Kohnen, S., & Hauser, C. (2017). Antimicrobial activity of *Olea europaea* Linné extracts and their applicability as natural food preservative agents. *International Journal of Food Microbiology, 251*, 48–66.

Visioli, F., Caruso, D., Grande, S., Bosisio, R., Villa, M., Galli, G., et al. (2005). Virgin olive oil study (VOLOS): Vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *European Journal of Nutrition, 44*, 121–127.