Food Chemistry 127 (2011) 1105–1113

Contents lists available at ScienceDirect

Food Chemistry

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

Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds

Fathia Aouidi [a,c,*], Samia Ayari [a,b], Hana Ferhi [a], Sevastianos Roussos [c], Moktar Hamdi [a]

[a] Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), 2 Boulevard de la Terre, B.P. 676, 1080 Tunis, University of Carthage, Tunisia
[b] National Center for Nuclear Sciences and Technologies (CNSTN), Tunis cedex, 2020, Tunisia
[c] Mediterranean Institute of Ecology and Palaeoecology UMR CNRS/IRD 193, IMEP Case 441, FST Saint Jérôme, Université Paul Cézanne, Av. Escadrille Normandie-Niemen, 13397 Marseille cedex 20, France

ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form 12 December 2010
Accepted 24 January 2011
Available online 2 February 2011

Keywords:
Olive leaf
Gamma irradiation
D_{10}-value
Phenolic compound
Antioxidant capacity

ABSTRACT

Olive leaves are commercialized for their antioxidative value due to their valuable phenolic compounds. The present study aimed to evaluate the effect of gamma irradiation on microbial load, on antioxidative properties and on phenolic compounds of air-dried olive leaves. Irradiation was applied up to 25 kGy (5 kGy intervals) to powdered and intact samples. Total aerobic bacteria, yeast and mold, and lactic acid bacteria were counted after gamma irradiation. Decontamination was obtained at 20 kGy. The radioresistance of microbial population was high with D_{10} values between 9.74 and 25.12 kGy. Besides, gamma irradiation up to 25 kGy was found to maintain the antioxidant capacity, molecular mass distribution of polyphenolics, total phenolics, *ortho*-diphenols, flavonoids, oleuropein, verbascoside and rutin contents.

To conclude, the improvement of the microbial quality of air-dried olive leaves, without affecting phenolic composition and antioxidative properties, can be successively achieved by the application of gamma irradiation treatment.

1. Introduction

The importance of functional foods, nutraceuticals, dietary supplements and other natural healthy products has been well recognized in connection with health promotion, disease risk reduction and reduction in health care costs. Therefore, there has been a growing interest in research, development and commercialization of these healthy products around the globe (Day, Seymour, Pitts, Konczak, & Lundin, 2009). Among nutraceuticals, phenolic compounds from natural sources have attracted great attention during the last decade, notably, due to their antioxidant contribution to human health (Benavente-Garcia, Castillo, Lorente, Ortuno, & Del Rio, 2000; Lee et al., 2009; Skerget et al., 2005).

Olive leaves from *Olea europaea* tree, native to the Mediterranean, could be considered as a nutraceutically valued material, owing to their high quality and quantity of their phenolic compounds. The main active phenolic constituents are oleuropein, verbascoside, luteolin-7-glucoside and rutin (Benavente-Garcia et al., 2000; Hayes, Stepanyan, Allen, O'Grady & Kerry, 2010; Lee et al., 2009).

Olive leaves have been reported to have antioxidative, antimicrobial, antiviral, anti-inflammatory, hypoglycemic, hypotensive and hypothyroidism properties (Anonymous, 2009). Historically, olive leaves had been used as a folk medicine for fever and malaria. Nowadays, this vegetable material is proved and recommended for treating many diseases, such as diabetes, cardiovascular diseases, viral and microbial infections (Anonymous, 2009).

Olive leaves can be used not only for therapeutic but also for cosmetic purpose and as food additive. In the past, powdered dried olive leaves were used as an ingredient for the preparation of a traditional food "Bsissa" in Tunisia. Recently, olive leaves extract has been investigated as an additive supplemented to food products, such as oils (Bouaziz, Fki, Jemai, Ayadi, & Sayadi, 2008) and meats (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2010), to extend shelf-life and impart the image of wholesomeness to consumers. Olive leaves are commercialized under several forms (intact leaves, powdered leaves, extract, etc.), for their benefits for human health.

Plant materials are highly susceptible to microbial contamination due to the medium (water and soil) in which they grow. Harvesting, storage and processing may cause additional contamination and microbial growth (Bhat, Sridhar, & Karim, 2010; Katusin-Razem, Novak, & Razem, 2001). Besides representing a

* Corresponding author at: Laboratory of Microbial Ecology and Technology, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), 2 Boulevard de la Terre, B.P. 676, 1080 Tunis, Tunisia. Tel.: +216 98 326 675; fax: +216 71 704 329.
E-mail address: aouidifathia@yahoo.fr (F. Aouidi).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.01.109

direct health hazard to consumers, such contaminated materials can also cause the spoilage of pharmaceuticals and food items to which they are added. Furthermore, the presence of microbial contaminants may also affect the efficacy and stability of active compounds of the plants during storage (Thomas, Senthilkumar, Kumar, Mandal, & Muraleedharan, 2008). Thus, an adequate technology for decontamination is required to improve the hygienic quality of plant materials and to make it suitable for human use and commercialization (Bhat et al., 2010; Katusin-Razem et al., 2001).

Conventional methods of microbial decontamination are fumigation with either gaseous ethylene oxide or methyl bromide, which are carcinogenic and hence are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons. Gamma irradiation is well known as a decontamination method for many foodstuffs and plant materials, being an environment friendly and effective technology to resolve technical problems in trade and commercialization (Khattak, Simpson, & Ihasnullah., 2009).

The phenolic composition of olive leaves and its antioxidant capacity have been well reported (Benavente-Garcia et al., 2000; Bouaziz et al., 2008; Lee et al., 2009).

To our knowledge, no studies have investigated the effect of gamma irradiation on phenolic composition and microbial load in olive leaves. Therefore, the present study aimed to evaluate the decontamination efficiency of gamma irradiation treatment of air-dried olive leaves. Powdered and intact samples were irradiated. The effect of this technology on the antioxidant capacity and phenolic content (total phenolics, *ortho*-diphenols, flavonoids, oleuropein, verbascoside and rutin contents and molecular mass distribution of polyphenolics) of olive leaves was also investigated.

2. Materials and methods

2.1. Chemical reagents and standards

Oleuropein, verbascoside and rutin were obtained from Extrasynthèse. ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, caffeic acid, gallic acid, formic acid, Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid), lysozym, blue dextran, diethylene glycol, sodium azide, sodium molybdate and sodium nitrate were obtained from Sigma. Sodium carbonate, ethanol and methanol were purchased from Riedel-de Haën. Methanol was of HPLC grade. Ethanol was of analytical grade. Folin–Ciocalteu phenol reagent, potassium persulfate and syringic acid were purchased from Fluka.

2.2. Sample preparation and irradiation treatment

Fresh olive leaves (*Olea europaea L.* var Chemlali North) were hand harvested randomly from a tree grown in National Institute of Applied Sciences and Technology (Tunis, Tunisia) in June 2009. Collected leaves (around 2 kg) were dried at room temperature (30 ± 5 °C) for one week in the dark and under ventilation. A part of the batch was powdered using a commercial grinder (Moulinex). 10 lots of dried intact olive leaves (IOL) (20 g each lot) and 10 lots of dried powdered olive leaves (POL) (50 g each lot) were packed hermetically in polyethylene bags.

Samples in duplicate were subjected to irradiation treatment at room temperature with a dose rate of 15.64 Gy min^{-1} for the POL samples and with a dose rate of 18.2 Gy min^{-1} for the IOL samples. The five doses used were 5, 10, 15, 20 and 25 kGy with a non-irradiated control.

After irradiation treatment, all samples were immediately stored at 4 °C until analysis.

Gamma irradiation was carried out using a carrier type irradiator equipped with a ^{60}Co source (C-188, MDS Nordion) at the National Center for Nuclear Sciences and Technologies (CNSTN), Tunis, Tunisia. The dose distribution was measured using a PMMA dosimeter (PMMA Instruments, Harwell, UK).

2.3. Microbial load analyses

Microbial load was evaluated by a plate count method. Samples (5 g) were decimal diluted serially with sterile solution (1% peptone, 0.5% sodium chloride). Total aerobic bacteria (TAB) were determined after 3 days incubation at 30 °C on the Plate Count Agar (PCA). Yeast and mold (YM) were determined on the Sabouraud Chloramphenicol Agar (SCA) after incubation for 3 days at 30 °C. Lactic acid bacteria (LAB) were determined on the Man, Rogosa and Sharpe agar (MRS) and were incubated for 24 h at 37 °C.

The microbial content of the samples was measured by observing the average number of viable cells, expressed as Colony forming units per gram (CFU g^{-1}), in the samples. The presented data are the average counts observed in three Petri dishes for each sample.

The percentage of the survival fraction was calculated according to the following formula:

$$\text{Survival fraction}\,(\%) = \frac{N}{N_0} * 100 \quad (1)$$

where N_0 is the microbial load in the control sample and N is the microbial load in the irradiated sample.

2.4. Determination of radiation D_{10}-values

The D_{10}-value was defined as the dose required inactivating one Log_{10} cycle of the initial population. In other words, The D_{10}-value is the dose required to inactivate 90% of a population.

CFU survivors following each gamma irradiation processing were plotted on a logarithmic scale as a function of gamma irradiation dose, resulting in survivor curves.A linear regression equation for the type $y = ax + b$ was derived.where y is Log_{10} (CFU) in each irradiation dose, x is the irradiation dose, and a is the slope.

$$a = \frac{-1}{D_{10}} \quad (2)$$

The D_{10}-value was determined by calculating the negative reciprocal of the survivor curve slope (Ayari, Dussault, Millette, Hamdi, & Lacroix, 2009).

2.5. Determination of total phenolic, ortho-diphenol and flavonoid contents in olive leaves extract

After irradiation, IOL samples were powdered. For all samples, the powders (1 g) were extracted by 50 ml methanol for 48 h. The extraction was performed at room temperature in the dark. The resulted extracts were centrifuged at 3500 rpm for 15 min. The supernatants were recovered and used to determine the total phenolic, *ortho*-diphenol and flavonoid contents.

The concentration of total phenolic content in extracts was measured by UV spectrophotometry, based on a colorimetric oxidation/reduction reaction (Skerget et al., 2005). The oxidizing agent used was Folin–Ciocalteu reagent. To 0.5 ml of diluted extract, 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) was added and, after 8 min, 2 ml of Na_2CO_3 (75 g l^{-1}) was added. The sample was incubated for 5 min at 50 °C and then cooled. For the blank, 0.5 ml of methanol was used instead of the extract. The absorbance was measured at 760 nm against the reagent blank. Gallic acid in methanol was used as an external standard to prepare calibration curve in the range 0–70 mg l^{-1}

($Y = 0.0142X$; Y: The absorbance at 760 nm; X: gallic acid concentration; $R^2 = 0.9936$). The results were expressed in milligram of gallic acid equivalents per gram of dry matter of olive leaves (mg GAE g^{-1} DM).

Ortho-diphenol content was also determined by colorimetric reaction according to Blekas, Psomiadou, Tsimidou, and Boskou (2002). An aliquot (0.2 ml) of the methanol extract was diluted with water to a volume of 1 ml and then a 0.1 M phosphate buffer (1 ml) and a 5% sodium molybdate solution (2 ml) were added. For the blank, 0.2 ml of methanol was used instead of the extract. Absorbance was measured after 15 min at 350 nm against the reagent blank. Caffeic acid in methanol served as a standard for preparation of calibration curve in the range 0–100 mg l^{-1} ($Y = 0.0049X$; Y: The absorbance at 350 nm; X: caffeic acid concentration; $R^2 = 0.9979$). The results were expressed in milligram of caffeic acid equivalents per gram of dry matter of olive leaves (mg CAE g^{-1} DM).

The content of total flavonoids was determined according to the method used by Lee et al. (2009). The sample solution (1 ml) was placed in a test tube with diethylene glycol (10 ml) and 1 N NaOH solution (1 ml) and allowed to stand for 30 min. For the blank, 1 ml of methanol was used instead of the extract. The absorbance was measured at 420 nm against the reagent blank.

Rutin in methanol served as a standard for the preparation of the calibration curve in the range 0–250 mg l^{-1} ($Y = 0.0031X$; Y: The absorbance at 420 nm; X: rutin concentration; $R^2 = 0.9991$). The results were expressed in milligram of rutin equivalents per gram of dry matter of olive leaves (mg RE g^{-1} DM).

All determinations of the total phenolic, *ortho*-diphenol and flavonoid contents were performed in triplicate for each extract of olive leaves.

2.6. Determination of total antioxidant capacity

The Trolox Equivalent Antioxidant Capacity (TEAC) assessment of methanol extract was determined in terms of radical scavenging ability by using improved $ABTS^+$ radical cation decolorization assay (Re et al. (1999). Stock solution of $ABTS^+$ radical cation was prepared by mixing 7 mM ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] and 2.45 mM potassium persulfate. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70 (± 0.03) at 734 nm and equilibrated at 30 °C. Ten microliters of sample was added to 1 ml $ABTS^+$ solution and the absorbance decrease at 734 nm was recorded after 6 min. Percentage inhibition of oxidation (PI) was calculated for each sample according to the following equation:

$$\mathrm{PI}(\%) = \frac{(\mathrm{OD}_0 - \mathrm{OD}_{6\,\mathrm{min}}) * 100}{\mathrm{OD}_0} \quad (3)$$

where OD_0 denotes the initial absorbance of diluted $ABTS^+$ and OD_{6min} denotes the absorbance of the sample after 6 min of reaction.

Series of dilutions were prepared such that they produced between 20% and 80% inhibition of the blank absorbance. Trolox was used as an external standard to prepare the calibration curve in the range 125–312.5 mg l^{-1} ($Y = 43.21X$; Y: percentage inhibition; X: Trolox concentration; $R^2 = 0.9961$).TEAC calculation was performed as following:

$$\mathrm{TEAC}(\mathrm{mM}) = \frac{PI * \mathrm{dilution}}{43.21} \quad (4)$$

The results were expressed as millimoles Trolox Equivalent Antioxidant Capacity (mM TEAC) in the methanol extract.

All determinations were performed in triplicate for each olive leaves extract.

2.7. HPLC analyses of phenolic compounds

HPLC analyses were performed with an analytical HPLC unit (Agilent technologies 1200 series), equipped with a diode array detector. The stationary phase was an Atlantis® Waters dC18 column (5 μm particle size; 250 mm; 4.6 mm).

The mobile phases were formic acid (19:1) (A) and methanol (B), starting with 5% B and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 35% B at 25 min, 45% B at 35 min, 50% B at 40 min, 100% B at 45 min, 5% B at 46 min, and finally, re-equilibration for 4 min to return to the initial composition.

The flow rate was 0.9 ml min^{-1} with elution at room temperature. The injection volume was 10 μl and chromatograms were recorded at 280 nm. The data were processed on the ChemStation Agilent technologies software.

Phenolic compounds in methanol extracts were identified by matching the retention time and the UV spectra of a peak in the extract chromatogram with the peak of a known standard compound. Identifications were confirmed by analyzing a sample supplemented by the corresponding standard.

Phenolic compounds quantification was achieved by calibration curves relative to external standards, developed by injecting different amounts of a known standard compound in the HPLC column (For oleuropein: $Y = 0.0032X$; $R^2 = 1.000$; for verbascoside: $Y = 0.001X$; $R^2 = 0.9990$; for rutin: $Y = 0.0012X$; $R^2 = 0.9989$. where Y: mass of the standard (μg) and X: air of corresponding peak).

2.8. Molecular mass distribution of polyphenolics

Chromatography gel filtration on Sephadex G-50 was used to analyze the polymeric aromatic fraction present in olive leaves aqueous extract. A powder (2 g) made from the olive leaves was mixed with water (20 ml). After 5 min, the mixture was centrifuged for 15 min at 3000 rpm. The supernatant was recovered and filtered through a 1.2 μm filter. Three milliliters of this aqueous extract was placed on a Sephadex coarse G-50 column (2.5–60 cm) previously equilibrated with $NaNO_3$ 0.05 M containing 0.02% sodium azide at a flow rate of 0.6 ml min^{-1}. Sample was collected on the basis of 3 ml per tube. The optic density of these fractions was measured spectrometrically at 280 nm. The column was calibrated with syringic acid (MM = 198 Da), lysozym (MM = 15 kDa) and blue dextran (MM = 200 kDa) (Asses et al., 2009).

2.9. Statistical analyses

All measurements were carried out at least in duplicate. The mean values and standard deviations were calculated using Excel Software Version 2003 for Windows. The results were statistically analyzed by analysis of variance (ANOVA) and multiple range tests using STATGRAPHICS Plus Software version 1.4 for Windows. For all analyses, parameters were considered significant at a 95% level when p-*value* <0.05.

3. Results and discussion

3.1. Effect of gamma irradiation on microbial load of dried olive leaves

3.1.1. Microbial load of dried olive leaves

Total aerobic bacteria (TAB), yeast and moulds (YM) and lactic acid bacteria (LAB) are present in the non-irradiated control samples, at levels of 2.5×10^2–6.65×10^2 CFU g^{-1}, 1.62×10^2–1.71×10^2 CFU g^{-1} and 0.25–3.51×10^2 CFU g^{-1}, respectively. These levels were within the range of levels reported in black tea (Thomas et al., 2008) and in lotus *Nelumbo nucifera* rhizome (Khattak et al., 2009). The contamination of plant materials depends on

various parameters; such as processing history, the composition and the available surface of the materials (Katusin-Razem et al., 2001).

The obtained results showed that air-dried olive leaves contain microbial contaminants. Therefore, decontamination could be required in order to obtain safe olive leaves.

3.1.2. Decontamination dose

Gamma irradiation was investigated in order to improve microbiological quality of olive leaves samples, presented for the treatment in two forms (POL and IOL). Different doses of (0, 5, 10, 15, 20 and 25 kGy) were applied.

The treatment of olive leaves samples with ionizing radiation had a substantial effect on the total microbial load. Gamma irradiation of olive leaves samples was found to cause a reduction in microbial load proportional to the dose delivered, as shown in Fig. 1. The fraction of surviving microorganisms markedly decreased with the increase of applied dose. Significant reduction (p-value < 0.05) was obtained at the dose of 5 kGy. For 10 kGy dose, the rates of lethal fraction of all studied populations and in all samples were more than 60%. Results obtained demonstrate that the contaminant microbes still survived at 15 kGy dose and treatment with doses from 20 kGy was proved sufficient to decrease the microbial load under the limit of the detection.

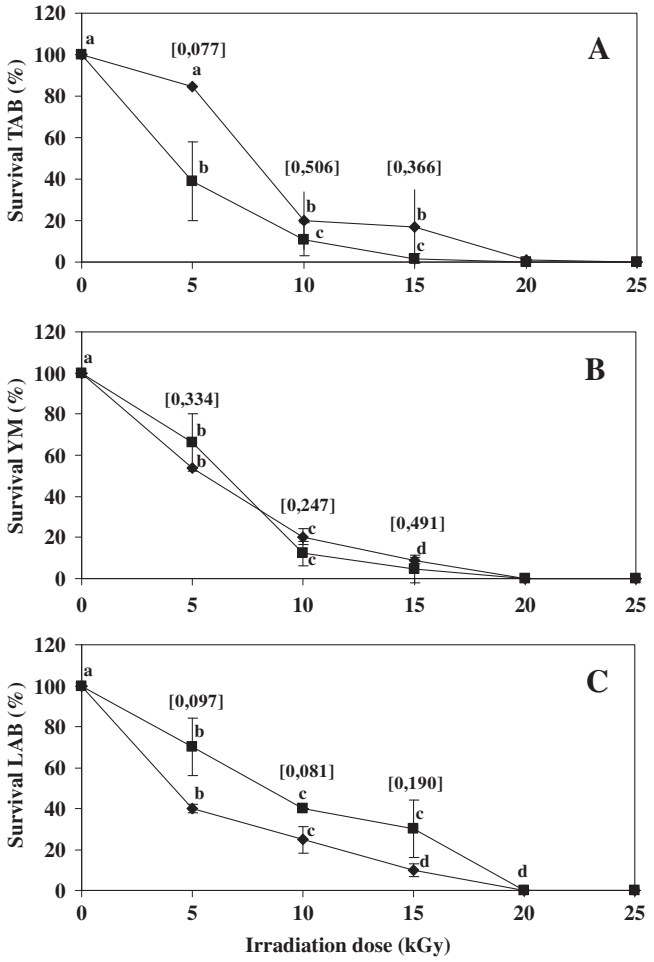

Fig. 1. Inactivation curves by gamma irradiation for total aerobic bacteria (TAB) (A), yeast and mold (YM) (B), and lactic acid bacteria (LAB) (C) in powdered olives leaves (POL) (-♦-) and in intact olive leaves (IOL) (-■-). All data were the mean of two replicates of samples and the vertical bars represent the standard deviations for each data point. Initial loads were 2.50 10^2 CFU g^{-1} of TAB, 1.71 10^2 CFU g^{-1} of YM and 3.51 10^2 CFU g^{-1} of LAB in POL and 6.65 10^2 CFU $g^{-1}$of TAB, 1.62 10^2 CFU g^{-1} of YM and 0.25 CFU $g^{-1}$of LAB in IOL. Values in square brackets are the *p-Values* determined by ANOVA between data for POL and IOL for each irradiation dose (the significance level was set at p-value < 0.05). Values in each curve with different letters differ significantly (p-value < 0.05).

According to WHO (1999), the 20 kGy irradiated olive leaves samples could be considered safe for human consumption. A joint FAO/IAEA/WHO experts committee was convened to assess the safety and nutritional adequacy of food irradiated to doses above 10 kGy. They indicated that irradiation at doses above 10 kGy does not produce any toxicological hazards or nutritional or microbiological problems in food. They also concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate (WHO, 1999).

Our results show that, like spices and tea, gamma irradiation could be successfully employed for olive leaves to reduce the contaminants of bacteria and fungal species, which may be responsible for the degradation of nutraceutic compounds.

No information in the literature is available on the effect of gamma irradiation on the microbial load of olive leaves, but decontamination of other plants materials following gamma irradiation are reported by several researchers. Khattak et al., 2009 indicated that the microbial load of *Nelumbo nucifera* rhizome were 3.8×10^2 CFU g^{-1} for bacterial count and 9.8×10^1 CFU g^{-1} for fungal count. A gamma irradiation treatment with a dose of 2 kGy was proved to be sufficient to decrease the microbial load of *Nelumbo nucifera* rhizome to less than 10 CFU g^{-1}. Thomas et al. (2008) reported that 7 kGy dose irradiation was effective to decontaminate black tea samples. However, an irradiation dose of $\geqslant$10 kGy was recommended to remove the microbial load of aniseed (*Pimpinella anisum*) (Al Bachir, 2007). In a recent study, Bhat et al. (2010) investigated the application of different doses of gamma irradiation (0, 2.5, 5, 7.5, 10, 15 and 30 kGy) to lotus seeds for decontamination purpose. A significant dose-dependent decrease in the fungal contaminants was revealed. However, the contaminant yeasts could survive up to 10 kGy dose, which could be completely eliminated at 15 kGy. From the results obtained, a dose range between 10 and 15 kGy is recommended for complete decontamination of lotus seeds. Similar to our results, Kikuchi, Todoriki, Saito, and Hayashi (2003) reported that doses of 5, 10 and 15 kGy of gamma irradiation were insufficient to eliminate the contaminant microbes in *Enrei* and *Vinton* varieties of soybeans and have recommended higher doses of gamma irradiation (20 kGy) to attain total decontamination.

The lethal action of gamma irradiation on microorganisms is owing to various reactive oxygen species (ROS) produced during the treatment of foodstuff. ROS introduces some heavy lesions in deoxyribonucleic acid (DNA) molecules, which are one of the principal targets for radiation damage, preventing the DNA from functioning normally and inducing alteration of protein expression. Thus, it contributes to cellular damage (Bonura, Smith, & Kaplan, 1975).

3.1.3. Radioresistance of microbial load in olive leaves

The resistance of microflora to irradiation is expressed as a dose required for the first 90% reduction i.e. the dose required to reduce the initial microbial load by one log cycle (D_{10}-value).

The D_{10}-values determined in the present work were arranged between 9.74 and 25.12 kGy, depending on the microbial population and the form of samples (Fig. 2 and Fig. 3). Our D_{10} results were remarkably higher than those reported in literature (Table 1). Katusin-Razem, Matic, Razem, and Mihokovic (1988) studied the decontamination of Mint leaves (*Folium menthae*) by treatment with gamma irradiation. D_{10}-value in Mint leaves (*Folium menthae*) were 1.10 kGy for total aerobic plate count and 1.30 kGy for mold propagula. These values are widely low, in comparison with our findings. Total aerobic plate count and mold propagula were found

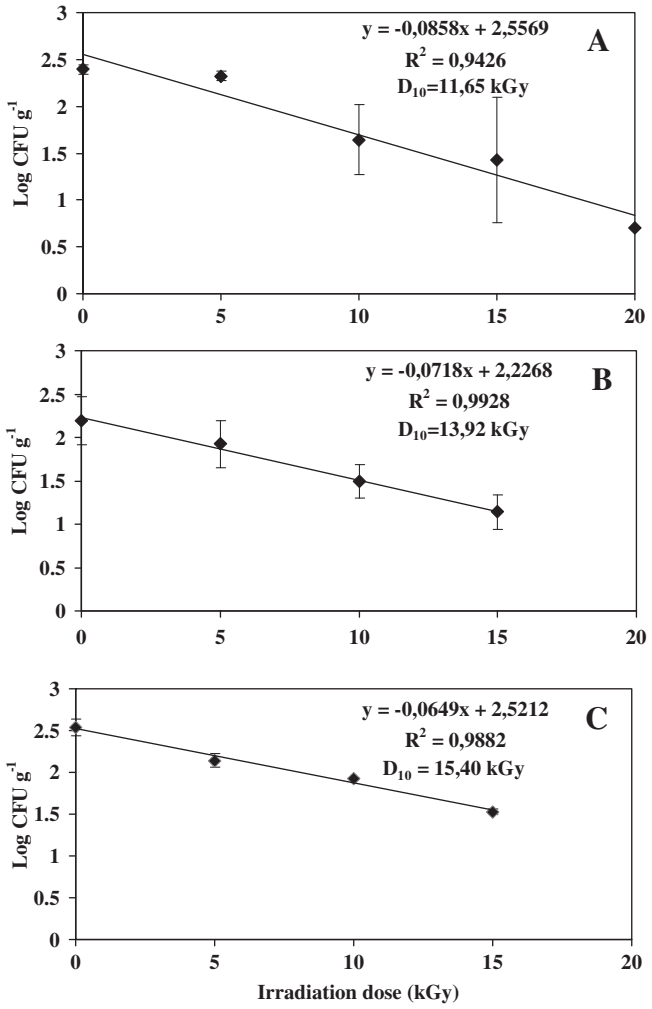

Fig. 2. Radioresistance curves of total aerobic bacteria (TAB) (A), yeast and mold (YM) (B), and lactic acid bacteria (LAB) (C) in powdered olives leaves (POL) and determination of D_{10}-values. All data were the mean of two replicates of samples and the vertical bars represent the standard deviations for each data point.

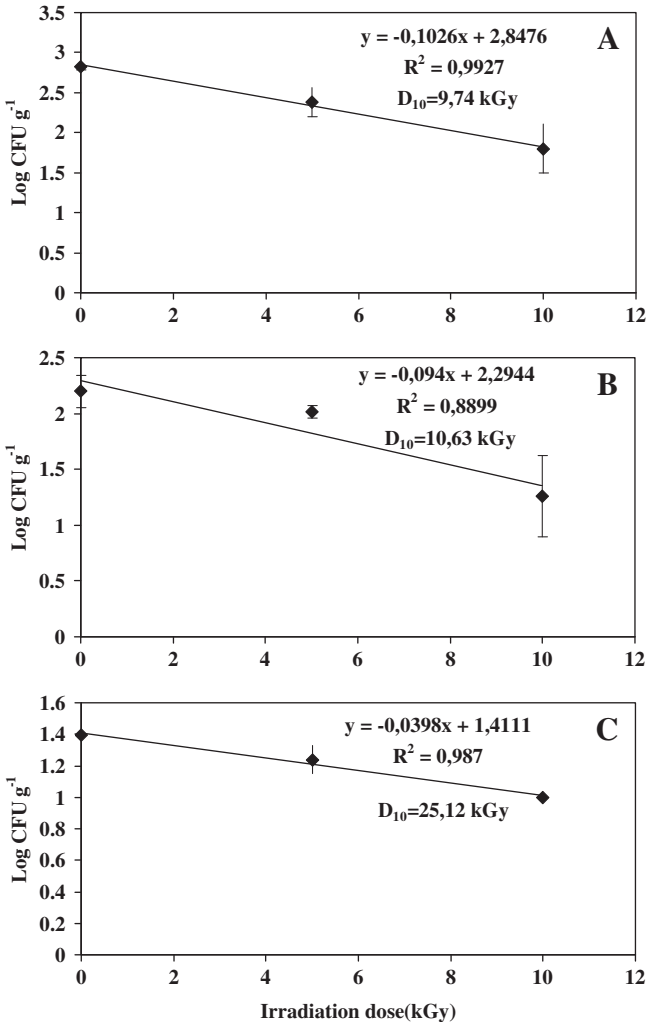

Fig. 3. Radioresistance curves of total aerobic bacteria (TAB) (A), yeast and mold (YM) (B), and lactic acid bacteria (LAB) (C) in intact olives leaves (IOL) and determination of D_{10}-values. All data were the mean of two replicates of samples and the vertical bars represent the standard deviations for each data point.

more radioresistant in Plantain seeds (*Semen plantaginis*), with D_{10}-value about 4.40 kGy and 3.80 kGy, respectively (Katusin-Razem et al., 2001). However, these results are also lower than ours findings.

It seemed that the high radioresistance of microorganisms in olive leaves is owing to the low availability of water (aw) and to the presence of phenolic compounds, among other factors. It was reported that various environmental factors affected the sensitivity of microorganisms to radiation. At a higher availability of water (aw), the microorganisms are more sensitive to radiation because of more free radicals generated by the irradiation. It has been reported that the efficiency of gamma irradiation on populations of microorganisms increased with increasing moisture content of the sewage sludge. D_{10} values were 1.55, 1.25, 1.16, 1.10 and 1.02 kGy for the sludge moisture content of 2%, 20%, 40%, 60% and 80%, respectively (Al-Bachir, Al-Adawi, & Asma, 2003). Besides, phenolic compounds in olive leaves act as scavengers of the radicals (Lee et al., 2009) leaving fewer radicals to react with the organism. Therefore, the radiosensitivity of microorganisms in olive leaves was low.

The resistance of microorganisms to gamma irradiation is dependant on the kinds of population. In both POL and IOL samples, LAB was found the most radioresistant population, followed by YM and then by TAB. Previously, it has been reported that gram (+) bacteria, such as LAB, are more radioresistant than gram (−) bacteria (Ayari et al., 2009). For each irradiation dose and each population, the difference between the radioresistance of miroorganisms on POL and on IOL was not statistically significant (*p-Values* > 0.05) (Fig. 1).

3.2. Effect of gamma irradiation on antioxidant capacity of olive leaves extract

Although irradiation is proven to be an adequate technology for the decontamination of olive leaves, the investigation of the effect of this treatment on antioxidative characteristics could be of interest when considering vegetable material as a food additive or medicinal plant.

The antioxidant capacity of methanol extract of irradiated and non-irradiated olive leaves samples was performed in terms of radical scavenging ability according to the ABTS/$K_2S_8O_2$ method. It is expressed as Trolox equivalent antioxidant capacity (TEAC). Methanol extract of non-irradiated samples showed a TEAC value of 1.546 and 1.324 mM for POL and IOL samples, respectively. These results confirmed the findings of Benavente-Garcia et al. (2000), who obtained a TEAC value of 1.58 mM.

In the present study, gamma irradiation treatment did not show any significant effect (*p-Values* > 0.05) on antioxidant capacity of

Table 1
Dose D_{10} required for decimal reduction of microbial load in various materials by gamma irradiation treatment.

Irradiated materials	Microbial population	D_{10}* (kGy)	Reference
Sewage sludge	Total microbial count	1.02–1.55	Al-bachir et al. (2003)
Mint leaves (*Folium menthae*)	Total aerobic plate count	1.10	Katusin-Razem et al. (1988)
Linden flowers (*Flos tiliae*)	Total aerobic plate count	1.40	Katusin-Razem et al. (1988)
Plantain seeds (*Semen plantaginis*)	Total aerobic plate count	4.40	Katusin-Razem et al. (2001)
Milled Plantain seeds (*Semen plantaginis*)	Total aerobic plate count	9.90	Katusin-Razem et al. (2001)
Intact olive leaves (*olea europaea)*	Total aerobic bacteria	9.74	This work
Powdered olive leaves (*olea europaea)*	Total aerobic bacteria	11.65	This work
Linden flowers (*Flos tiliae*)	Mold propagula	0.80	Katusin-Razem et al. (1988)
Mint leaves (*Folium menthae*)	Mold propagula	1.30	Katusin-Razem et al. (1988)
Plantain seeds (*Semen plantaginis*)	Mold propagula	3.80	Katusin-Razem et al. (2001)
Intact olive leaves (*olea europaea*)	Yeast and mold	10.63	This work
Powdered olive leaves (*olea europaea*)	Yeast and mold	13.92	This work
Powdered olive leaves (*olea europaea)*	Lactic acid bacteria	15.40	This work
Intact olive leaves (*olea europaea*)	Lactic acid bacteria	25.12	This work

* D_{10} is the dose required to inactivate a $1Log_{10}$ cycle of the initial population.

both POL and IOL samples (Table 2). This could be attributed to the low water content, which limited the possibility of free radicals being formed (Murcia et al., 2004). In fact, radiolysis of water results in the production of radicals such as hydrated electrons, hydroxyl radicals and hydrogen atoms. These radicals may break some bonds, leading to the possible structure modifications of some active principles that have antioxidant capacity.

Though various researchers have worked on the antioxidant capacity of olive leaves (Altiok, Baycin, Bayraktar, & Ulku, 2008; Benavente-Garcia et al., 2000; Kiritsakis et al., 2010; Lee et al., 2009), nothing was reported on the effect of gamma irradiation on this property. However, the effect of the irradiation technology on antioxidant capacity was reported for other plants and spices. Our finding was in agreement with that of Lee, Jo, Sohn, Kim, and Byun (2006), who reported the absence of a significant effect of gamma irradiation at 20 kGy on antioxidant capacity of green tea byproducts and green tea leaf extracts. Murcia et al. (2004) also revealed that irradiation up to 10 kGy did not have any effect on the antioxidant capacities of seven dessert spices.

Nevertheless, some studies indicated an enhancement of antioxidant capacity by gamma irradiation processing of some vegetable materials, such as almond skin treated up to a 16 kGy dose (Harrison & were, 2007) and *Nelumbo nucifera* rhizome treated up to a 6 kGy dose (Khattak et al., 2009). Gamma irradiation could also reduce the antioxidant capacity as observed in *Glycyrrhiza glabra* root treated up to a 25 kGy dose (Khattak & Simpson, 2010).

3.3. Effect of gamma irradiation on phenolic compounds content in dried olive leaves

The nutraceutical property of olive leaves is attributed to phenolic compounds, which play an antioxidant role by reducing the free radicals and the reactive oxygen species. Gamma irradiation could modify the structure of phenolic compounds, depending on the environmental conditions. Therefore, it is interesting to investigate the effect of gamma irradiation on phenolic compounds content in treated olive leaves.

Table 2
Antioxidant capacity in olive leaves presented in intact and powdered forms to gamma irradiation at different doses.

Irradiation dose (kGy)	Antioxidant capacity (mM TE)	
	Powdered olive leaves (POL)	Intact olive leaves (IOL)
0	1.54 ± 0.09ab	1.32 ± 0.30a
5	1.57 ± 0.13 a	1.79 ± 0.29a
10	1.60 ± 0.15a	1.55 ± 0.48a
15	1.36 ± 0.17b	1.81 ± 0.27a
20	1.55 ± 0.07ab	1.37 ± 0.52a
25	1.49 ± 0.15ab	1.47 ± 0.29a

TE: Trolox equivalents.
Results were expressed as Mean ± standard deviation. All results were the mean of at least 4 replicates.
Values in each column with different letters differs significantly (*p-Values* < 0.05).

3.3.1. Total phenolics content

The total phenolics content of irradiated and control samples of olive leaves was determined in methanol extracts using the Folin–Ciocalteau's phenol reagent (Table 3). The total phenolics contents were 53.05 and 63.98 mg g^{-1} DM in the POL and IOL control samples, respectively. An earlier study by Kiritsakis et al. (2010) reported that total phenolics contents in olive leaves of three Greek cultivars were 60.9, 55.7 and 61.9 mg g^{-1} DM. While, Lee et al. (2009) found 148 mg g^{-1} of total phenolics in 80% ethanol extract of olive leaves.

Gamma irradiation at all the tested doses (5, 10, 15, 20 and 25 kGy) did not affect significantly the total phenolics content (*p-Values* > 0.05) in both POL and IOL samples, in comparison with non-irradiated control samples.

This result was in agreement with that of Koseki et al. (2002) who did not observe any significant changes in the total phenolics content of sweet basil (*Ocimum basilicum Linné*) at doses up to 30 kGy. A similar observation was reported for green tea byproducts and green tea leaf extracts treated by gamma irradiation at dose of 20 kGy (Lee et al., 2006). However, gamma irradiation treatments have been shown to either increase or decrease the total phenolics content of plants materials, which is dependent on the dose delivered and the raw material used. Khattak et al. (2009) reported that gamma irradiation treatment at dosage levels of 1, 2, 4 and 6 kGy increased the yield of total phenolics in *Nelumbo nucifera* rhizome. A similar effect was observed on irradiated almond skin (Harrison & Were, 2007). In contrast to this, it has been found that gamma irradiation, up to 30 kGy (10 intervals), decreased total phenolics in dehydrated rosemary (*Rosmarinus officinalis Linné*) (Koseki et al., 2002).

3.3.2. Ortho-diphenols content

Total phenolics content in olive leaves has been reported in the past (Boudhrioua, Bahloul, Ben Slimen & Kechaou, 2009; Kiritsakis et al., 2010; Lee et al., 2009), but to our knowledge, no previous studies were carried out on *ortho*-diphenols content in olive leaves. *Ortho*-diphenols, especially hydroxytyrosol and caffeic acid, are the most active antioxidants among the olive phenols. *Ortho*-diphenols levels (Table 3) were 9.46 and 8.52 mg g^{-1} DM in POL and IOL control samples, respectively.

The effect of gamma irradiation on the *ortho*-diphenols contents in olive leaves depends on the delivered dose and on the form of

Table 3
Total phenolics, *ortho*-diphenols and flavonoids contents in olive leaves presented in intact and powdered forms to gamma irradiation at different doses.

Form of presentation	Irradiation dose (kGy)	Total phenolics content (mg GAE g^{-1} DM)	*Ortho*-diphenols content (mg CAE g^{-1} DM)	Flavonoids content (mg RE g^{-1} DM)
Powdered olive leaves (POL)	0	53.05 ± 1.61ab	9.46 ± 0.72a	18.68 ± 0.27a
	5	50.11 ± 1.56a	8.19 ± 0.68b	14.62 ± 0.96c
	10	54.21 ± 1.44b	11.57 ± 0.68c	17.75 ± 0.29ab
	15	50.66 ± 4.53ab	10.05 ± 0.19a	16.71 ± 1.09b
	20	53.46 ± 3.37ab	9.31 ± 0.20ab	20.19 ± 1.13d
	25	53.18 ± 2.02ab	9.98 ± 1.52a	20.16 ± 1.43d
Intact olive leaves (IOL)	0	63.98 ± 3.47a	8.52 ± 2.91ab	18.65 ± 0.44a
	5	56.61 ± 1.39b	7.47 ± 1.50ab	15.03 ± 0.52a
	10	59.12 ± 3.18ab	9.03 ± 0.56ab	16.05 ± 0.27a
	15	60.96 ± 7.61ab	10.20 ± 1.49a	15.23 ± 2.23a
	20	59.23 ± 2.93ab	7.34 ± 1.94b	18.07 ± 3.74a
	25	62.09 ± 1.86ab	7.82 ± 2.01ab	18.11 ± 4.19a

GAE: gallic acid equivalents; CAE: caffeic acid equivalents; RE: rutin equivalents; DM: dry matter.
Results were expressed as mean ± standard deviation. All results were the mean of at least 4 replicates.
Values in each column with different letters differs significantly (*p-Values* < 0.05).

Table 4
Effect of gamma irradiation on oleuropein, verbascoside and rutin contents in intact and powdered olive leaves samples.

Form of presentation	Irradiation dose (kGy)	Oleuropein (mg g^{-1} DM)	Verbascoside (mg g^{-1} DM)	Rutin (mg g^{-1} DM)
Powdered Olive leaves (POL)	0	27.47 ± 0.06a	0.79 ± 0.12a	0.60 ± 0.04a
	5	27.96 ± 3.12a	0.77 ± 0.10a	0.80 ± 0.12a
	10	25.16 ± 3.05a	0.66 ± 0.06a	0.72 ± 0.04a
	15	22.78 ± 1.79a	0.55 ± 0.08a	0.67 ± 0.06a
	20	24.21 ± 2.13a	0.60 ± 0.15a	0.71 ± 0.08a
	25	23.17 ± 2.11a	0.58 ± 0.08a	0.67 ± 0.11a
Intact olive leaves (IOL)	0	19.86 ± 0.54a	0.20 ± 0.04a	0.94 ± 0.13a
	5	20.79 ± 1.70a	0.14 ± 0.01a	0.61 ± 0.08a
	10	17.11 ± 1.80a	0.15 ± 0.01a	0.62 ± 0.04a
	15	17.46 ± 2.28a	0.15 ± 0.04a	0.64 ± 0.11a
	20	18.13 ± 1.30a	0.20 ± 0.01a	0.86 ± 0.22a
	25	18.96 ± 2.11a	0.20 ± 0.04a	0.95 ± 0.19a

DM: dry matter.
Results were expressed as mean ± standard deviation. All results were the mean of 2 replicates.
Values in each column with different letters differs significantly ($p < 0.05$).

samples presentation to treatment (POL or IOL). Statistical analysis by ANOVA showed a significant variation (*p-Values* < 0.05) in *ortho*-diphenols content in POL samples, depending on irradiation dose. While no significant effect (*p-Values* > 0.05) was observed for irradiated IOL samples, in comparison with the control. *Ortho*-diphenols in IOL seemed to be protected from the action of gamma irradiation, owing to the compartmentalization.

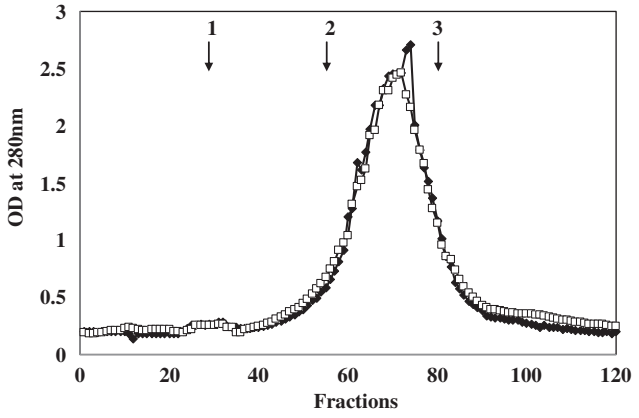

Fig. 4. Effect of gamma irradiation of powdered olive leaves on molecular mass distribution of polyphenolics: The elution pattern of aqueous extract from non-irradiated (-♦-) and 20 kGy irradiated (-□-) sample, obtained by gel filtration chromatography on Sephadex G50. 1: Blue dextran (MM = 200 kDa), 2: Lysosym (MM = 15 kDa), 3: Syringic acid (MM = 198 Da).

3.3.3. Flavonoids content

The flavonoids contents were determined in methanol extracts. The non- irradiated olive leaves showed 18.68 and 18.65 mg g^{-1} DM of flavonoids in POL and IOL samples, respectively (Table 3).

It should be mentioned that the levels of flavonoids contents in this investigation were lower than that found in an 80% ethanol extract (58 mg g^{-1}) (Lee et al., 2009). However, when considering the abundance percent of flavonoids against total phenolics, our finding (about 29%-35%) was in the range of level reported by Lee et al. (2009) (39%).

One of the prominent and medically most useful properties of many flavonoids is their ability to scavenge free radicals (Benavente-Garcia et al., 2000).

The gamma irradiation treatment did not have any detrimental effect on flavonoids contents in IOL samples. However, treated POL samples showed significant variation (*p-Values* < 0.05) of flavonoids content, comparing to the control. This finding is in agreement with that of *ortho*-diphenols contents.

There is no information available in the literature on the effect of gamma irradiation on the flavonoids content of olive leaves. However for other plant materials, diverse effects of radiation have been reported.

Koseki et al. (2002) showed that there was no significant effect of irradiation, up to 30 kGy, of three herbs: sweet basil (*Ocimum basilicum Linné*), Rosemary (*Rosmarinus officinalis Linné*) and Artichoke (*Cynara scolymus Linné*). Also, gamma irradiation (1–6 kGy) did not affect some flavonoids, notably ellagic acid derivatives and quercitin concentrations, in strawberries (Breitfellner, Solar,

& Sontag, 2002). Nevertheless, this treatment noticeably diminished some other flavonoids, notably catechin and kaempferol components, in strawberries materials (Breitfellner et al., 2002).

3.3.4. Molecular mass distribution of polyphenolics

Gel filtration chromatography on Sephadex G50 was used to analyze the polymeric aromatic fraction present in aqueous extract of olive leaves. The elution pattern of untreated POL sample (Fig. 2) showed one peak, corresponding to a family of aromatics with molecular mass between 198 Da (molecular mass of syringic acid) and 15,000 Da (molecular mass of lysosym).

The elution pattern of irradiated POL sample at a 20 kGy dose coincides with that of the untreated POL sample. Thus, it can be conclude that gamma irradiation did not affect the molecular mass distribution of polyphenolics in olive leaves. According to this result, it could be suggested that gamma irradiation of air-dried olive leaves does not lead to polymerization, hydrolysis or oxidation of polyphenolic compounds.

3.3.5. HPLC analyses of methanol extract

HPLC analyses were carried out in order to quantify three phenolic compounds (Oleuropein, verbascoside and rutin) in the methanol extract of olive leaves samples. Table 4 showed the content of oleuropein, verbascoside and rutin in the control and the irradiated samples of POL and IOL.

Oleuropein was found to be the major phenolic compound in olive leaves as reported by many authors (Benavente-Garcia et al., 2000; Kiritsakis et al., 2010; Lee et al., 2009).

Oleuropein contents in the control sample were 27.47 and 19.86 mg g^{-1} DM in POL and IOL, respectively. These levels were consistent with the finding of Japon-Lujan, Luque-Rodriguez, and de Castro (2006) who extracted up to 23 mg g^{-1} of oleuropein. Altiok et al. (2008) extracted 2 to 91 mg g^{-1} of oleuropein, depending on extraction solvent. Verbascoside (0.79 mg g^{-1} DM in POL and 0.20 mg g^{-1} DM in IOL) and rutin (0.60 mg g^{-1} DM in POL and 0.94 mg g^{-1} DM in IOL) were minor phenolic compounds in olive leaves. The level of verbascoside is within the range of levels reported by Japon-Lujan et al. (2006) (0.4 mg g^{-1}). However, the level of rutin is markedly lower than those showed by Altiok et al. (2008) (around 0.5–20 mg g^{-1}, depending on extraction solvent).

Gamma irradiation did not have any significant effect (*p-Values* > 0.05) on oleuropein, verbascoside and rutin contents in both the POL and IOL samples. In a previous study (Schindler, Solar, & Sontag, 2005), it was shown that gamma irradiation treatment (2, 4, and 6 kGy) markedly reduced the concentration of rutin in tomato.

4. Conclusion

The results of our investigations showed that the gamma irradiation treatment effectively reduces the numbers of microorganisms present in air-dried olive leaves. A dose of 20 kGy was required to decontaminate the studied materials. Microbial populations in this material are highly radioresistant (D_{10}: 9.74–25.12 kGy). The high radioresistance seemed to be attributed to the presence of phenolic compound and to the low availability of water in dried olive leaves.

Despite the high doses, the irradiation dose up to 25 kGy didn't alter the antioxidant capacity, molecular mass distribution of polyphenolics, total phenolics, *ortho*-diphenols, flavonoids, oleuropein, verbascoside and rutin contents in olive leaves.

To conclude, gamma irradiation is a promising technology for decontamination of air-dried olive leaves. In fact, it improves microbial quality of olive leaves without altering the phenolic composition and the antioxidative properties. (Fig. 4).

Acknowledgments

This research was financially supported by the Tunisian Ministry of Scientific Research, Technology and Competences Development. The authors gratefully acknowledge the National Center for Nuclear Sciences and Technologies for the irradiation operations. The authors are also thankful to the Mediterranean Institute of Ecology and Palaeoecology UMR CNRS/IRD for providing all the facilities to conduct HPLC analyses.

References

Al-Bachir, M. (2007). Effect of gamma irradiation on microbial load and sensory characteristics of aniseed (*Pimpinella anisum*). *Bioresource Technology, 98*, 1871–1876.

Al-Bachir, M., Al-Adawi, M. A., & Asma, M. (2003). Synergetic effect of gamma irradiation and moisture content on decontamination of sewage sludge. *Bioresource Technology, 90*, 139–143.

Altiok, E., Baycin, D., Bayraktar, O., & Ulku, S. (2008). Isolation of polyphenols from the extracts of olive leaves (*Olea europaea L.*) by adsorption on silk fibroin. *Separation and Purification Technology, 62*(2), 342–348.

Anonymous (2009). Olive leaf. *Alternative medicine review, 14*(1), 62–66.

Asses, N., Ayed, L., Bouallagui, H., Ben Rejeb, I., Gargouri, M., & Hamdi, M. (2009). Use of *Geotrichum candidum* for olive mill wastewater treatment in submerged and static culture. *Bioresource Technology, 100*(7), 2182–2188.

Ayari, S., Dussault, D., Millette, M., Hamdi, M., & Lacroix, M. (2009). Changes in membrane fatty acids and murein composition of *Bacillus cereus* and *Salmonella Typhi* induced by gamma irradiation treatment. *International Journal of Food Microbiology, 135*, 1–6.

Benavente-Garcia, O., Castillo, J., Lorente, J., Ortuno, A., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea L*-leaves. *Food Chemistry, 68*(4), 457–462.

Bhat, R., Sridhar, K. R., & Karim, A. A. (2010). Microbial quality evaluation and effective decontamination of nutraceutically valued lotus seeds by electron beams and gamma irradiation. *Radiation Physics and Chemistry, 79*(9), 976–981.

Blekas, G., Psomiadou, E., Tsimidou, M., & Boskou, D. (2002). On the importance of total polar phenols to monitor the stability of Greek virgin olive oil. *European Journal of Lipid Science and Technology, 104*, 340–346.

Bonura, T., Smith, K. C., & Kaplan, H. S. (1975). Enzymatic induction of DNA double strand breaks in gamma irradiated *Escherichia coli K-12*. *Proceedings of the National Academy of Sciences of the USA, 72*, 4265–4269.

Boudhrioua, N., Bahloul, N., Ben Slimen, I., & Kechaou, N. (2009). Comparison on the total phenol contents and the color of fresh and infrared dried olive leaves. *Industrial Crops and Products, 29*, 412–419.

Bouaziz, M., Fki, I., Jemai, H., Ayadi, M., & Sayadi, S. (2008). Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from *Chemlali* olive leaves. *Food Chemistry, 108*, 253–262.

Breitfellner, F., Solar, S., & Sontag, G. (2002). Effect of gamma irradiation on flavonoids in strawberries. *European Food Research and Technology, 215*, 28–31.

Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., & Lundin, L. (2009). Incorporation of functional ingredients into foods. *Trends in Food Science & Technology, 20*, 388–395.

Harrison, K., & Were, L. M. (2007). Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts. *Food Chemistry, 102*, 932–937.

Hayes, J. E., Stepanyan, V., Allen, P., O'Grady, M. N., & Kerry, J. P. (2010). Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Meat Science, 84*(4), 613–620.

Japon-Lujan, R., Luque-Rodriguez, J. M., & de Castro, M. D. L. (2006). Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. *Journal of Chromatography A, 1108*(1), 76–82.

Katusin-Razem, B., Matic, S., Razem, D., & Mihokovic, V. (1988). Radiation decontamination of tea herbs. *Journal of Food Science, 53*, 1120–1126.

Katusin-Razem, B., Novak, B., & Razem, D. (2001). Microbiological decontamination of botanical raw materials and corresponding pharmaceutical products by irradiation. *Radiation Physics and Chemistry, 62*, 261–275.

Khattak, K. F., & Simpson, T. J. (2010). Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of *Glycyrrhiza glabra* root. *Radiation Physics and Chemistry, 79*, 507–512.

Khattak, K. F., Simpson, T. J., & Ihasnullah (2009). Effect of gamma irradiation on the microbial load, nutrient composition and free radical scavenging activity of *Nelumbonucifera* rhizome. *Radiation Physics and Chemistry, 78*, 206–212.

Kikuchi, O. K., Todoriki, S., Saito, M., & Hayashi, T. (2003). Efficacy of soft-electron (low-energy electron beam) for soybean decontamination in comparison with gamma-rays. *Journal of Food Science, 68*, 649–652.

Kiritsakis, K., Kontominas, M. G., Kontogiorgis, C., Hadjipavlou-Litina, D., Moustakas, A., & Kiritsakis, A. (2010). Composition and antioxidant activity of olive leaf extracts from greek olive cultivars. *Journal of the American Oil Chemists' Society, 87*(4), 369–376.

Koseki, P. M., Villavicencio, A. L. C. H., Brito, M. S., Nahme, L. C., Sebastiao, K. I., Rela, P. R., et al. (2002). Effects of irradiation in medicinal and eatable herbs. *Radiation Physics and Chemistry, 63*, 681–684.

Lee, N. Y., Jo, C., Sohn, S. H., Kim, J. K., & Byun, M. W. (2006). Effects of gamma irradiation on the biological activity of green tea byproduct extracts and a comparison with green tea leaf extracts. *Journal of Food Science, 71*, 269–274.

Lee, O. H., Lee, B. Y., Lee, J., Lee, H. B., Son, J. Y., Park, C. S., et al. (2009). Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. *Bioresource Technology, 100*, 6107–6113.

Murcia, M. A., Egea, I., Romojaro, F., Parras, P., Jiménez, A. M., & Martinez-Tomé, M. (2004). Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. *Journal of Agriculture and Food Chemistry, 52*, 1872–1881.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Schindler, M., Solar, S., & Sontag, G. (2005). Phenolic compounds in tomatoes. Natural variations and effect of gamma-irradiation. *European Food Research and Technology, 221*, 439–445.

WHO. (1999). *High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy, Report of a joint FAO/IAEA/WHO study group. WHO technical Report Series 890*. Geneva: World Health Organization.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A. R., Simonic, M., & Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry, 89*, 191–198.

Thomas, J., Senthilkumar, R. S., Kumar, R. R., Mandal, A. K. A., & Muraleedharan, N. (2008). Induction of γ-irradiation for decontamination and to increase the storage stability of black teas. *Food Chemistry, 106*, 180–184.