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Effect of polyphenols extracted from (*Olea europaea. L*) solid residues and leaves on the oxidative stability of a commercial olive oil

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ABSTRACT

In this study, polyphenols have been extracted from solid residues and leaves of olive tree (*Olea europaea L*) in order to assess this vegetable biomass by evaluation of their capacity as a natural antioxidants. The anti-oxidative activity was tested by addition of these extracts on commercial olive oil samples. The olive oil stability has been measured by accelerated oxidation under a simulated storage conditions (60°C/15 days). The change in the oxidation state was followed by measuring of the free acidity, peroxide value (PV) and the specific extinction at 270 nm. The assessment of enriched oils showed that polyphenols from olive by-products play a key role in reducing of oxidative deterioration of the oil. The results suggest the introduction of olive leaves and solid residues extracts in food preparation, both during storage or heating food, for better preservation.

Keywords: *Olive by-products, commercial olive oil, polyphenols, antioxidants, oxidative stability.*

1. INTRODUCTION

In the olive-growing Mediterranean countries, the extraction of olive oil at an industrial scale requires a huge amount of water and raw materials. This extraction process generates a large amount of by-products such as crude olive cake, solid residues, vegetation water, twigs and leaves (10% of the total weight of the olives), which are rich in lipids and other bioactive organic compounds [1-6]. These by-products are generally used for cattle feed or often removed in nature and they are considered as toxic residues. Their toxicity is mainly due to a higher COD (Chemical Oxygen Demand) [7], which makes these wastes as a recalcitrant compounds (different molecules that could be removed in soil, air and water when the disposal by-products were not treated) in the bio-degradation that generate both a soil pollution, air and water [8-11]. Moreover, the untreated disposal of olive wastes (liquid and solid forms) onto lands causing the inhibition of numerous microorganisms, a reduction in seed germination and the alteration of several soil characteristics such as porosity and humus concentration [12]. It also increases the average diameter of the soil aggregates, bulk density and slows down hydraulic conductivity [13]. The olive wastewater is also characterized by dark color due to chromophoric lignin related materials with different degrees of polymerization and a sharp characteristic odor [14]. Olive solid residues are also characterized by its phytotoxicity, hydrophobicity, salinity, low pH and polyphenols [15].

Despite their potential pollution, these by-products are proving a very rich source of phenolic compounds. Moreover, being natural antioxidants, these polyphenols are attracting more and more interest for the prevention of many diseases. They can be used in other applications with higher added value such as cosmetic, therapeutic and food industries mainly to stabilize vegetable oils and dispersed mediums (emulsions, foams, creams). Many studies demonstrated that numerous bioactive components such as oleuropein, hydroxytyrosol and biophenols were present in olive leaves [3].

In food industry, olive leaves can extend the shelf life of foods. Indeed, they were used to increase the oxidative stability of the eggs enriched with very very-long-chain n-3 fatty acids [16]. The protective effects of olive leaf extracts on protein and lipid oxidation of pork patties during refrigerated storage [17] and frozen storage have been studied too [18].

The olive leaves extract has a great potential as effective natural antioxidants and may have applications in the development of new functional healthy products. Several studies highlighted the beneficial effects of the addition of natural oxidants to vegetable oils [19]. Extracts of olive leaves are also revealed to be effective stabilizers in vegetable oils under oxidative induction [20, 21].

These bioactive compounds can be incorporated into edible films or food packaging materials to maximize their additional properties such as their antioxidant capacities [6, 22, 23].

Currently, scientific community as well as consumers have a tendency to avoid synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which are limited by Codex Alimentarius (FAO/WHO Food Standards, 2005) as well as by European Regulation [24, 25] and FDA Food Additive Status List [26], because they are suspected for toxic, allergenic and carcinogenic effects. Natural antioxidants being recognized by their efficiency and their substitution to the synthetic additives are highly used in the bio-industry.

The impact of the olive phenol components in the olive oil stability is well known. Therefore, in the present study, our objective was to assess the potential of the by-products of olive oil industry and the effect of adding leaves and solid residues extracts from Picholine olive tree variety to commercial olive oil. The thermal oxidation of the commercial olive oil enriched with olive leaves and solid residues extracts was time monitored by measuring free acidity, peroxide value and specific extinction.

2. EXPERIMENTAL SECTION

2.1. Olive raw materials. The plant materials used in this study consists of:

- The solid residues (SR) were obtained from olives tree (*Olea europaea L*) of the *Picholine* variety, pressed into a semi-automatic oil mill (Society (HBS) Agadir-Morocco).
- Olive leaves (OL) come from an old olive tree variety *Picholine* (Essaouira, Morocco).
- A Commercial Olive Oil or lampante olive oil is a commercial product that's furnished from a local market (Béthune, France) and produced and conditioned in Spain.

2.2. Extraction of polyphenols from olive by-products

Solid residues: An operation of lipids removal or "delipidation" was previously performed on the solid residues. It consists in mixing in a separator funnel of 100 g of solid residues with 100 mL of hexane as a solvent. This extraction lasts 10 min and repeated twice.

Subsequently, the extraction of phenolic compounds is carried out in a second separating funnel of capacity of 500 mL. A delipidated solid residues extract (100 mL) are mixed under stirring with 100 mL of diethyl ether followed by decanting. The extraction operation was repeated 4 times in order to recover the maximum amount of phenolic compounds. Evaporation of the solvent is carried out under vacuum at 40°C.

Leaves: To make easy the extraction of phenolic compounds from olive leaves, two operations of material pre-treatment were carried out such as drying (oven heated to 40°C) and electric grinding. A 30 g of olive leaves powder is mixed with 120 mL of ethyl ether. This mixture was transferred in an Erlenmeyer of capacity of 250 mL under magnetic stirring for 5 min. Filtration and vacuum evaporation were then conducted in order to recover the polyphenol-rich extract.

2.3. Total phenolic content (TPC). The polyphenol content of olive coproducts was determined using the Folin-Ciocalteu spectrophotometrically according to the Singleton method [26] using gallic acid as standard. In an alkaline medium, polyphenols reduce phosphomolybdic acid of Folin-Ciocalteu reagent; this reduction is reflected by the appearance of a dark blue colour. The colour produced (whose maximum absorption is between 725 and 765 nm) is proportional to the amount of polyphenols present in plant extracts [27]. Therefore, to 1 mL of each extract, 7 ml of distilled water was added and then 0.5 ml of Folin-Ciocalteu reagent and allowed to set one minute. It's then added 1 ml of saturated solution of sodium carbonate (Na_2CO_3), the blue colour start to appear. The absorbance is then carried out at 750 nm after incubation for 1 h in the dark using a spectrophotometer.

2.4. Enrichment of olive oil by extracts of olive by-products. Three samples were prepared from the same commercial olive oil with the addition of each extract:

- A control commercial olive oil (without addition): "c-COO"
- Commercial olive oil enriched with 100 ppm of polyphenols extracted from solid residue: "COO-PhSR"
- Commercial olive oil enriched with 100 ppm of polyphenols extracted from leaves "COO-PhL".

2.5. Accelerated oxidation: SCHAAL oven stability test. Oxidative stability, or storage life until development of rancidity, is an important factor in the processing and marketing of fats, oils, and fat-containing foods. Methods for determining oxidative stability are therefore essential, particularly where antioxidants are being evaluated for effectiveness in retarding rancidity in these products. One widely used method involves storage tests (SCHAAL test) in which oil is stored under controlled conditions and evaluated periodically for organoleptic and physicochemical changes.

Oil storage experiments were conducted in an oven according to the Schaal Oven test. Oils were stored in loosely sealed glass containers at 60°C [28]. At fixed intervals, oil samples were withdrawn, and all quality parameters were determined. This method has the advantage of being closer to the real storage conditions.

2.6. Physicochemical analysis of oil oxidative stability. To assess changes in the oxidation of enriched oils, three main parameters (acidity, peroxide value and extinction at 270 nm) were determined according to standard methods (ISO 660, ISO 3960, ISO 3656). The rancimat test is not considered here because the lack of an appropriate device.

a) Acidity (ISO 660: 2009(E)): The acidity, expressed as percentage of oleic acid, is the amount of free fatty acids in oil. Free acidity is an important factor in assessing the quality of oil, and it is widely used both as a classification criterion of vegetable oils and a factor that provides information on the alteration by hydrolysis by oil. Hydrolysis of triglycerides generates fatty acids, so their determination helps to have an idea about the progress of the oil degradation like oxidation.

b) Peroxide value (ISO 3960:2007): The peroxide value of an oil is the number of micrograms of the active peroxide contained in one gram of product. In acidic medium, hydroperoxides react with iodide ion, to generate iodine, which is titrated with a sodium thiosulfate solution in the presence of starch paste.

c) Specific Extinction at 270 nm (ISO 3656:2011): Determining the absorbance of oil sample at 270 nm allows the detection and the evaluation of secondary oxidation products. This method is based on spectrometric measurement of the absorbance of a sample in a specified field of wavelength in the UV range.

2.7. Statistical analysis of data. Data were expressed as means ± s.d. Tukey-Kramer multiple comparison tests were conducted to check differences between mean values pairs.

3. RESULTS SECTION

3.1. Total Phenolic Components of olive by-products. TPC was determined according a gallic acid calibration curve. The gallic acid calibration was fitted using linear regression. Therefore, TPC content was reported in Table 1. It was found that the content of

total phenol compounds in the leaves being about 6.32 ± 0.12 g/L, is almost twice of that of solid residues. Olive leaves are considered as a major source of natural bioactive antioxidants [20, 29-31]. We can note that in our case, the phenolic compounds content obtained either from olive leaves and solid residues are comparable and sometimes much higher than those found in literature [31, 32]. This variability seems to be related to the genotype characteristics of the variety of olive trees, agro-climatic factors, and cultural conditions.

3.4. Oxidative stability during incubation at 60°C. The effect of addition of 100 ppm of polyphenols extract from olive by-products on the oxidative stability of the olive oil at 60 ± 0.1°C was studied according to assessing some quality parameters.

3.4.1. Acidity index. The results of the free acidity of control and enriched oil samples as function of incubation time at 60°C are given in Table 2.

Table 2 indicates a time change (throughout the duration of storage) of the acidity index of both the control and the treated samples. The examination of the table 2 show that the analysis of analytical data by statistical procedure of Tukey-Kramer exhibits that variation of treatment by phenolic coumpond or additive at a given incubation time follow two tendencies.

- At initial time (0 days), analytical data exhibit a none significantly differences whatever the nature of additive;
- The treatment of oil samples with additive show a significantly differences on free acidity at 4, 11 and 15 days of storage.

Moreover, the comparison of the effect of storage time for a fixed additive treatment shows an increase of free acidity with significant differences.

It is noted that at the first incubation period, about 4 days, the acidity index remains constant. This can be explained by the fact that the oil initially contains intrinsic phenolic fraction molecules. In addition, stabilization of the treated oil is observed in terms of their acidity compared to the untreated oil (c-COO). This stability is due to the pronounced effect of the phenolic compounds contained in extracts of these olive co-products.

After two weeks of storage at 60°C, acidity index of samples recorded an increase of about 23%, 8% and 3% respectively for the untreated oil (c-COO), the enriched oil by polyphenols extracts from solid residues (COO-PhSR) and the enriched by polyphenols from leaves (COO-PhL) of the olive tree. The hydrolysis of triglycerides is favored at 60°C, which results in the increase of free fatty acids in both oils studied. However, this increase appears more in the control oil (c-COO) compared to those enriched by olive co-products extracts.

The results of acidity index change of oil samples “COO-PhSR and COO-PhL” being lower, highlights the oxidative stability provided by the antioxidant effect of the molecules contained in these by-products. This antioxidative effect is most remarkable when using leaves than solid residues. This trend has been mentioned by many studies on the oxidative stability of vegetable oils [1, 21, 33].

3.4.2. Peroxide value (PV). Oxidation and the formation of peroxides, occurs during oil extraction and processing and can continue after bottling and during storage. Peroxides are intermediate oxidation products of oil, which in presence of light or temperature lead to the formation of a complex mixture of volatile compounds (aldehydes, ketones, hydrocarbons, alcohols and esters) responsible for the deterioration of quality proprieties [34].

The peroxide value measured in the obtained oil samples based on the storage time and additive treatment is presented in Table 3.

With the exception of the initial conditions for the all oil samples, the peroxide value has also found to vary significantly according to both the treatment method and storage period. The increase of incubation period of oil samples allow to increase the contact time with the additive. Thereby it has a significant preservation effect which may be attributable to the nature of additive and seems to be more pronounced when leaves are used.

According to Table 3, the initial peroxide value of c-COO is about 22.5 meq O_2/kg. After 15 days of storage, this parameter reaches 36.17 meq O_2/kg. Furthermore, for oil enriched with polyphenols of solid residues (COO-PhSR), it is shown that the PV pass from 22.5 to 32.70 meq O_2/kg and for oil enriched with polyphenols of leaves extract (COO-PhL), this index rises from 22.5 to 27.17 meq O_2/kg.

Indeed, under the same operating conditions, the evolution of PV values of COO-PhL sample remains low compared both to "COO-PhSR" and “c-COO” samples (Table 3). According to the results of the PV, it is noted that the impact of by-product extracts is important on the oil oxidation. In fact, more PV is low, the better the oxidative stability of the oil during storage. Phenolic compounds of leaves exert a better effect on trapping the active oxygen by reducing primary and secondary compounds generated during oxidation.

The results of our study are comparable or even lower than the results obtained by [31, 35, 36], who found that the stability of the oils is well correlated with total phenols content and the presence of high levels of natural antioxidants, the most important being tocopherols. According to literature data, the effect of phenolic compounds of the leaves and seeds of the olive tree on the oxidative stability of different varieties of olive oil samples showed the same trends [1-3, 34, 37].

We notice that the values of the peroxide values (PV) of the present samples containing antioxidants are significantly lower than those reported by El Kateb [5] for different varieties of Tunisian olive leaves at only about 4 days of oxidative exposure and at the same concentration of oil enrichment.

3.4.3. Coefficient of specific extinction K270. After the formation of hydroperoxides in the early stages of oxidation, which are highly active, they quickly turn into secondary oxidation products especially unsaturated ketones and diketones that absorb light in the vicinity of 270 nm. Table 4 shows the evolution of specific extinction parameter.

The same trends were observed on the significant effect of operating conditions (storage time and nature of additive treatment) on the specific extinction E270 (Table 4) of oil tested oil. The impact of preservation of oil samples against the quality degradation can also significantly differ based on the additive treatment coupled to the time of storage. It exhibited a significant antioxidant activity when using leaves and solid residues as a source of phenolic compounds.

There is an increase in the coefficient of extinction during the storage period at 60°C for all tested oils. This change, reflecting the formation of secondary oxidation products depends on the nature of the added extract. After 2 weeks of incubation at 60°C, the coefficient K270 pass from 0.82 to 1.37; 0.82 to 1.17 and 0.82 to 1.05 respectively for the samples "c-COO", "COO-PhSR" and "COO-PhL" (Table 4). Indeed, oils enriched with phenolic compounds extracts show a resistance against oxidation. As for free fatty acids and peroxide value, the results of the specific extinction of oils enriched with phenolic compounds of the olive co-products show that these extracts increased stability against the oxidation of oils and reduce the formation of oxidation by-products compared with untreated oil (c-COO). Moreover, phenols from leaves (case of COO-PhL) seem to act significantly better than those from solid residues (COO-PhSR). Our results are in concordance with literature data mentioned [34-37].

Table 1. TPC of olive by-products

	Olive Leaves	Olive Solid residues
TPC (g/L)	6.32 ± 0.12	3.56 ± 0.23

Table 2. Change of acidity index of enriched oils as function of incubation time at 60°C. Values reported are the means ± sd of three replications.

Variation of treatment at a given incubation time →

Variation of incubation time at a given treatment ↓

Code		a	b	c	Comparison
	Incubation time (d) / treatment	c-COO	COO-PhSR	COO-PhL	
a'	0	3.653 ± 0.020	3.653 ± 0.020	3.653 ± 0.020	ab : NSD ac : NSD bc : NSD
b'	4	3.789 ± 0.047	3.657 ± 0.008	3.691 ± 0.016	ab : SD ac : SD bc : NSD
c'	11	4.118 ± 0.023	3.783 ± 0.007	3.726 ± 0.01	ab : SD ac : SD bc : SD
d'	15	4.467 ± 0.015	3.952 ± 0.013	3.778 ± 0.006	ab : SD ac : SD bc : SD
Comparison		a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : NSD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : SD a'c' : SD a'd' : SD b'c' : NSD b'd' : SD c'd' : SD	

NSD: Not Significantly Different; SD: Significantly Different. The analytical results were compared statistically at the significant level at $P < 0.05$. The letters (a, b, c, a',…) are used here only as a codes for the statistical purposes.

Table 3. Change of peroxide value as function of treatment of oil enrichment and incubation time at 60°C. Values reported are the means ± sd of three replications.

Variation of treatment at a given incubation time →

Variation of incubation time at a given treatment ↓

Code		a	b	c	Comparison
	Incubation time (d) / treatment	c-COO	COO-PhSR	COO-PhL	
a'	0	22.575 ± 0.323	22.575±0.323	22.575 ± 0.323	ab : NSD ac : NSD bc : NSD
b'	4	25.426 ± 0.242	21.664±0.070	19.352 ± 0.016	ab : SD ac : SD bc : SD
c'	11	33.561 ± 0.056	28.771±0.063	25.106 ± 0.112	ab : SD ac : SD bc : SD
d'	15	36.175 ± 0.237	32.717±0.062	27.171 ± 0.011	ab : SD ac : SD bc : SD
Comparison		a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	

NSD: Not Significantly Different; SD: Significantly Different. The analytical results were compared statistically at the significant level at $P < 0.05$. The letters (a, b, c, a',…) are used here only as a codes for the statistical purposes.

Table 4. Change of specific extinction parameter E270 as function of treatment of oil enrichment and incubation time at 60°C. Values reported are the means ± sd of three replications.

Variation of treatment at a given incubation time →

Variation of incubation time at a given treatment ↓

Code		a	b	c	Comparison
	Incubation time (d) / treatment	c-COO	COO-PhSR	COO-PhL	
a'	0	0.823 ± 0.003	0.823 ± 0.003	0.823 ± 0.003	ab : NSD ac : NSD bc : NSD
b'	4	0.982 ± 0.004	0.869 ± 0.005	0.832 ± 0.004	ab : SD ac : SD bc : SD
c'	11	1.261 ± 0.005	1.132 ± 0.006	0.987 ± 0.003	ab : SD ac : SD bc : SD
d'	15	1.378 ± 0.008	1.168 ± 0.006	1.053 ± 0.009	ab : SD ac : SD bc : SD
Comparison		a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : SD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	a'b' : NSD a'c' : SD a'd' : SD b'c' : SD b'd' : SD c'd' : SD	

NSD: Not Significantly Different; SD: Significantly Different.
The analytical results were compared statistically at the significant level at $P < 0.05$.
The letters (a, b, c, a',…) are used here only as a codes for the statistical purposes.

4. CONCLUSIONS

In our present study, we conclude that under forced oxidation of oils, polyphenols extracted from the leaves and solid residues of the Picholine olive tree variety are regarded as effective antioxidants for these processed oils. At a concentration of about 100 ppm, polyphenols have conferred resistance against the oxidative stress during storage in an oven at 60°C. This behavior is revealed by the low values of the acidity index, peroxide value and extinction coefficient of the enriched oil samples compared with the control one. The use of by-products of olive oil industry has a double advantage. On the one hand, being natural compounds can substitute synthetic additives, which are involved in health risks. Furthermore, the valorization of these co-products is not only a pathway against pollution but also it is the contribution to a green approach in producing countries of olive oil. This study is focused in part to evaluate mainly the phenolic fraction but there remains another cellulosic fraction and an oil fraction to be treated that could be developed as substrates for other processes (biodiesel, fermentations).

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