



The use of gamma radiation for extractability improvement of bioactive compounds in olive oil wastes

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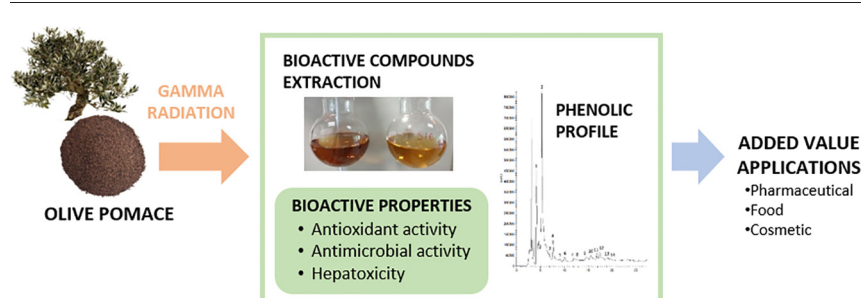
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HIGHLIGHTS

- Olive pomace is a source of phenolic compounds.
- Two types of olive pomace, crude and extracted, were evaluated for its bioactive potential.
- Hydroxytyrosol was the major compound in extracted and crude olive pomaces.
- Gamma radiation promoted the increase of phenolic compounds content in olive pomaces.
- Extracted olive pomace had higher bioactivity than crude olive pomace.

GRAPHICAL ABSTRACT



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ABSTRACT

Olive pomace is an environmentally detrimental waste from olive oil industry, containing large amounts of bioactive compounds that might be used by the food industry. In this work, the effects of gamma radiation on phenolic compounds and bioactive properties (antioxidant, antimicrobial activities and hepatotoxicity) of Crude Olive Pomace (COP) and Extracted Olive Pomace (EOP) extracts were evaluated. Hydroxytyrosol was the main phenolic compound identified in both olive pomace extracts (24–25 mg/g). The gamma radiation treatment of olive pomace improved at least 2-fold the extractability of phenolic compounds. Moreover, results suggested that gamma radiation at 5 kGy increased the antioxidant activity in EOP, while keeping the ability to protect erythrocytes against oxidation-induced haemolysis. Gamma radiation at 5 kGy could be a suitable technology for olive oil pomaces waste valorization, contributing to enhance extraction of phenolic compounds and bioactive properties, especially when applied on extracted material.

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1. Introduction

The olive oil production is an important industry, mainly of the Mediterranean region countries (Ammar et al., 2017; Suárez et al., 2010; Zorpas and Costa, 2010). In Portugal, this industry produces >600,000 t of wastes, whose discharge without treatment would have a negative impact in the environment. Due to the high content in phosphorous, potassium and organic matter, these wastes have been applied as amendment on the agricultural soils, not only because of its low cost but also for its potential to enhance soil fertility and reduce the risks of soil degradation (Federici et al., 2017; Regni et al., 2017). On the other hand, these wastes contain high amounts of organic substances (fibers, sugars, volatile fatty acids, polyalcohols, pectins and fats) and a variety of phenolic compounds such as hydroxytyrosol and tyrosol, secoiridoid derivatives, phenolic acids and flavonoids (Papaioannou et al., 2013), thus being also a promising source of valuable compounds. During the olive oil extraction process, the majority of these phenolic compounds remain in the olive pomace waste, as only 1% are found in the olive oil (Fernández-Bolaños et al., 2006).

The recovery of phenolic compounds from olive pomace have been studied through the application of several techniques, such as hydrothermal treatment (Fernández-Bolaños et al., 2002), membrane technology (Sygouni et al., 2019), centrifugation (Cioffi et al., 2010), Superheated Liquid Extraction (Peralbo-Molina et al., 2012), Pressurized Liquid Extraction (Pavez et al., 2019), Multi-frequency Multimode Modulated ultrasonic technique (Nunes et al., 2018) and solid-liquid extraction (Suárez et al., 2010, 2009; Vitali Čepo et al., 2017; Zuurro, 2014). Hydroxytyrosol has been reported as the main phenolic compound in olive pomace, being an effective natural antioxidant with beneficial properties such as cancer prevention, skin protection, anti-inflammatory activity (Fernández-Bolaños et al., 2008), and in the protection against cardiovascular diseases (Bulotta et al., 2014; Robles-Almazan et al., 2018). Other authors reported oleuropein as one of the most abundant phenolic compound in olive pomace, being the major one present in olive pomace from the Cilento National Park, in Italy (Cioffi et al., 2010). Tyrosol, verbascoside, caffeic acid, vanillin, vanillic acid, apigenin, luteolin and rutin are also found in olive pomace but in lower concentrations (Suárez et al., 2010). Verbascoside was identified as the most potent antioxidant in Australian olive mill waste extracts (Obied et al., 2008).

Nowadays, consumers prefer natural additives to be added to food products (Carocho et al., 2015) as they are considered safer than the artificial counterparts. Phenolic compounds are considered promising bioactive molecules to be used in the replacement of some of these additives. This is seen by the food industry as an opportunity to find new and more efficient natural ingredients, while also fighting to reduce the overall addition of artificial additives and producing minimally processed goods (Carocho et al., 2015). Phenolic compounds obtained from olive pomace could be suitable candidates to provide bioactive properties and bring additional value to food products, as they have been considered to lack toxicity (Bulotta et al., 2014).

Ionizing radiation is a clean and environment friendly technology. It is a physical treatment that does not rely on the addition of chemicals and have the capability of degrading complex molecules into low-molecular weight compounds. Recently, the scientific community has aroused considerable interest in the enhancement of beneficial properties by irradiation and in the ionizing radiation effects on bioactive compounds. Our previous studies have reported the use of this technology to improve phenolic content and antioxidant activity on industrial wastewater (Madureira et al., 2017), fresh fruits such as cherry tomatoes (Guerreiro et al., 2016) and raspberries (Cabo Verde et al., 2013), and dry medicinal plants (Pereira et al., 2017a), as well as its efficiency for decomposition of recalcitrant compounds (Madureira et al., 2018).

The aim of this work was to characterize the phenolic compounds profile of olive pomace waste from Portuguese olive oil industry and to assess the impact of gamma radiation on these compounds selecting

the optimal radiation dose to improve their extractability. Furthermore, the extracts were evaluated in terms of their biological activity, namely antimicrobial and antioxidant properties and hepatotoxicity. To our knowledge, this study represents the first application of ionizing radiation technologies with a view to improving the recovery of phenolic compounds in order to valorize olive pomace industrial waste.

2. Material and methods

2.1. Standards and reagents

HPLC-grade acetonitrile was obtained from Fisher Scientific (Lisbon, Portugal). Methanol and formic acid were acquired from Sigma-Aldrich (St. Louis, USA) and Honeywell (Charlotte, USA), respectively. Vanillic acid ($\geq 95\%$), apigenin-7-*O*-glucoside ($\geq 99\%$), *p*-hydroxybenzoic acid ($\geq 99\%$), caffeic acid ($\geq 99\%$) and syringic acid ($\geq 95\%$) were purchased from Extrasynthese (Genay, France), whereas hydroxytyrosol ($\geq 99\%$) and tyrosol ($\geq 98\%$) were obtained from Applichem (Darmstadt, Germany) and TCI (Tokyo, Japan), respectively. Verbascoside ($\geq 99\%$), oleuropein ($\geq 98\%$), trolox, ellipticine and sulforhodamine B were acquired from Sigma-Aldrich (St Louis, USA). Water was treated in a Milli-Q water purification system (Merck Millipore, USA).

2.2. Olive pomace samples

Olive pomace samples were collected, in 2018, from UCASUL - União de Cooperativas Agrícolas do Sul, located in Alentejo region, in Portugal. This is a cooperative industrial unit that receives the olive pomace produced in the olive oil mills of the region to extract olive pomace oil that has commercial value. The majority of the region mills use the two-phase process for olive oil production. Pomace enters UCASUL with high humidity (68% in average) and is first dried to 8%. UCASUL waste is the defatted or extracted olive pomace (EOP). This is considered biomass and disposed for energy production. The samples collected at UCASUL are of two different types: non-defatted or Crude Olive Pomace (COP) that consists of dried pomace before the extraction of pomace oil and defatted or Extracted Olive Pomace (EOP) that is the pomace left after the extraction of pomace oil.

2.3. Irradiation experiments

Irradiation experiments were carried out in a Co-60 semi-industrial unit (with an activity of 187 kCi) located at Technological Unit of Radiosterilization - UTR of IST, University of Lisbon. Sealed bags (10 cm \times 7 cm) containing 30 g of olive pomace, COP and EOP, were irradiated at room temperature at doses ranging from 4.9 to 21.8 kGy using a dose rate of 16 kGy h⁻¹. The absorbed doses were measured by Amber Perspex routine dosimeters (Whittaker and Watts, 2001) (dose uniformity DUR = 1.03). For simplicity, the absorbed doses will be referred as 5, 10, 16 and 22 kGy. The irradiations were performed in triplicate. In order to analyze the effect of gamma radiation in olive pomace, non-irradiated (0 kGy) samples submitted to the same experimental procedure were used as control.

2.4. Phenolic compounds extraction

After irradiation, all olive pomace samples were immediately lyophilized (Heto CD8, Allerod, Denmark) and stored until used. The olive pomace extracts were prepared by a solid-liquid extraction as previously described (Pinela et al., 2016). Briefly, 1 g of freeze-dried olive pomace was stirred with a methanol:water mixture (80:20, v/v; 30 mL) for 1 h at room temperature. The supernatant was then filtered (Whatman No. 4 filter paper) and the residue was re-extracted with an additional portion of solvent (30 mL). The combined extracts were then evaporated at 40 °C under reduced pressure (rotary evaporator Büchi R-

210, Flawil, Switzerland) to remove the methanol and the aqueous phase was lyophilized to obtain dry extracts.

A schematic diagram of the procedure steps carried out for the extraction of phenolic compounds from olive pomaces and subsequent analysis of their bioactive properties is represented in Fig. 1.

2.5. Analysis of phenolic compounds

After preparation, the dry extracts (~10 mg) were dissolved in a methanol:water mixture (20:80 v/v, 2 mL) and filtered through 0.22 µm disposable LC filter disks. Then, the extracts were analyzed by HPLC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA) using a methodology previously described by the authors (Bessada et al., 2016). Detection was performed in a Diode Array Detector (DAD) with 280, 330 and 370 nm as preference wavelengths, and in a mass spectrometer with an ESI source operating in negative mode (Linear Ion Trap LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA). Data acquisition was carried out with Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA).

The phenolic compounds were identified based on their chromatographic behavior and UV-vis and mass spectra, and comparison with standard compounds, when available, and data reported in the literature (Ammar et al., 2017; Cardoso et al., 2005; Obied et al., 2007). Calibration curves for quantitative analysis were prepared for each available phenolic standard based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed from the calibration curve of the most similar standard. The results were expressed in mg per g of extract.

2.6. Evaluation of bioactive properties

2.6.1. Antioxidant activity

The extracts were dissolved in distilled water at a concentration of 5 mg/mL and then successive dilutions were carried out (2500 to 2 µg/mL).

The antioxidant activity was measured through the thiobarbituric acid reactive substances (TBARS) assay and anti-haemolytic activity. The TBARS assay was performed following a methodology described by Pinela et al. (2012) and the results were expressed in IC₅₀ values (sample concentration providing 50% of antioxidant activity). The anti-haemolytic activity of the extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA), as previously described by Lockowandt et al. (2019). The results were presented as IC₅₀ values (extract concentration that delayed the haemolysis time for 60 and 120 min, with 50% of intact erythrocytes). In both assays, trolox was used as positive control. All determinations were performed in triplicate.

2.6.2. Hepatotoxicity

The extracts were re-dissolved in water at 8 mg/mL and further diluted in the range (400 to 1.56 µg/mL). The hepatotoxicity of olive pomace extracts was evaluated in non-tumor liver cells primary culture (PLP2) using the sulforhodamine B assay (SRB, Sigma-Aldrich, St. Louis, MO, USA) (Abreu et al., 2011). The results were expressed in GI₅₀ values (sample concentration that inhibited 50% of the cell growth) and ellipticine was used as positive control. Analyses were performed in triplicate.

2.6.3. Antimicrobial activity

2.6.3.1. Antibacterial activity. The bacterial isolates used on antibacterial activity assay intend to embrace a spectrum of different types of aerobic bacteria with reported association to food outbreaks, including Gram-negative and Gram-positive, spore forming bacteria, as well as a biofilm forming bacteria due to documented difficulty to control biofilms in food environments. The antibacterial activity was evaluated using three Gram-negative bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas fluorescens* (ATCC 13525) and *Salmonella enterica* serotype Typhimurium (ATCC 14028), and three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (SSI C1/1) and *Listeria monocytogenes* (ATCC 19111). The bacterial suspension was adjusted

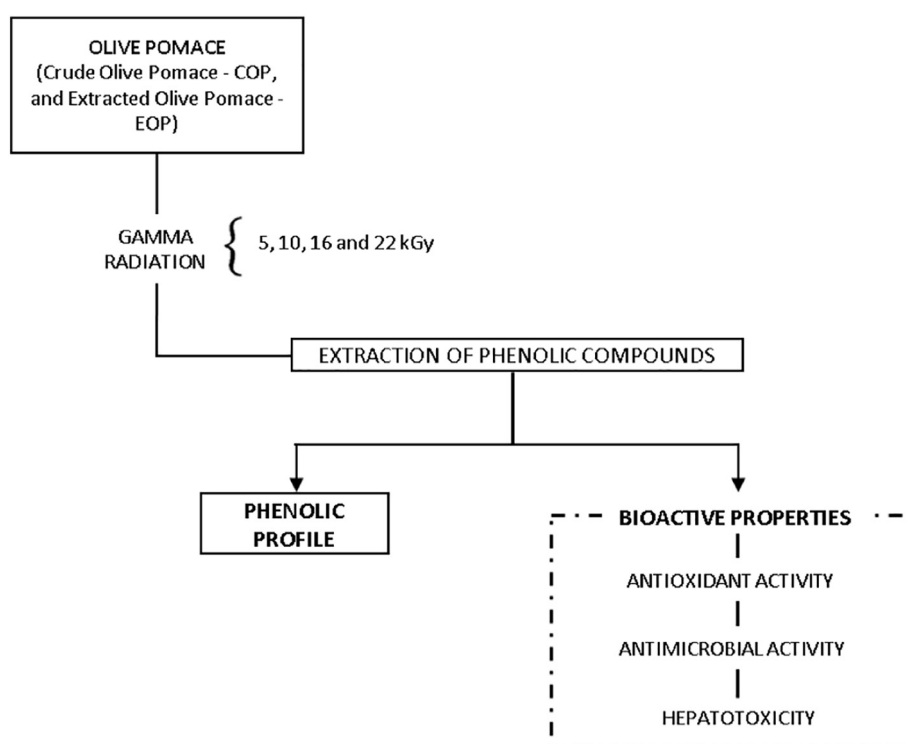


Fig. 1. Schematic diagram of the different steps of the experimental procedure for the extraction and analysis of bioactive properties of olive pomaces.

with sterile saline to a concentration of 1.0×10^5 CFU/mL. The extracts (EOP and COP) were dissolved in Tryptic Soy Broth (TSB) at a final concentration of 100 mg/mL. Then, several concentrations (10–60 mg/mL for Gram-negative bacteria and 1.25–20 mg/mL for Gram-positive bacteria) were prepared directly in the well and added 100 μ L of the respective inoculum (1.0×10^4 CFU per well). The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by the microdilution method (Sokovic et al., 2010). Streptomycin was used as a positive control. Samples were tested in triplicate and each experiment was repeated two times.

2.6.3.2. Antifungal activity. For the antifungal assays, two fungi were used: *Penicillium spinulosum* (environment isolate) and *Aspergillus fumigatus* (environment isolate). These mycotoxin producing fungi were selected since are recognized food deteriorating agents also present in food wastes. The fungal spores were washed from the surface of agar plates with sterile 0.9% saline solution. The spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The extracts (EOP and COP) were dissolved in Tryptic Soy Broth (TSB) at a final concentration of 100 mg/mL. Then, several concentrations (10–60 mg/mL) were prepared directly in the well and added 100 μ L of the respective inoculum (1.0×10^4 CFU per well). Minimum inhibitory concentration (MIC) determination was performed by a serial dilution technique (Gomes Corrêa et al., 2015). Ketoconazole was used as positive control. Samples were tested in triplicate and each experiment was repeated two times.

2.7. Statistical analysis

Data results were expressed as mean \pm standard deviation. In data analysis, confidence intervals for means values were estimated considering a significance level of $p < 0.05$ and the number of replicates for each assay. The differences among treatments were analyzed using the one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$.

3. Results and discussion

Two types of olive pomace, Crude Olive Pomace (COP) and Extracted Olive Pomace (EOP), were analyzed in order to find out the best conditions to improve the extractability of its natural bioactive compounds. The extraction of phenolic compound was performed by conventional solid-liquid technique due to its feasibility on the studied substrate allied to the use of eco-friendly solvents.

3.1. Phenolic compounds characterization

The phenolic profile of olive oil pomaces was similar for non-treated and treated samples and both types of extracts (COP and EOP). Thus, to illustrate the phenolic profiles, examples of non-irradiated (control) and irradiated at 5 kGy of EOP olive pomace samples, recorded at 280 nm, are shown in Fig. 2.

Peak characteristics and tentative identities of the detected compounds are presented in Table 1. Nine phenylethanoid derivatives (peaks 1, 2, 3, 4, 5, 7, 8, 11 and 12), two phenolic acids (peak 6 and 10), one flavonoid (peak 13) and one unknown compound (peak 14) were detected in both EOP and COP samples.

Hydroxytyrosol-1- β -glucoside (peak 1), hydroxytyrosol (2), tyrosol (4), caffeic acid (6), vanillin (9), verbascoside (11), 4-hydroxyphenylacetic acid (12) and luteolin-7-O-rutinoside (13) were positively identified by comparison with commercial standards, all of them being previously described for olive pomace (Cardoso et al., 2005; De Marco et al., 2007; Obied et al., 2007; Suárez et al., 2010). Peaks 3 and 5 presented the same pseudomolecular ion $[M-H]^-$ at m/z 377, which fits the oleuropein aglycon (i.e., 3,4-(dihydroxyphenyl)ethanol elenolic acid ester, 3,4-DHPEA-EA). The presence of several

oleuropein aglycon derivatives in olive oil and by-products is well reported (Obied et al., 2007; Olmo-García et al., 2018) and explained by the enzymatic loss of the glucose residue of oleuropein and further structural arrangement involving ring opening and keto-enol equilibria during olive oil processing (Obied et al., 2007). Compound 5 shows the same MS^2 fragments as reported by Cardoso et al. (2005) for the oleuropein aglycon derivative detected in olive pulp and pomace. The MS^2 fragmentation pattern of compound 3 is completely different, although its main product ions (m/z at 179 and 119) have also been described for oleuropein and its component elenolic acid (Ammar et al., 2017; Obied et al., 2007). All in all, an identity as oleuropein aglycon isomers has been assumed for peaks 3 and 5. Compounds 7 and 8 ($[M-H]^-$ at m/z 639) showed a molecular weight 16 Da higher than verbascoside and similar UV spectra to this latter. Up to four compounds with the same mass characteristics were also recovered by Cardinali et al. (2012) and Ammar et al. (2017) from *Olea europaea* by-products and identified as β -hydroxyverbascoside diastereoisomers, so that that identity was also assigned the compounds detected herein. Peak 10 presented a pseudomolecular ion $[M-H]^-$ at m/z 197 and a fragment ion at m/z 153 (-44 u; loss of a carboxyl residue), which would match syringic acid, a benzoic acid frequently found in olive fruits and by-products (Obied et al., 2007; Olmo-García et al., 2018), an identity that is given as tentative. A compound with the same pseudomolecular ion as peak 14 ($[M-H]^-$ at m/z 337) was detected by Ammar et al. (2017) in olive by-products and identified as ascorbyl-hexoside. Nevertheless, the late chromatographic elution of the peak and the UV spectral characteristics seem to not support that identification, so that the peak remains as unknown.

In non-irradiated samples, the individual contents of hydroxytyrosol-1- β -glucoside, hydroxytyrosol, tyrosol, oleuropein aglycon isomers 1 and 2, β -hydroxyverbascoside isomer 1, vanillin and 4-hydroxyphenylacetic acid were not significantly different ($p > 0.05$) between EOP and COP (Table 2). However, COP extracts contained significant higher levels of caffeic acid, β -hydroxyverbascoside isomer 2, syringic acid, verbascoside and luteolin-7-O-rutinoside. Hydroxytyrosol was the most abundant compound in both samples (25 ± 1 mg/g extract in EOP samples, and 23.9 ± 0.3 mg/g extract in COP ones), followed by hydroxytyrosol-1- β -glucoside, tyrosol, luteolin-7-O-rutinoside, oleuropein aglycon isomer 1 and verbascoside. Hydroxytyrosol is a phenyl alcohol for which antioxidant, anti-inflammatory and antimicrobial activities have been described (Bulotta et al., 2014; Fernández-Bolaños et al., 2008; Robles-Almazan et al., 2018). The extraction of phenolic compounds was significantly increased ($p < 0.05$) after gamma radiation in both olive pomace samples, obtaining the highest yield at 10 kGy for EOP and at 22 kGy for COP. At these doses, the concentrations of total phenolic compounds in the obtained extracts were 143 ± 4 mg/g in the EOP and 140 ± 2 mg/g in the COP ones (Table 2), representing an increase in extractable phenolic compounds of 2.4 and 2.3 fold, respectively, compared to non-irradiated samples. However, there was no significant difference between the total phenolic compounds content extracted from EOP at 10 kGy and from COP at 22 kGy. Contrary to these results, Aouidi et al. (2011) did not find that gamma radiation induced significant changes on total phenolic content in olive leaves even at doses as high as 25 kGy.

Regarding individual compounds behavior with gamma radiation, a significant decrease ($p < 0.05$) in the concentrations of some phenolics was observed at some irradiation doses in EOP samples, namely tyrosol and verbascoside at 16 kGy, 4-hydroxyphenylacetic acid and syringic acid from 10 kGy, and oleuropein aglycon isomer 2 and luteolin-7-O-rutinoside from 16 kGy. By contrast, the contents of vanillin and β -hydroxyverbascoside isomers 1 and 2 significantly increased in those samples at 10 kGy to decrease significantly at 16 kGy. In COP extracts, gamma radiation also led to different effects on different compounds. Thus, the concentration of oleuropein aglycon isomer 2 increased at 5 kGy to decrease significantly at 10 and to increase significantly again at 16 kGy, while vanillin significantly decreased at 10 kGy and increased

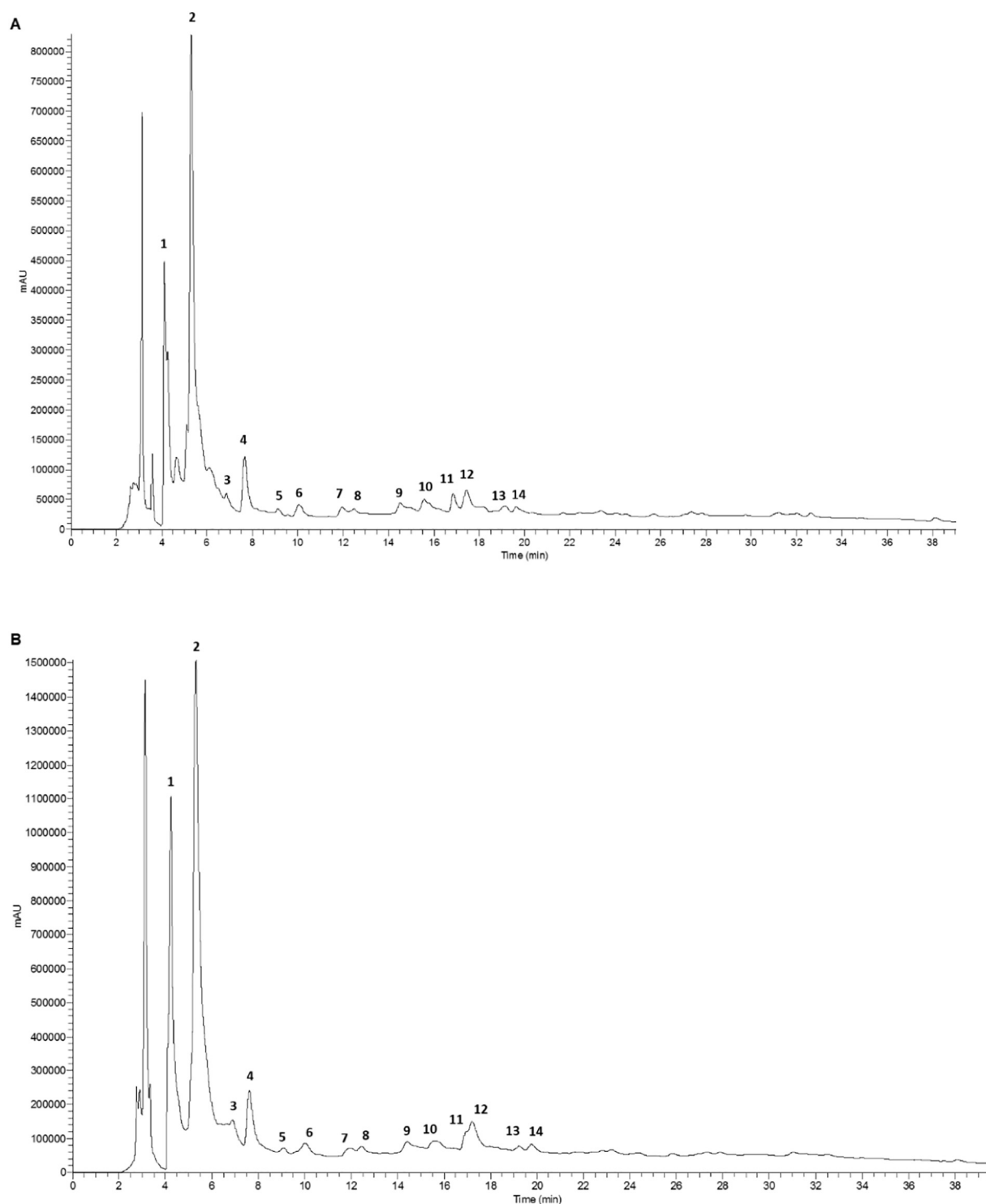


Fig. 2. Chromatographic profile of non-irradiated (A) and irradiated at 5 kGy (B) of EOP samples, recorded at 280 nm.

significantly at 16 kGy to values similar to 5 kGy. On the other hand, significant increases were produced in the concentrations of caffeic acid and oleuropein alkycon isomer 1 at 16 and 22 kGy, β -hydroxyverbascoside isomers 1 and 2 at 22 kGy, and tyrosol with increasing irradiation doses. Also, the concentrations of verbascoside and luteolin-7-*O*-rutinoside increased at higher doses (16 and 22 kGy) after a significant decrease at 10 kGy, whereas a significant decrease was observed in syringic acid contents at 10 kGy comparatively to 5 kGy and higher applied doses (16 kGy and 22 kGy). As for the most abundant compound (hydroxytyrosol), a sharp increase was produced

at all the applied irradiation doses in both olive pomace extracts compared to non-irradiated samples, without significant differences among the different doses, except for EOP at 16 kGy for which a punctual decrease was observed in relation to the other doses. Considering the overall results, the highest improvement (≥ 2 fold) in the extractability of phenolic compounds in COP was induced by irradiation at 22 kGy (Table 2). For EOP, the extractability was enhanced (≥ 2 fold) for the majority of the compounds after irradiation at 10 kGy, although at 5 kGy the extractability of 9 out of 13 compounds did not differ significantly from the obtained at 10 kGy. For illustrative purposes, Fig. 3 shows the

Table 1
Chromatographic and mass spectral characteristics and tentative identification of phenolic compounds in olive pomace extracts.

Peak	Rt (min)	λ_{\max} (nm)	Pseudomolecular ion [M-H] ⁻ (m/z)	MS ² (m/z)	Tentative identification
1	4.25	229, 277	315	179(3), 161(1), 153(100), 135(58)	Hydroxytyrosol-1- β -glucoside
2	5.31	281	153	123(100)	Hydroxytyrosol
3	6.90	220, 267	377	331(26), 287(42), 179(80), 161(62), 143(64), 131(25), 119(100), 113(68), 101(51)	Oleuropein aglycon isomer 1
4	7.59	220, 277	137	106(100)	Tyrosol
5	8.35	220, 267	377	197(100), 179(5), 153(20), 135(5)	Oleuropein aglycon isomer 2
6	10.02	324	179	135(100)	Caffeic acid
7	11.96	284, 326	639	621(100), 529(14), 459(5), 179(5)	β -Hydroxyverbascoside isomer 1
8	12.38	285, 327	639	621(100), 529(13), 459(5), 179(5)	β -Hydroxyverbascoside isomer 2
9	14.41	262, 295	151	136(100)	Vanillin
10	15.39	288	197	153(100)	Syringic acid
11	16.93	285, 326	623	461(50), 315(100)	Verbascoside
12	17.21	281	151	123(100), 107(5)	4-Hydroxyphenylacetic acid
13	19.24	266, 340	593	447(100), 285(95)	Luteolin-7-O-rutinoside
14	19.77	223, 282, 323	337	183(38), 157(100), 139(24)	Unknown

tendency of the major compounds (hydroxytyrosol-1- β -glucoside, hydroxytyrosol and tyrosol) and total phenolic compounds in EOP extracts where greater effect on their extractability was obtained with gamma irradiation.

To our knowledge, this is the first report describing the effect of gamma radiation on individual phenolic compounds of olive pomaces. Nonetheless, the influence of this technology on total phenolic composition and bioactive properties has been explored in different plant materials by other authors. Namely, Khattak et al. (2009) found that the concentration of total phenolics increased in *Nelumbo nucifera* rhizomes with the application of increasing doses of gamma radiation from 0 to 6 kGy. Further, Zhu et al. (2010) reported an increase of phenolic acids content in black rice extracts irradiated at 8 kGy, and a similar effect was observed for infusions and extracts of *Ginkgo biloba* L. (Pereira et al., 2015) and for lemon verbena and peppermint infusions (Pereira et al., 2017b) at 10 kGy irradiation dose. The increase in phenolic concentrations on irradiated samples could be related to the release of these compounds from matrix structures, increasing their extractability (Pereira et al., 2015), and/or to the radiolytic degradation of larger compounds into smaller ones (Hussain et al., 2016).

Although there are some reports in the literature concerning the recovery of phenolic compounds from olive pomace (Cioffi et al., 2010; Nunes et al., 2018; Pavez et al., 2019; Peralbo-Molina et al., 2012; Sygouni et al., 2019), most of these studies did not quantify the phenolic compounds present in the samples. Table 3 present the quantification of some compounds observed in EOP irradiated at 5 kGy in comparison with a work developed by Suárez et al. (2009) using solid-liquid extraction at atmosphere and high pressures. In this table, only the five compounds identified in both works are showed, although the total phenolic compounds content refers to all the compounds and not only to those five represented. The observed results prove that the applied methodology in this work, and especially gamma irradiation, allowed to extract higher concentrations of individual phenolic compounds than the methodologies used by Suárez et al. (2009). The total phenolic compounds concentration was higher when applying solid-liquid extraction at atmosphere pressure using ethyl acetate as solvent. Nevertheless, it is important to emphasize that these authors identified

twenty-four compounds instead of our fourteen, being expectable that the total phenolic concentration is higher.

All in all, the obtained results pointed that the use of ionizing radiation can be an environmentally friendly innovative approach to improve the extractability of bioactive compounds from olive residues, helping the olive oil industry to adopt clean processes and promoting sustainable development.

3.2. Bioactive properties of olive pomace extracts

3.2.1. Antioxidant activity

The results of the antioxidant activity assays are presented in Table 4. Data are expressed as IC₅₀ values, meaning the extract concentration able to provide 50% of antioxidant activity (TBARS assay) or to protect 50% of the erythrocyte population from haemolysis caused by an oxidizing agent (OxHLIA assay). The IC₅₀ values in the OxHLIA assay were obtained at two times (Δt 60 min and Δt 120 min) considering that natural extracts contain different antioxidant molecules capable of interacting with each other and offering protection at different time periods. In both assays, the lower the IC₅₀ values, the higher the antioxidant capacity of the olive pomace extracts.

Erythrocytes are blood cells especially susceptible to oxidation due to their high lipid content and their rich oxygen supply and presence of transition metals. Reactive oxygen species can attack the erythrocytes membrane, compromise cell integrity and induce oxidation of lipids and protein, which results in haemolysis (Lockowandt et al., 2019). Hydrophilic peroxy radicals generated by thermal decomposition of AAPH may attack the erythrocytes membrane from the outside and the extent of haemolysis is proportional to their amount (Miki et al., 1987). Phenolic compounds can be incorporated by erythrocyte membranes, being located at the polar-unpolar interface of the membrane or entering cells (e.g., quercetin), providing antioxidant protection through the interaction with some membrane and intracellular components, such as glutathione or ascorbic acid (Alvarez-Suarez et al., 2012).

In the OxHLIA assay, the haemolysis curves for the extracts of EOP and COP at different concentrations (data not shown) revealed that higher concentrations protected the erythrocyte population from

Table 2

Quantification of phenolic compounds in EOP and COP extracts from non-irradiated and irradiated samples.

Compound	Quantification (mg/g extract)									
	EOP					COP				
	0 kGy	5 kGy	10 kGy	16 kGy	22 kGy	0 kGy	5 kGy	10 kGy	16 kGy	22 kGy
Hydroxytyrosol-1- β -glucoside ¹	9.8 \pm 0.4 ^d	27 \pm 1 ^{a,b,c}	29 \pm 1 ^{a,b}	25 \pm 1 ^c	26.6 \pm 0.8 ^{b,c}	9.94 \pm 0.08 ^d	29.3 \pm 0.8 ^a	28.9 \pm 0.6 ^{a,b}	27.0 \pm 0.2 ^{a,b,c}	28.2 \pm 0.3 ^{a,b}
Hydroxytyrosol ¹	25 \pm 1 ^d	62 \pm 1 ^a	63 \pm 2 ^a	55 \pm 3 ^{b,c}	60 \pm 1 ^{a,b}	23.9 \pm 0.3 ^d	52 \pm 2 ^c	51 \pm 2 ^c	51 \pm 1 ^c	52.4 \pm 0.8 ^c
Oleuropein aglycon isomer 1 ²	3.0 \pm 0.3 ^c	8.7 \pm 0.5 ^a	8.7 \pm 0.6 ^a	7.4 \pm 0.3 ^a	7.5 \pm 0.6 ^a	3.1 \pm 0.1 ^c	5.0 \pm 0.2 ^b	4.9 \pm 0.1 ^b	8.4 \pm 0.3 ^a	8.0 \pm 0.2 ^a
Tyrosol ³	5.9 \pm 0.2 ^c	14.1 \pm 0.6 ^a	13.9 \pm 0.3 ^a	12.2 \pm 0.8 ^b	12.5 \pm 0.4 ^{a,b}	5.9 \pm 0.4 ^c	11.9 \pm 0.5 ^b	12.0 \pm 0.2 ^b	12.6 \pm 0.6 ^{a,b}	14.1 \pm 0.5 ^a
Oleuropein aglycon isomer 2 ²	1.80 \pm 0.08 ^f	3.57 \pm 0.06 ^a	3.8 \pm 0.1 ^a	3.2 \pm 0.1 ^b	2.80 \pm 0.08 ^{c,d}	1.7 \pm 0.1 ^f	2.54 \pm 0.09 ^d	2.19 \pm 0.06 ^e	2.95 \pm 0.09 ^{b,c}	3.2 \pm 0.1 ^b
Caffeic acid ⁴	0.55 \pm 0.01 ^f	1.36 \pm 0.04 ^c	1.32 \pm 0.03 ^c	1.46 \pm 0.05 ^{c,d}	1.62 \pm 0.03 ^d	0.89 \pm 0.03 ^e	1.73 \pm 0.06 ^c	1.85 \pm 0.07 ^c	2.0 \pm 0.1 ^b	2.52 \pm 0.07 ^a
β -Hydroxyverbascoside isomer 1 ⁵	1.70 \pm 0.05 ^e	3.5 \pm 0.1 ^d	4.2 \pm 0.2 ^c	3.4 \pm 0.1 ^e	3.41 \pm 0.02 ^d	2.27 \pm 0.03 ^e	4.8 \pm 0.2 ^{a,b}	4.8 \pm 0.1 ^b	4.8 \pm 0.3 ^b	5.3 \pm 0.2 ^a
β -Hydroxyverbascoside isomer 2 ⁵	1.62 \pm 0.03 ^e	3.50 \pm 0.06 ^c	4.4 \pm 0.1 ^b	3.14 \pm 0.08 ^c	3.15 \pm 0.06 ^{c,d}	2.66 \pm 0.06 ^d	4.55 \pm 0.07 ^b	4.2 \pm 0.1 ^b	4.6 \pm 0.3 ^b	5.4 \pm 0.2 ^a
Vanillin ⁶	1.22 \pm 0.01 ^d	2.36 \pm 0.07 ^{b,c}	3.1 \pm 0.1 ^a	2.06 \pm 0.04 ^c	2.3 \pm 0.1 ^{b,c}	1.43 \pm 0.06 ^d	3.49 \pm 0.07 ^a	2.5 \pm 0.1 ^b	3.2 \pm 0.2 ^a	3.5 \pm 0.2 ^a
Syringic acid ⁷	0.93 \pm 0.03 ^f	3.05 \pm 0.12 ^{a,b}	2.24 \pm 0.05 ^c	1.89 \pm 0.09 ^d	2.02 \pm 0.12 ^{c,d}	1.32 \pm 0.08 ^e	3.04 \pm 0.08 ^{a,b}	2.05 \pm 0.02 ^{c,d}	2.8 \pm 0.2 ^b	3.20 \pm 0.08 ^a
Verbascoside ⁵	2.29 \pm 0.03 ^f	3.4 \pm 0.1 ^{d,e}	4.01 \pm 0.06 ^d	3.02 \pm 0.05 ^{e,f}	3.44 \pm 0.07 ^{d,e}	3.4 \pm 0.2 ^{d,e}	8.6 \pm 0.3 ^a	6.5 \pm 0.2 ^c	7.5 \pm 0.4 ^b	9.4 \pm 0.4 ^a
4-hydroxyphenylacetic acid ⁸	1.60 \pm 0.08 ^c	4.3 \pm 0.1 ^a	3.6 \pm 0.2 ^b	3.6 \pm 0.1 ^b	3.7 \pm 0.1 ^b	1.70 \pm 0.03 ^c	nd	2.01 \pm 0.04 ^c	nd	nd
Luteolin-7-O-rutinoside ⁹	3.1 \pm 0.1 ^e	5.1 \pm 0.3 ^c	5.0 \pm 0.2 ^c	4.55 \pm 0.1 ^d	4.4 \pm 0.1 ^d	4.1 \pm 0.2 ^d	6.4 \pm 0.3 ^b	2.30 \pm 0.02 ^f	5.5 \pm 0.2 ^{b,c}	7.8 \pm 0.2 ^a
Unknown	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
Total phenylethanoid derivatives	53 \pm 2 ^d	129 \pm 2 ^{a,b}	135 \pm 4 ^a	116 \pm 5 ^c	121 \pm 2 ^{b,c}	54.6 \pm 0.4 ^d	119 \pm 3 ^c	117 \pm 3 ^c	118 \pm 3 ^c	126 \pm 2 ^{a,c}
Total phenolic acids	1.48 \pm 0.02 ^g	4.4 \pm 0.1 ^c	3.53 \pm 0.04 ^{d,e}	3.28 \pm 0.08 ^a	3.7 \pm 0.2 ^{c,d}	2.21 \pm 0.05 ^f	4.8 \pm 0.1 ^b	3.90 \pm 0.05 ^c	4.7 \pm 0.1 ^b	5.73 \pm 0.05 ^a
Total flavonoids	3.1 \pm 0.1 ^e	5.1 \pm 0.3 ^c	5.0 \pm 0.2 ^c	4.5 \pm 0.1 ^d	4.4 \pm 0.1 ^d	4.1 \pm 0.2 ^d	6.4 \pm 0.3 ^b	2.30 \pm 0.02 ^f	5.5 \pm 0.2 ^c	7.8 \pm 0.2 ^a
Total phenolic compounds	57 \pm 2 ^f	139 \pm 2 ^{a,c}	143 \pm 4 ^a	124 \pm 6 ^{d,e}	129 \pm 2 ^{b,c,e}	61.0 \pm 0.5 ^f	130 \pm 4 ^{b,d}	123 \pm 3 ^{d,e}	128 \pm 3 ^{b,e}	140 \pm 2 ^{a,b,c}
Other compounds [†]	1.220 \pm 0.005 ^d	2.36 \pm 0.07 ^b	3.1 \pm 0.1 ^a	2.06 \pm 0.04 ^c	2.3 \pm 0.1 ^{b,c}	1.43 \pm 0.06 ^d	3.5 \pm 0.1 ^a	2.5 \pm 0.1 ^b	3.2 \pm 0.2 ^a	3.5 \pm 0.2 ^a

Values within a row with similar letters do not differ significantly ($p > 0.05$). nd—not detected. nq—not quantified. Calibration curves used for quantification: ¹Hydroxytyrosol ($y = 124,154x + 17,393$, $R^2 = 0.9999$), ²Oleuropein ($y = 32,226x + 12,416$, $R^2 = 0.9999$), ³Tyrosol ($y = 91,708x + 9398.5$, $R^2 = 0.9999$), ⁴Caffeic acid ($y = 388,345x + 406,369$, $R^2 = 0.9939$), ⁵Verbascoside ($y = 124,233x - 18,873$, $R^2 = 1$), ⁶Vanillin ($y = 29,751x - 28,661$, $R^2 = 0.9999$), ⁷Syringic acid ($y = 376,056x + 141,329$, $R^2 = 0.9995$), ⁸p-hydroxybenzoic acid ($y = 208,604x + 173,056$, $R^2 = 0.9995$), ⁹Apigenin-7-O-glucoside ($y = 10,683x - 45,794$, $R^2 = 0.996$).

[†] Other compounds: vanillin.

haemolysis for a longer time period. The extract of the non-irradiated EOP provided higher antihaemolytic protection (IC_{50} values of $20.0 \pm 0.8 \mu\text{g/mL}$ and $35 \pm 2 \mu\text{g/mL}$, at 60 min and 120 min, respectively) than the non-irradiated COP extract ($32.2 \pm 0.6 \mu\text{g/mL}$ and $61 \pm 2 \mu\text{g/mL}$). These results could suggest that phenolic compounds in the

EOP samples (defatted pomace) could be more bioaccessible to protect the erythrocyte membrane from the AAPH-induced oxidation due to the higher hydrophilicity of the medium. The presence of some fat content in the extracts obtained from the COP samples could somehow hamper contact between the phenolic antioxidants and the erythrocyte

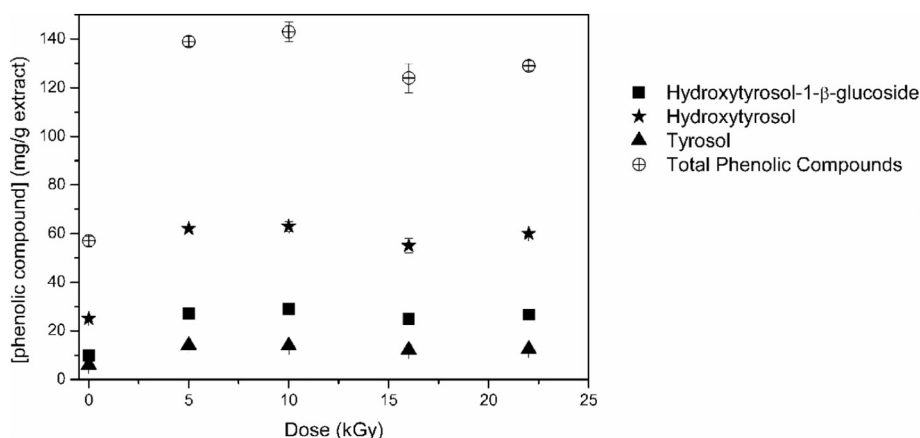


Fig. 3. Extractability of hydroxytyrosol-1- β -glucoside, hydroxytyrosol and tyrosol and total phenolic compounds in EOP extracts during the irradiation treatment.

Table 3

Comparison of the results obtained in this work with others reported in the literature (mg/g extract).

	This work	Suárez et al. (2009)	
	Extracted olive pomace, 5 kGy	Solid-liquid extraction at atmosphere pressure	Solid-liquid extraction at high pressure
Hydroxytyrosol	62	2.79	2.54
Tyrosol	14.1	0.32	0.09
Caffeic acid	1.36	0.43	0.09
Vanillin	2.36	0.06	0.05
Verbascoside	3.4	2.18	0.56
Total phenolic compounds	139	233.35	76.53

membrane or the generated radicals, thus hindering the formation of a protective barrier against the diffusion of free radicals or its elimination from the reaction system. This may justify the higher IC₅₀ values of the non-defatted extracts in the OxHLIA assay.

On the other hand, since lipophilic radicals can result from the AAPH-induced oxidation of the fat present in the extracts, the haemolytic response may have been intensified. If this happened in the system, it also justifies the largest amount of COP extract (higher IC₅₀ values) needed to protect the erythrocyte population from oxidation.

Interestingly, despite the increase in phenolic compounds content, gamma radiation promoted a negative effect on the antihaemolytic activity of the extracts since the IC₅₀ values increased with the increase of irradiation doses. For EOP extracts, however, the differences in the IC₅₀ values were not significant ($p > 0.05$) at the lower doses (i.e., 5 and 10 kGy), which did not differ from those of the non-irradiated samples and the standard antioxidant compounds, Trolox, used positive control. These findings indicated the existence of relevant antioxidant capacity in the prevention of cellular oxidative processes of the defatted pomace extracts. Similar observations were made by Lins et al. (2018) in extracts obtained from olive leaves.

Table 4

Antioxidant activity (IC₅₀ values, µg/mL) and hepatotoxicity (GI₅₀ values, µg/mL) of the olive pomace extracts and positive controls (trolox or ellipticine, respectively).

	Antioxidant activity ¹			Hepatotoxicity
	OxHLIA, Δt = 60 min	OxHLIA, Δt = 120 min	TBARS	
EOP samples				
0 kGy	20.0 ± 0.8 ^h	35 ± 2 ^g	49 ± 1 ^a	>400
5 kGy	20.6 ± 0.4 ^h	37 ± 1 ^g	22 ± 1 ^e	>400
10 kGy	21.8 ± 0.4 ^{g,h}	36.2 ± 0.6 ^g	28 ± 2 ^d	>400
16 kGy	23.6 ± 0.5 ^g	47 ± 2 ^{e,f}	38 ± 2 ^c	>400
22 kGy	27.6 ± 0.7 ^f	51 ± 2 ^c	43 ± 2 ^b	>400
COP samples				
0 kGy	32.2 ± 0.6 ^e	61 ± 2 ^d	20 ± 1 ^f	>400
5 kGy	36.1 ± 0.7 ^d	69 ± 5 ^c	19 ± 1 ^f	>400
10 kGy	56 ± 1 ^c	82 ± 2 ^b	23 ± 1 ^e	>400
16 kGy	61 ± 2 ^b	88 ± 3 ^b	24 ± 1 ^e	>400
22 kGy	68 ± 1 ^a	99 ± 2 ^a	27 ± 1 ^d	>400
Trolox	19.6 ± 0.6 ^h	41.1 ± 0.8 ^{e,g}	5.4 ± 0.3 ^g	
Ellipticine	–	–	–	2.3 ± 0.2
Homoscedasticity ²	0.590	0.220	0.000	
1-way ANOVA ³	<0.001	<0.001	<0.001	

Values within a column with similar letters do not differ significantly ($p > 0.05$).

¹ The results are given as mean ± standard deviation.

² Homoscedasticity among samples, $p > 0.05$; heteroscedasticity among samples, $p < 0.05$.

³ In each column, $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case, multiple comparison tests were performed).

Contrary to the results obtained in the OxHLIA assay, the COP extracts were more effective than the EOP ones to inhibit TBARS formation. In this assay, the reaction mechanisms occur in a lipophilic environment, where the presence of fat will not lead to the possible bioaccessibility issues mentioned for OxHLIA. However, it has been reported that thiobarbituric acid (TBA) not only reacts with malondialdehyde (MDA), which is a secondary oxidation product of lipid peroxidation, but also with other aldehydes that can be generated in the system, especially from fat-containing samples. Thus, substances that overlap with the MDA-TBA complex absorption peak are generated and may lead to an overestimation of the antioxidant capacity (Semeniuc et al., 2016). On the other hand, gamma irradiation improved the antioxidant capacity of the EOP extracts, with particular efficiency at the lowest assayed doses (5 kGy), whereas no improvement in the antioxidant effect was observed after irradiation in COP samples.

The overall results obtained in these assays suggested that gamma radiation could be a suitable technique to improve the antioxidant activity in EOP when applied at low doses (i.e., 5 kGy), at which an increase in antioxidant potential was produced as evaluated by the TBARS assay, while keeping the ability to protect erythrocytes against oxidation-induced haemolysis.

3.2.2. Hepatotoxicity

The assessment of the cytotoxicity for PLP2 (non-tumor cells) is essential considering that mammalian hepatocytes constitute a required step in the verification of toxic compounds (Abreu et al., 2011). Although the use of the PLP2 culture could not give an absolute answer about hepatotoxicity, in fact none in vitro assay can do that, it is considered a feasible method to perform preliminary hepatotoxicity studies (Abreu et al., 2011). The effects of olive pomace (EOP and COP) extracts on the growth of non-tumor liver primary cell line (PLP2) represented as the concentration that caused 50% of cell growth inhibition (GI₅₀), are also summarized in Table 4. The obtained results indicated that none of the extracts, irradiated or non-irradiated, had an inhibitory effect on the cell growth (GI₅₀ > 400 µg/mL), suggesting no hepatotoxicity of the extracts.

3.2.3. Antimicrobial activity

Antimicrobial activity of the olive pomace extracts against three Gram-negative (*E. coli*, *S. Typhimurium*, *P. fluorescens*) and three Gram-positive bacteria (*B. cereus*, *S. aureus*, *L. monocytogenes*) and two fungi (*A. fumigatus* and *P. spinulosum*) was evaluated (Table 5).

All olive pomace extracts had similar potential antibacterial activity against the studied Gram-positive bacterial strains (MIC 20 mg/mL), whereas EOP samples showed higher antibacterial activity against Gram-negative bacteria, namely *E. coli* and *P. fluorescens* (MIC 20 mg/mL), than COP (MIC 40 mg/mL). It is to highlight the ability to inhibit the *P. fluorescens* biofilm formation after 72 h by defatted olive pomace extracts. Comparatively to COP extracts, the EOP ones presented the higher hydroxytyrosol content (Table 2), suggesting a correlation between antimicrobial activity and a rich concentration in this phenolic compound. According to a previous study, the hydroxytyrosol-rich composition sample from olive mill wastewater inhibited the growth of Gram-negative bacteria (Yangui et al., 2009). The authors attributed the effectiveness of hydroxytyrosol to its capability of chelating transition metals, lowering the reactivity of metal iron by forming an inert metal–ligand complex, reducing the bioavailability for microbial growth (Wong and Kitts, 2006; Yangui et al., 2009). In agreement with our results, Brenes et al. (2007) also observed higher sensitivity of Gram-positive than Gram-negative bacteria to olive oil polyphenols. Furthermore, the olive pomace extracts also revealed some potential antifungal against the studied fungi (MIC 40 mg/mL). In general, gamma radiation seemed to have no effect on the antimicrobial potential of the studied olive pomace extracts, but for EOP sample in the case of *A. fumigatus* (Table 5), for which the MFC value varied from 40 mg/mL in non-irradiated to ≥40 mg/mL in irradiated pomaces. Other

Table 5
Antimicrobial activity of olive pomace extracts (MIC, mg/mL).

	<i>B. cereus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>S. Typhimurium</i>	<i>P. fluorescens</i>	<i>A. fumigatus</i>	<i>P. spinulosum</i>
	MIC (mg/mL)							
EOP samples								
0 kGy	20	20	20	20	40	20	40	40
5 kGy	20	20	20	20	40	20	40	40
10 kGy	20	20	20	20	40	20	40	40
16 kGy	20	20	20	20	40	20	40	40
22 kGy	20	20	20	20	40	20	40	40
COP samples								
0 kGy	20	20	20	40	40	40	40	40
5 kGy	20	20	20	40	40	40	40	40
10 kGy	20	20	20	40	40	40	40	40
16 kGy	20	20	20	40	40	40	40	40
22 kGy	20	20	20	40	40	40	40	40
	<i>B. cereus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>E. coli</i>	<i>S. Typhimurium</i>	<i>P. fluorescens</i>	<i>A. fumigatus</i>	<i>P. spinulosum</i>
	MBC (mg/mL)				MFC (mg/mL)			
EOP samples								
0 kGy	20	≥20	≥20	≥20	≥40	≥20	40	≥40
5 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
10 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
16 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
22 kGy	20	≥20	≥20	≥20	≥40	≥20	≥40	≥40
COP samples								
0 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
5 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
10 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
16 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40
22 kGy	20	≥20	≥20	≥40	40	≥40	≥40	≥40

authors have demonstrated the capability of a saprophytic fungus to grow on dry olive-mill residue (Sampedro et al., 2005), which can envisage the inefficacy of pomace extracts against fungi. Furthermore, Sousa et al. (2006) observed no antifungal activity at the tested concentrations in table olive extracts.

As far as we know, this is the first report concerning the antimicrobial potential of olive pomace and the evaluation of gamma radiation effect on this activity. There are, however, some studies reporting high antimicrobial activity in table olives (Sousa et al., 2006), olive oil (Brenes et al., 2007) and olive mill wastewaters (Yakhlef et al., 2018; Yanguí et al., 2009). These findings point to the possibility of using olive pomace extracts as a food preservative in order to prevent the growth of foodborne pathogens or to delay the food spoilage. Nevertheless, further studies have to be performed to identify which are the compounds responsible for this antimicrobial activity.

4. Conclusions

This work focused on the impact of gamma radiation on the phenolic composition and bioactive properties of olive pomace extracts (EOP and COP samples). HPLC-DAD-MS analyses demonstrated that olive pomace is a rich source of hydroxytyrosol, and also contains significant amounts of other phenolics, such as hydroxytyrosol-glucoside, tyrosol, syringic acid or luteolin-rutinoside. The gamma radiation treatment of olive pomace improved the extractability of phenolic compounds. The extracts of EOP olive pomaces were more efficient in protecting erythrocytes against oxidatively-induced haemolysis, while those from COP showed greater ability to inhibit TBARS formation, which might be related with fat content of the samples. In general, gamma radiation did not improve the antioxidant capacity of the olive pomaces in relation to non-irradiated ones, except in the case of TBARS inhibition of EOP samples, which was significantly increased, especially at low irradiation doses. The treatment of EOP olive pomace extracts at a dose of 5 kGy could be considered a good compromise for gamma irradiation, as it

increases phenolic extractability at the same time that improves the antioxidant activity of the extracts, as evaluated by the TBARS assay, and preserves the antihaemolytic activity, maintaining it in values similar to the reference antioxidant Trolox. Both extracts presented antimicrobial activity against the studied bacterial and fungi strains, but EOP extracts were more effective against *E. coli* and *P. fluorescens*. No toxicity in non-tumor hepatic cells (PLP2) was observed, either for EOP or COP extracts. Gamma radiation treatment seemed to cause no alteration in the antimicrobial activity and hepatotoxicity of the extracts. Based on the obtained results, the olive pomace extracts could be considered a suitable alternative for the food industry to be used as food preservatives.

All in all, the observations made in the present study indicate that gamma radiation at low doses (5 kGy) could be applied as a ground-breaking technology in the valorization of olive oil by-products, with better results when extracted material is used. The olive pomace extracts could be considered a suitable alternative for the food industry to be used as food preservatives. Although, further studies are needed to evaluate the effect of gamma radiation on olive pomace individual compounds to assess its bioactivity. This work should contribute to enhance the sustainability of the agro-industrial sector considering both economic and environmental aspects.

CRedit authorship contribution statement

Joana Madureira: Investigation, Writing – original draft. **Maria Inês Dias:** Investigation. **José Pinela:** Investigation. **Ricardo C. Calhella:** Investigation. **Lillian Barros:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Celestino Santos-Buelga:** Writing – review & editing, Supervision. **Fernanda M.A. Margaça:** Writing – review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Methodology, Supervision. **Sandra Cabo Verde:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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