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RESEARCH PAPER

Effect of Olive Pruning Wood Extract on Lipid Oxidation in Sunflower Oil

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Abstract

P. González-Fuentes, M. Zuñiga, C. Olea-Azar, C. Loyola, and C. Folch-Cano. 2017. Effect of the Olive Pruning Wood Extract on Lipid Oxidation in Sunflower Oil. *Cien. Inv. Agr.* 44(3): 262-271. The production of high quality natural Antioxidants from olive pruning wood extracts and their application to prevent the oxidation of vegetable oils were evaluated. The production of antioxidants from branches of olive pruning wood (OPW) using Soxhlet extraction and ethanol was optimized, evaluating the use of dry and fresh OPW, with extraction of an initial mass of 1 and 3 g and extraction times of 2, 4 and 8 h. For the evaluation of the antioxidant properties of the OPW extracts (OPWEs), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical bleaching and ORAC-FL assays were performed. Additionally, the phenolic profile of the OPWEs was obtained by using HPLC-DAD, and the total phenolic composition was quantified by the Folin-Ciocalteu assay. The OPWE obtained from dry OPW using 1 g of initial mass showed the highest content of phenolic compounds and better antioxidant capacity compared to the OPWE obtained from fresh OPW. Both extracts showed the same profiles, but the OPWE obtained from dry OPW showed twice the concentrations of catechin (1.00 g 100 g⁻¹ OPWE) and oleuropein (12.59 g 100 g⁻¹ OPWE). Low concentrations of optimized OPWE showed activity against lipid oxidation of sunflower oil, measured by the peroxide value obtained by microwave and forced air oven heating. Under high temperature conditions and concentrations, the results suggest that OPWE may present the opposite effect.

Keywords: Antioxidants, catechin, lipid oxidation, oleuropein, olive wood pruning.

Introduction

Biomass from olive tree pruning is the result of the operation applied to the trees after harvesting the fruit to keep their productive and

reproductive functions balanced. In 2014, it was estimated that 29,600 tons of by-product from pruning olive trees was generated in Chile (Larrañaga and Osoreo, 2014), and these by-products from olive trees are often treated as waste and disposed of on the agricultural land by incineration; however, this practice is linked with environmental problems.

Studies of the chemical composition of the different parts of olive trees report the presence of phenolic compounds, mainly oleuropein (OL) and hydroxytyrosol (HT), not only in the olives but also in the leaves and wood (Pérez-Bonilla *et al.*, 2006; Luque de Castro and Japon-Lujan, 2006; Altiok *et al.*, 2008; Pérez-Bonilla *et al.*, 2011).

The leaves have oleuropeosides such as OL and verbascoside, flavonoids such as luteolin, luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, diosmetin, rutin, and catechin (C). Also present are simple phenolic compounds such as tyrosol, HT, vanillin, vanillic acid, and caffeic acid. Of all the compounds mentioned above, the most abundant has been shown to be OL (Pérez-Bonilla *et al.*, 2006; Altiok *et al.*, 2008).

The chemical composition of olive wood has also been studied, but these studies focused only on robust tree parts (trunk). The compounds reported are HT, tyrosol, OL, cyclooolivil, 7-deoxyloganic acid, and ligustroside. The most abundant compound is OL (Pérez-Bonilla *et al.*, 2006; Pérez-Bonilla *et al.*, 2011).

The phenolic compounds present in olive leaf extracts are known to be strong antioxidants. Pharmacologically, they present anti-inflammatory activity, lowering of cholesterol (they help regulate total serum cholesterol), and antiviral, anti-ischemic, neuro-protective, and cardio-protective properties. The HT extracted from olive leaves was recognized in 2011 as a compound with high added value by the European Food Safety Authority (EFSA) (Pérez-Bonilla *et al.*, 2006; Altiok *et al.*, 2008; Peralbo-Molina and Luque de Castro, 2013; Rahmanian *et al.*, 2015).

In recent years, interest in obtaining antioxidants from natural sources has increased because of the need to replace currently used synthetic homologs. Many of the synthetic antioxidants have been associated with the development of degenerative diseases (Pérez-Bonilla *et al.*, 2011; Rahmanian *et al.*, 2015).

Some applications of olive leaf extract have been evaluated in beef to control its microbial load, and olive leaf extracts are also used for the enrichment of table olives and have been added to vegetable oils to inhibit their oxidation (Kiritsakis *et al.*, 2010; Malheiro *et al.*, 2013).

The present work focuses on the evaluation of the production of a high-quality extract with natural antioxidants from branches from olive pruning (olive pruning wood: OPW) and the application of this extract to prevent oxidation of vegetable oils. For this purpose, different extraction conditions were tested, and the composition and antioxidant activity of the extracts were evaluated by measuring the total phenolic compounds, their phenolic profile by HPLC-DAD and the ORAC-FL assay, and the bleaching of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

Materials and methods

Plant material

The by-products of pruning Arbequina variety olive trees (*Olea Europaea* L.) were collected in early June 2015 after harvesting of the fruits from the olive tree farm. These by-products were brought to Arauco's cellulose plant in the Bio-Bio region (South Chile), UTM coordinates 5940650 N 726602 E DATUM WGS84 18.

The by-products of pruning olive were separated into leaves, branches and trunks. The branches had a diameter less than 5 cm. The branches, hereinafter olive pruning wood (OPW), were dry ground to a size smaller than 0.5 mm. A fraction of the sample was dried to a constant mass in a conventional oven at 40 ± 4 °C for 72 h.

Preparation of extracts

Solid-liquid extractions were performed continuously in a Soxhlet extractor in the dark

with 200 mL of ethanol. The use of fresh and dried OPW in the extractions was evaluated, with initial masses of 1 g and 3 g of OPW and extraction times of 2, 4, and 8 h. To determine the yield of each extraction, the extracts were concentrated to dryness. The extraction yields were expressed as dry mass percentage of extract in 100 g of OPW.

Total phenolic (TP) compounds by the Folin-Ciocalteu assay

TP analyses were carried out on a Synergy HT multidetection microplate reader from Bio-Tek Instruments, Inc. (Winooski, USA), using 96-well polystyrene microplates purchased from Nunc (Denmark). To each cell, 200 μ L of Folin-Ciocalteu reagent, 15 μ L of OPW extract (OPWE), 40 μ L of Na_2CO_3 at 20% (m/v), and 45 μ L of distilled water were added. The samples were incubated for 30 min at 37 °C in a Synergy HT, Bio-Tek microplate reader and measured at 725 nm (Singleton *et al.*, 1999). The process was controlled by the Gen5 software. The phenolic content was expressed as mg equivalent of gallic acid per 100 g of OPWE.

DPPH radical bleaching

The ability of phenolic compounds to scavenge DPPH radicals was determined according to the method of Bendary *et al.* (2013). Five aliquots of each OPWE (from 20 μ L to 60 μ L) were added to a 1.0 mM DPPH ethanolic solution. The reaction mixture was shaken vigorously and kept in the dark for 15 min. The absorbance, registering the decrease of the DPPH concentration, was measured at 517 nm against a blank solution. The results were expressed as the IC_{50} kinetic parameter (concentration of antioxidant necessary to decrease the initial DPPH radical concentration to 50%). The bleaching percentages were calculated by

$$\% \text{ bleaching DPPH} = \frac{\text{ABS}_{\text{DPPH}} - \text{ABS}_S}{\text{ABS}_{\text{DPPH}} A}$$

where

ABS_S : absorbance of the OPWE sample.

ABS_{DPPH} : DPPH absorbance.

Absorption capacity of oxygen radicals-fluorescein (ORAC-FL)

ORAC analyses were carried out on a Synergy HT multidetection microplate reader from Bio-Tek Instruments, Inc. (Winooski, USA), using 96-well polystyrene microplates purchased from Nunc (Denmark).

Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen5® software. The oxygen radical absorbance capacity was determined as described by Folch-Cano *et al.* (2010).

The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 μ L of FL (150 μ L; 40 nM, final concentration) and OPWE (25 μ L) solutions, which were placed in the wells of the microplate. The mixture was pre-incubated for 15 min at 37 °C before rapidly adding the AAPH solution (2,2'-azobis(2-methylpropionamide) dihydrochloride), 25 μ L, to an 18 mM final concentration using a multichannel pipette. The microplate was immediately placed in the reader and automatically shaken prior to each reading.

The fluorescence was recorded every minute for 90 min. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution and five calibration solutions using Trolox as an antioxidant were also used in each assay. The inhibition capacity was expressed as Trolox

equivalents (n_{TX}), and the inhibition capacity was quantified by integration of the area under the curve (AUC). All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample.

The area under the fluorescence decay curve (AUC) was calculated by integrating the decay of the fluorescence, where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at a specified time. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using MATLAB software.

Characterization of antioxidants

Selected OPWEs were characterized using a PerkinElmer Flexar HPLC equipped with a UV-vis detector with a diode array. A Chromolith RP 18 (100 x 4.6 mm) high resolution column (Merck) was used. Chromatograms were obtained at 230, 280 and 340 nm, using the Chromera® software. The injection flow was 1 mL min⁻¹. The mobile phases for chromatographic analysis were A: acetonitrile and B: 5% aqueous orthophosphoric acid. A linear gradient was run from 2 to 12% A for 15 min. The gradient changed to 17% A in 25 min, to 58% A in 45 min, and finally to 70% A in 50 min.

OL, HT and C in OPWE were identified by comparing their retention times with those of the corresponding standards. OL and C in OPWE were quantified by means of calibration solutions.

Lipid oxidation

Lipid oxidation was evaluated by two methods. In the first study, to simulate conventional times used in microwave home cooking, 10 mL of sunflower oil was mixed with 10, 50 and 100 µL of the selected OPWE. The mixtures were placed in a microwave oven on high (1200 W) and heated for 1, 3, 5, 10, and 15 min (Malheiro *et al.*, 2013).

The peroxide value (PV) of the oil was evaluated according to AOAC (1997).

In the second study, 10 mL of sunflower oil was mixed with 10 and 100 µL of the selected OPWE (Taghvaei *et al.*, 2014). PV was evaluated according to AOAC 1997, and the mixture was then heated for 2, 4, 8, 16, 24, and 72 h in a forced air oven at a temperature of 55 °C.

Statistical analysis

The data were analyzed with infoStat statistical software. All assays were performed in triplicate. The results were expressed as the mean ± standard deviation.

The extraction yields obtained using 1 and 3 g of initial mass and fresh and dry OPW were analyzed using ANOVA. Tukey's HSD test was performed by comparing means for each extraction time.

The TP content (m_{EAG}) of OPWE was analyzed using ANOVA and Tukey's HSD test, executed by comparing all means obtained (regardless of whether OPWE was obtained from dry or fresh OPW or the extraction time). The results of antioxidant activity against DPPH free radicals (IC_{50}) and the ORAC-FL index (n_{TX}) of OPWE were analyzed in the same way as the TP content.

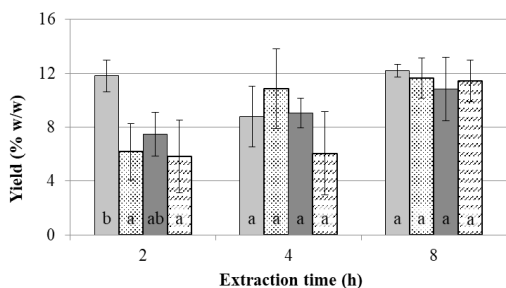
Normalized PVs obtained in the first and second studies of lipid oxidation were analyzed using ANOVA and Tukey's HSD test, performed by comparing means for each extraction time.

Tukey's HSD test considered statistically significant at $p < 0.05$.

Results and discussion

The by-products of olive pruning wood represented 39% (w/w) of OPW. Figure 1 shows the extraction yields obtained for fresh and dry OPW

(with humidities of 14.82 and 1.23%, respectively), with different initial mass (1 and 3 g) and different extraction times (2, 4 and 8 h). Through one-way ANOVA of the use of fresh or dry OPW, the results showed that the use of fresh or dry OPW did not affect the extraction significantly



□ 1g fresh OPW ▨ 3g fresh OPW ■ 1g dry OPW ▩ 3g dry OPW

Figure 1. Extraction yields obtained using 1 and 3 g of initial mass, fresh and dry OPW, and three extraction times. Yields were expressed as dry mass percentage of extract in 100 g of OPW. Different letters for the same extraction time show means with significant differences (Tukey's HSD, $p=0.05$).

($p=0.05$), with average yields of $10.24 \pm 2.72\%$ and $8.79 \pm 2.96\%$, respectively.

Through the same analysis, the use of an initial mass of 1 g ($10.14 \pm 2.27\%$) or 3 g ($8.66 \pm 3.40\%$) did not influence the extraction yield significantly, suggesting that the solvent reaches the maximum extraction capacity with 1 g of OPW under these conditions.

Extraction time positively influences the extraction yields in all the tested samples. Extraction times of 2 and 4 h showed no significant differences,

with average yields of $7.81 \pm 3.01\%$ and $8.68 \pm 2.77\%$, respectively. The extraction time of 8 h showed the significantly highest average yield ($11.39 \pm 1.72\%$).

Perez-Bonilla *et al.* (2006) worked with olive wood trunk and aqueous ethanol as the solvent to extract phenolic compounds. The highest extraction yield obtained by them was 14.5%, close to the maximum extraction yield achieved in the present study with an initial OPW of 1 g and an extraction time of 8 h.

Based on these results, the initial OPW extraction mass of 1 g was selected to continue the study and to evaluate the influence of extraction time and of the use of fresh or dry OPW on obtaining an OPWE with the highest TP and best antioxidant activity.

The OPWEs obtained from dry OPW with an extraction time of 2 and 4 h showed TP significantly higher than all OPWEs obtained from fresh OPW (Table 1). In OPWEs from fresh OPW, we found an increase of the TP value with respect to extraction time. The opposite effect was observed with the OPWEs obtained from dry OPW, with a significant decrease of TP after an extraction time of 4 h. This result may be attributed to the degradation of phenolic compounds after prolonged exposure to heat (during drying and extraction times of 2, 4 and 8 h).

Table 1. TP content (m_{EAG}), antioxidant activity against DPPH free radicals (IC_{50}) and ORAC-FL index (n_{TX}) of OPWE obtained from fresh and dry OPW at different extraction times.

OPW	Time (h)	m_{EAG}^{\dagger} (mg 100 g ⁻¹ OPWE)	IC_{50}^{\dagger} (μ g mL ⁻¹ OPWE)	n_{TX}^{\dagger} (mol 100 g ⁻¹ OPWE)
Fresh	2	60.15 \pm 7.57 ^a	137.04 \pm 3.7 ^{ab}	3032.72 \pm 619.36 ^a
Fresh	4	95.20 \pm 1.98 ^b	170.60 \pm 3.86 ^b	9106.53 \pm 971.00 ^{ab}
Fresh	8	135.25 \pm 7.14 ^c	143.95 \pm 6.73 ^{ab}	8094.95 \pm 1048.03 ^{ab}
Dry	2	178.80 \pm 15.13 ^d	93.30 \pm 10.07 ^a	19830.37 \pm 4318.26 ^{bc}
Dry	4	170.10 \pm 1.70 ^d	136.74 \pm 29.92 ^{ab}	40274.73 \pm 4253.16 ^d
Dry	8	125.97 \pm 9.94b ^{bc}	121.97 \pm 17.74 ^{ab}	24218.90 \pm 4784.32 ^c

[†]Results were expressed as dry mass averages of three replicates \pm standard deviation. Different letters in a column show means with significant differences (Tukey's HSD, $p=0.05$).

The maximum theoretical yield of the TP content determined by the Folin–Ciocalteu assay was 250.2 mg EAG 100 g⁻¹ dry weight of olive leaf extract (Hayes *et al.*, 2011; Rahmanian *et al.*, 2015), a value that is very close to the maximum obtained in the present study for OPWE.

In another study, Xie *et al.* (2015) obtained olive leaf extracts using aqueous ethanol as the solvent with ultrasound and temperature in the extraction process. The TP value obtained by the Folin–Ciocalteu assay of this extract was 24520 mg EAG 100 g⁻¹.

The previous reports correspond to olive leaf extracts, and the OPWE corresponds to another tree part that possibly has a different phenolic composition with different properties, not related to the amount of phenolics. In addition, several studies have shown that TP is strongly influenced by environmental conditions (Kiritsakis *et al.*, 2010; Bilgin and Şahin, 2013; Rahmanian *et al.*, 2015).

All the OPWEs showed antioxidant activity (Table 1). The OPWE obtained from dry OPW showed a trend toward better antioxidant activity against the DPPH radical than the OPWE obtained from fresh OPW, in agreement with results obtained by the TP assay. Extraction time did not significantly affect the antioxidant activity of the OPWEs measured by this assay.

Hayes *et al.* (2011) determined the antioxidant activity of olive leaf extracts through DPPH radical quenching, finding an IC₅₀ value of 28.6 µg mL⁻¹. In other research, Bouaziz *et al.* (2008) obtained an IC₅₀ value of 1.5 µg mL⁻¹ for olive leaf extracts by the same assay. In both studies, the olive leaf extracts showed lower values of IC₅₀, i.e., higher antioxidant activity, than the best OPWE obtained from dry OPW (93.3 µg mL⁻¹). The differences in the antioxidant activity by DPPH radical quenching are probably due to the different extraction methods used and the origins in the olive tree (Kiritsakis *et al.*, 2010; Bilgin and Şahin, 2013; Rahmanian *et al.*, 2015; Xie *et al.*, 2015).

The antioxidant capacity of all samples was obtained, and the antioxidant capacities are in agreement with the results shown by the TP assay. The ORAC values of fresh OPWE were significantly lower than the values obtained from dry OPW (Table 1). The extraction time did not significantly affect the antioxidant capacity of OPWE. The OPWE of dry OPW with a 4 h extraction time showed an ORAC-FL index significantly higher than all the other OPWEs.

No correlations between the antioxidant activity, evaluated through bleaching the DPPH stable radical, and the ORAC-FL index of the OPWE were found.

Hayes *et al.* (2011) determined the ORAC index for olive leaf extract, obtaining 17.43 g Tx 100 g⁻¹ dry leaf. This value is considerably lower than the value reported in the present study (Table 1).

According to the results shown, extracts with significantly improved antioxidant capacity were obtained from dry OPW with an extraction time of 4 h (40274.73 ± 4253.16 mol Tx 100 g⁻¹ of OPWE).

Three samples were selected for the HPLC assay: OPWE obtained from dry and fresh OPW, both with an extraction time of 4 h, and OPWE obtained from dry OPW with an extraction time of 2 h.

The profiles recorded for all the OPWEs tested by HPLC have a very similar pattern. The standards showed a retention time of 15.29 min for C and 30.49 min for OL.

Based on the retention times of the standards, all samples of OPWE measured by HPLC were determined to have C and OL. The absence of HT in the OPWE may be because that compound is more polar than OL and C. The extraction process used was more efficient for the extraction of less polar phenolic compounds.

The OPWE obtained from dry OPW with an extraction time of 4 h presented 1.00 g of C and 12.59 g of OL in 100 g of OPWE, while fresh OPWE registered 0.48 g of C and 6.35 g of OL in 100 g of OPWE. Thus, the OPWE obtained from dry OPW presented 2 times more OL and C than the fresh extract.

The results of TP, antioxidant activity, and ORAC index are consistent with the results obtained in the characterization of the OPWE obtained from dry OPW with an extraction time of 4 h and the OPWE obtained from fresh OPW with an extraction time of 4 h. Thus, the OPWE obtained from dry OPW with an extraction time of 4 h presented significantly higher TP, a significantly higher ORAC value, a lower IC_{50} , and significantly higher concentrations of C and OL with respect to the OPWE obtained from fresh OPWE with the same extraction time.

The OPWE obtained from dry OPW with 2 h of extraction time was concentrated four times more than the dry OPWE obtained from dry OPW with 4 h of extraction time and presented

3.90 g of C and 49.45 g of OL in 100 g of OPWE. These results showed that the concentrated OPWE presented 3.9 times more OL and C than the unconcentrated extract with the same initial concentration of these compounds, in agreement with the trend shown by the TP value.

The antioxidant effect of the olive leaf extracts may be due to their hydrogen donor ability to form stable free radicals, preventing oxidation and further propagation (Xie *et al.*, 2015). In addition, mixtures of different phenolic compounds in olive leaf extracts have higher antioxidant activity than the individual compounds because of the synergy that occurs between them (Ramos *et al.*, 2013). Similar phenomena may be occurring in the OPWE obtained.

For the lipid oxidation assay, two samples were selected: OPWE obtained from dry OPW and from fresh OPW, both with extraction times of 4 h. The first OPWE was considered the best due to the significantly higher phenolic content and better antioxidant activity measured by the different assays.

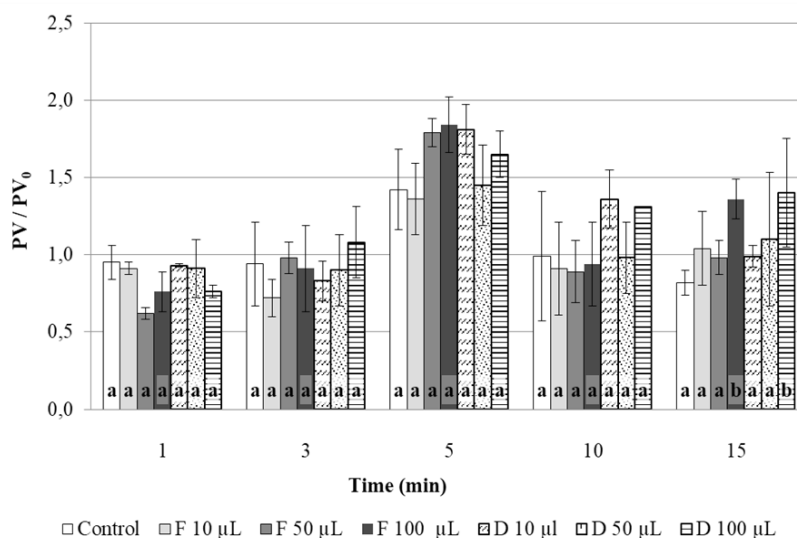


Figure 2. Normalized PVs of sunflower oil that is pure (control) and with increasing amounts of OPWE with an extraction time of 4 h. Microwave heating for 1, 3, 5, 10 and 15 min. D corresponds to extracts obtained from dry OPW and F to fresh OPW. The volumes represent the aliquot added; i.e., D 10 µL represents oil + 10 µL of OPWE obtained from dry OPW. Different letters for the same time show means with significant differences (Tukey's HSD, $p=0.05$).

To show variations more clearly, the results of the peroxide were normalized. Normalization was done by dividing the PV in time by the initial value achieved by each series.

The PVs obtained during the 15 min of microwave heating are presented in Figure 2. In the first minutes of microwave heating (3 min), there was a slight decrease in lipid oxidation of the oils with OPWE addition, but PV did not present significant differences. After 3 min of heating, the results showed a strong increase (5 min) of PV in all samples, including the control, reaching values significantly higher than at all other times. At 10 min of heating, the results showed a strong decrease in the PV of all samples, without significant differences between oils with and without addition of OPWE. At 15 min of heating, the oils with addition of 100 μL of OPWE from dry or fresh OPW reached PVs significantly higher than the controls and the oils with 10 and 50 μL of OPWE addition.

Malheiro *et al.* (2011) described the oxidation of oils by the change in the PV, and they reported that this index reaches a maximum due to the initial formation of hydroperoxides and then decreases due to volatilization of these compounds at the same time as when the secondary products (aldehydes and ketones) increase. This information agrees with the behavior described for oils evaluated in the present study.

Additionally, Cerretani *et al.* (2009) compared the effect of microwave heating for 15 min, for various types of olive oils, at medium power (720 W). They quantified the phenolic compounds by HPLC, finding a 40% loss of phenolic compounds at 6 minutes, and at 15 min, they reported a total decrease of the phenolic compounds, showing the temperature effect on the main phenolic compounds found in olive oil, which are probably the same as those contained in the OPWE. Based on this background, we propose that the phenolic compounds present in the OPWE are sensitive to temperature, losing part of their effectiveness

after 3 min of microwave heating. Furthermore, when a 100- μL aliquot of OPWE was added to the oils, there was an increase of PV at longer heating times (after 5 min).

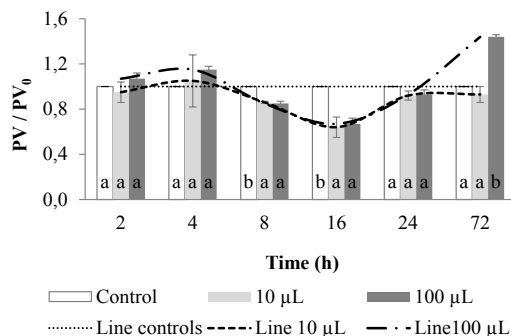


Figure 3. Normalized PVs obtained from sunflower oil that is pure (control) and with high (100 μL) and low (10 μL) OPWE amounts of dry OPWE added for 2, 4, 8, 16, 24 and 72 h in a forced air oven at 55 °C. Different letters for the same time show means with significant differences (Tukey's HSD, $p=0.05$).

In a second study (Figure 3), we evaluated the effect of OPWE concentration for longer heating times at 55 °C. Initially, the results showed a slight increase of lipid oxidation due to the larger amounts of OPWE added, followed by a decrease of PVs from 8 until 16 h for both concentrations, a time in which the oils with OPWE addition presented PVs significantly lower than the controls. Then, there was an increase of PV until 24 h, which remained constant for the oil with 10 μL of OPWE added. The oil with the higher amount of OPWE added (100 μL) showed a linear increase of PV and reached a value significantly higher than oils with 10 μL of OPWE and controls. We believe that this effect is related to the higher content of phenolic compounds that can generate more secondary compounds with a pro-oxidant effect due to the longer heating time of the oils, in agreement with Malheiro *et al.* (2011).

The main conclusions are as follows. OPWE obtained from 1 g of dry OPW using ethanol and an extraction time of 4 h yields higher TP content, antioxidant activity and antioxidant ability. The extract also has twice as much OL

and C than the OPWE obtained from fresh OPW in the same extraction time. The OPWE showed activity against lipid oxidation of sunflower oil in short periods of microwave heating. Under forced oxidation conditions and longer heating times, high OPWE concentrations may have the opposite effect.

Acknowledgments

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Resumen

P. González-Fuentes, M. Zuñiga, C. Olea-Azar, M. Loyola, y C. Folch-Cano. 2017. Efecto del extracto de madera de poda de olivo sobre la oxidación lipídica en aceite de girasol. Cien. Inv. Agr. 44(3): 262-271. Se evaluó la producción de antioxidantes naturales de alta calidad desde extractos de madera de poda de olivo y su aplicación para prevenir la oxidación de aceites vegetales. La extracción de antioxidantes desde las ramas de poda de olivo (OPW), fue optimizada usando extractor Soxhlet y etanol, evaluando el empleo de OPW seca y fresca, masas iniciales de extracción de 1 y 3 g y tiempos de extracción de 2, 4 y 8 h. Para la evaluación antioxidante de los extractos de OPW (OPWE) se realizaron los ensayos de Inhibición del radical DPPH y ORAC-FL. Adicionalmente, el perfil fenólico de los OPWE fue obtenido por HPLC-DAD y el contenido total de fenoles fue cuantificado a través del ensayo de Folin-Ciocalteu. El OPWE obtenido desde OPW seca y usando 1 g de masa inicial mostró el más alto contenido de compuestos fenólicos y la mejor capacidad antioxidante, comparado con los obtenidos desde OPW fresca. Ambos extractos mostraron perfiles similares, pero el OPWE obtenido de OPW seca mostro dos veces la concentración de catequina ($1.00 \text{ g } 100 \text{ g}^{-1}$ OPWE) y oleuropeína ($12.59 \text{ g } 100 \text{ g}^{-1}$ OPWE). Bajas concentraciones del OPWE optimizado mostraron actividad contra la oxidación lipídica del aceite de girasol, medida a través del índice de peróxidos durante el calentamiento en microondas y en estufa de aire forzado. En condiciones de mayores temperaturas y concentraciones, los resultados sugieren que el OPWE puede presentar el efecto contrario.

Palabras clave: Antioxidantes, madera de poda, oleuropeína, oxidación de lípidos.

References

- Altioek, E., D. Baycin, O. Bayraktar, and S. Ulku. 2008. Isolation of polyphenols from the extracts of olive leaves (*Olea europaea* L.) by adsorption on silk fibroin. *Sep Purif Technol.* 62:342–348.
- AOAC Association of official Analytical Chemistry. 1997. Official methods of analysis. Arlington, EEUU.
- Bendary, E., R. Francis, H. Ali, M. Sarwat, and S. Hady. 2013. Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *AOAS.* 58:173–181.
- Bilgin, M., and S. Şahin. 2013. Effects of geographical origin and extraction methods on total phenolic yield of olive tree (*Olea europaea*) leaves. *J Taiwan Inst Chem Eng.* 44:8–12.
- Bouaziz, M., I. Fki, H. Jemai, M. Ayadi, and S. Sayadi. 2008. Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. *Food Chem.* 108:253–262.
- Cerretani, L., A. Bendini, M. Rodriguez-Estrada, E. Vittadini, and E. Chiavaro. 2009. Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative

- stability indices and phenolic compounds. *Food Chem.* 115:1381–1388.
- Folch-Cano, C., C. Jullian, H. Speisky, and C. Olea-Azar. 2010. Antioxidant activity of inclusion complexes of tea catechins with β -cyclodextrins by ORAC assays. *Food Res Int.* 43:2039–2044.
- Hayes, J., P. Allen, N. Brunton, M. O'grady, and J. Kerry. 2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. *Food Chem.* 126:948–955.
- Kiritsakis, K., M. Kontominas, C. Kontogiorgis, D. Hadjipavlou-Litina, A. Moustakas, and A. Kiritsakis. 2010. Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. *J Am Oil Chem Soc.* 87:369–376.
- Larrañaga, P., and M. Osoreo. 2014. Catastro frutícola, principales resultados, Región de Valparaíso, julio 2014. Informe técnico ODEPA (Chile) y Ciren (Chile).
- Luque de Castro, M., and Japon-Lujan R. 2006. State of the art and trends in the analysis of oleuropein and derivatives. *Trends Anal. Chem.* 25:501–510.
- Malheiro, R., S. Casal, E. Ramalhosa, and J. Pereira. 2011. Microwave heating: a time saving technology or a way to induce vegetable oils oxidation? In: Grundas, S (ed) *Advances in Induction and Microwave Heating of Mineral and Organic Materials*, INTECH-Open 597–614.
- Malheiro, R., N. Rodrigues, G. Mancke, A. Bento, J. Pereira, and S. Casal. 2013. The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating. *Ind Crops Prod.* 44:37–43.
- Peralbo-Molina, A., and M. Luque de Castro. 2013. Potential of residues from the Mediterranean agriculture and agrifood industry. *Trends Food Sci Technol.* 32:16–24.
- Pérez-Bonilla, M., S. Salido, T. Van Beek, P. Linares, A. Sanchez, and J. Altarejos, M. Noguera, and A. Sanchez. 2006. Isolation and identification of radical scavengers in olive tree (*Olea europaea*) wood. *J. Chromatogr.* 1112:311–318.
- Pérez-Bonilla, M., S. Salido, T. Van Beek, P. Waard, P. Linares-Palomino, A. Sánchez, and J. Altarejos, 2011. Isolation of antioxidative secoiridoids from olive wood (*Olea europaea* L.) guided by on-line HPLC–DAD–radical scavenging detection. *Food Chem.* 124:36–41.
- Rahmanian, N., S. Jafaric, and T. Ahmed. 2015. Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. *Trends Food Sci Technol.* 42:150–172.
- Ramos, P., S. Santosc, Â. Guerra, O. Guerreiro, L. Felício, E. Jerónimo, A. Silvestre, C. Pascoal, and M. Duarte. 2013. Valorization of olive mill residues: Antioxidant and breast cancer antiproliferative activities of hydroxytyrosol-rich extracts derived from olive oil by-products. *Ind Crops Prod.* 46:359–368.
- Singleton, V., R. Orthofer, and R. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Meth. Enzymol.* 299:152–178.
- Taghvaei, M., S. Jafari, A. Mahoonak, A. Nikoo, N. Rahmanian, J. Hajitabar, and N. Meshginfar. 2014. The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. *LWT Food Sci Technol.* 56:124–130.
- Xie, P., L. Huang, C. Zhang, and Y. Zhang. 2015. Phenolic compositions, and antioxidant performance of olive leaf and fruit (*Olea europaea* L.) extracts and their structure–activity relationships. *J Funct Foods.* 16:460–471.