Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activities of Algerian pomace olive oil


Biotechnology of Bioactive Molecules and Cellular Physiopathology Laboratory (LBMBPC), Batna -1- University, Algeria
Food Science Laboratory (LSA), Batna -1- University, Algeria

Abstract

The present study was aimed to investigate the effect of extraction solvent on oil yield, phenolic, flavonoid content and antioxidant activities. For this purpose, two-phases pomace olive were subjected to solvent extraction using solvents of different polarities (Acetone, Hexane, methanol, Petroleum ether and mixture of Chloroform/Methanol). The phenolic compound in pomace olive oil were extracted by methanol-water. The Folin-Ciocalteu and Aluminium trichloride (AlCl3) method was employed to calculate the total phenolic and flavonoid content respectively, while antioxidant capacity was assessed with DPPH, β-carotene/linoleic acid and FRAP. The oil content of pomace olive obtained after extraction with solvents of different polarities was in the range of 7.55 - 16.92 g/100 g. The best oil yield (16.92%) was obtained with the mixture Chloroform/Methanol 25/75. POO8 (extracted with pure Methanol) has the highest content of total phenolic and flavonoid which was 136.78 mg GAE/100g oil and 27.66 mg QE/100g oil, respectively. On the other hand, POO7 (extracted with mixture Chloroform/ Methanol 25/75) exhibited the highest radical DPPH scavenging power (71.40%), and POO5 (extracted with mixture Chloroform/Methanol 75/25) presented the highest bleaching rate of β-carotene (83.84%). High positive significant correlation (r=0.880, p<0.01) was found between total phenolic content and FRAP. However, no significant correlation was found between total flavonoid content and β-carotene bleaching. The results obtained demonstrated that pomace olive oil could have to be used as natural a potential source of bioactive compounds (antioxidants).

Introduction

Olea genus represents about 35 species comprising evergreen shrubs and trees in which Olea europaea L. is mostly grown throughout the world for the production of oil (Pervez et al., 2013) and table olives (Ahmad-Qasem et al., 2013). Olive oil possesses excellent nutritional, sensory and functional properties and is an agricultural product with major economic importance in the Mediterranean area. In fact, Algeria is a rich country in olive oil. In 2014, it was classified as the 8th in the world (International Olive Council, 2014). Virgin olive oil is particularly appreciated for its high content in healthy constituents, such as mono-unsaturated fatty acids and phenolic compounds (Pervez et al., 2013).

Nowadays, the olive oil industry generates a great environmental impact due to the production of high polluting residues (Baeta-Hall et al., 2005). Several studies have stated the negative effects of these forms of waste on soil’s microbial populations, aquatic ecosystems (DellaGreca et al., 2001) and even on the air (Rana et al., 2003). Olive pomace is the solid waste whose production can reach up to 30% of olive oil manufacturing, depending on the milling process, after oil extraction. It still retains a certain quantity of olive oil and mainly consists of vegetable water and pieces of skin, pulp and pit of the olive fruit. Olive pomace is usually used as natural fertilizers, combustible biomass and additives in animal feeding (Abu-Qudais, 1996; Khrisha et al., 1999; Pagnanelli et al., 2003; Meziane et al., 2009) and in the production of chemical compounds as soil conditioner and activated carbon (Melloul et al., 1998; Baçaoui et al., 2001; Montané et al., 2002).

In many Mediterranean countries, olive pomace is also used for the solvent extraction of residual oil. This grade of olive oil, named crude olive pomace oil, is often used in soap making, because of the high content of unsaponifiable matters that it contains. It is also commercialized for human consumption after refined and blended with a proper amount of virgin olive oil. Oil thus obtained is classified as “olive-pomace oil”. However, the extraction of this
oil, as one knows, is affected by many independent variables related to the extraction process (Meziane et al., 2008, 2013). In the past decades, several researchers have studied the oil yield of solid olive residues from different milling processes. Hexane (Salem, 1972), acidic hexane (Kniesielak et al., 1991) and the acetone-trichloroethylene (75-25%) mixture (Moussaoui and Youyou, 2006) were successfully used for efficient oil extraction from olive pomace.

Until now, only a few papers in the literature have focused on the rich content and high-added value compounds that can be extracted from pomace olive oil. Pomace olive oil provide a rich source of natural antioxidants. These include a diversity of phenolic compounds which may act, by different mechanisms, as an effective defence system against free radical attacks. Extraction is a very important phase in the evaluation, isolation and recovery of phenolic compounds; many authors have investigated different phenolics extraction techniques from different matrices. Boudissa and Kadi (2013) have studied the transfer of the phenolic compounds from olive mill wastewater to oil extracted under microwave from olive cake. Refined olive oil and olive-pomace oil were also enriched with olive leaf phenolic compounds in order to enhance its quality and bring it closer to virgin olive oil (Bouaziz et al., 2010).

Therefore, in the present study, we aimed to optimize the extraction of oil from pomace olive (obtained from 2-phases system) using the easily obtainable solvent with different polarities. Also, the influence of this extraction parameter on total phenolic, flavonoid contents and antioxidant activity was also investigated.

Materials and Methods

Plant material

The raw material used in this work was olive pomace from two-phases centrifugation separation process for obtaining olive oil, provided by an oil factory located in Ain Touta area (Batna, Algeria). The pomace was collected just after the pressing operation. The initial moisture content was determined by drying in a vacuum chamber at 70°C until reaching constant weight (International Olive Council, 2006). After cooling, the olive pomace was immediately packaged in plastic boxes and stored at 4°C.

Reactants

The chemical reactants were used as well as their purity so, ascorbic acid (Vitamin C), hexane and petroleum ether were purchased from “Biochem-Chemopharma”. Acetone, aluminum chloride, BHA (butylated hydroxyanisol), β-carotene, chloroform, DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric chloride, Folin-Ciocalteu’s reagent, gallic acid, linoleic acid, methanol, potassium ferricyanide, quercetin, sodium carbonate, trichloroacetic acid, Tween 40 were purchased from “Sigma-Aldrich”. All Chemicals and reagents used in this work were of analytical grade.

Extraction oil

The olive pomace was defatted by extraction with polar organic solvents (Acetone and methanol), non-polar organic solvents (Hexane, Petroleum ether and Chloroform) and mixture of polar and non-polar organic solvents (Chloroform/Methanol with the following proportion respectively: 75-25, 50-50, 25-75%). The oil extraction was carried out by “Soxhlet” method for the determination of fat in dried solid foods (Mandana et al., 2012) with slight modifications. 20 g of pomace olive (Initial water content is 63 ± 0.36%) was put into cellulose extraction thimbles which covered with cotton and then transferred into a Soxhlet apparatus “Gerhardt Soxtherm 2000”. 150 ml of different extraction solvents was added to each flask, which was connected to the extractor. Each extraction (one cycle) was performed in triplicate during 3 hours. The extraction temperatures of the different solvents in use are: 150°C (Petroleum ether), 180°C (Acetone and Hexane) and 190°C (Chloroform and Methanol). After extraction was completed, the excess of solvent was eliminated by drying at 40°C to constant weight.

Preparation of the methanol extracts

The liquid/liquid extraction was performed according to the procedure described by Ollivier et al. (2004). 1 g of olive oil was weighed into a centrifuge tube, to which 1 ml of methanol/water (80/20, v/v) was added. The mixture was stirred for 10 min in a vortex apparatus, and the tube was centrifuged at 3800 rpm for 15 min. The methanol layer was then separated and the extraction repeated twice. The methanolic extracts were combined to be used for colorimetric determination of total phenols and flavonoids.

Determination of total phenolic content (TPC)

Total phenolic content of the methanol extracts was determined by employing the method given in the literature (Ollivier et al., 2004) involving Folin–Ciocalteu reagent and gallic acid as standard. 0.5 ml of methanolic extract solution was added to a test tube.
5 ml distilled water and 1 ml Folin–Ciocalteu reagent was added and test tube was shaken vigorously. After 4 min, a 0.8 ml of Na$_2$CO$_3$ (7.5%) solution was added and the mixture was allowed to stand for 2 h by intermittent shaking. Absorbance was measured at 640 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph:

$$Absorbance = 0.006 \text{gallic acid (mg)} - 0.021 \quad (R^2=0.969)$$

**Determination of total flavonoid content (TFC)**

Total flavonoid content was determined using the method as adapted by Bahorun et al. (1996). Briefly, 1 ml of 2% (AlCl$_3$) in methanol was mixed with the same volume of the methanolic extracts. Absorption readings at 430 nm were taken after 10 min against a blank sample consisting of 1 ml extract solution with 1 ml methanol without AlCl$_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

$$Absorbance = 0.026 \text{quercetin (mg)} - 0.018 \quad (R^2=0.992)$$

**Determination of antioxidant activity**

**DPPH free radical-scavenging assay**

The free radical scavenging activity was determined spectrophotometrically by the DPPH assay (Sanchez-Moreno, 2002). Briefly, 50 µl of sample methanolic solution was initiated by the addition of 1.95 ml of DPPH (0.025 mg/ml) prepared in methanol. After thirty minutes, the absorbance was measured at 515 nm. Methanol (80%) was used as a control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following equation:

$$\frac{DPPH \text{ radical scavenging effect (µg/g)}}{A_{120}(\text{Sample}) - A_{120}(\text{Control}) \times 100}$$

Where: $A_{120}$ is the initial concentration of the DPPH and $A_{Sample}$ is the absorbance of the remaining concentration of DPPH in the presence of the extract and positive controls. Gallic acid, BHA, quercetin and vitamin C were used as antioxidant standard for comparison of the activity.

**β-carotene/linoleic acid bleaching assay**

In this method a model system of b-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant activity. The free linoleic acid radical formed upon the abstraction of a hydrogen atom from one of its methylene groups attacked the β-carotene molecule, which lost the double bonds and therefore, its characteristic orange color (Juntachote and Berghofer, 2005).

The total antioxidant activity was evaluated using β-carotene-linoleic acid test system (Kulisic et al., 2004; Gursoy et al., 2009) with a little modification. Briefly, β-Carotene (2 mg) in 4 ml of chloroform was added to 25 µl of distilled water saturated with oxygen was added by vigorous shaking to form emulsion A. 2.5 ml of this mixture were transferred into 0.5 ml of the samples. A control negative (without antioxidant) consisting of 0.5 ml of methanol and 2.5 ml of emulsion A was prepared. A second emulsion (B) consisting of 25 µl of linoleic acid, 200 mg of Tween 40 and 100 ml of distilled water saturated with oxygen was also prepared. Methanol (0.5 ml), to which 2.5 ml of emulsion B was added, was used to zero the spectrophotometer. Absorbance was measured at 0, 30, 60, 90 and 120 min on a Shimadzu UV-120-01 spectrophotometer at 490 nm. All determinations were performed in triplicate.

Antioxidative activities of the oils were compared with those of BHA (0-100 µg/ml), gallic acid (0-500 µg/ml), quercetin (0-100 µg/ml), vitamin C (0-500 µg/ml), and all results were expressed as a microgram standard equivalent antioxidant capacity per gram of oil (µg SEAC/g oil).

The antioxidant activity (percentage inhibition) of β-carotene was calculated according to the following equation (Bourkhiss et al., 2010):

$$\text{AA (Inhibition %)} = \frac{A_{t=0}(\text{Sample}) - A_{t=120}(\text{Control})}{A_{t=0}(\text{Control}) - A_{t=120}(\text{Sample})} \times 100$$

Ferric Reducing Power Assay (FRAP)

The reducing power was determined according to the method of (Gulçin, 2006). 1 ml of each extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added. 2.5 ml of this mixture was added to 2 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank. Gallic acid, BHA, quercetin and vitamin C were
used as antioxidant standard for comparison of the activity. Increased absorbance of the reaction mixture indicated an increase of reduction capability.

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± standard deviation and analyzed by SPSS statistical software (Version 20.0. Armonk, NY: IBM Corp.). The data obtained are treated statistically by analysis of variances, multiple comparisons of Duncan test and p<0.05 was regarded as significant. Pearson’s correlation coefficients (r) between TPC, TFC total of the pomace olive oil and antioxidant activity calculated values in each antioxidant assay were determined.

Results and Discussion

Oil yield

The oil yield of different pomace olive coming from two-phases system mill are shown in Table 1. Results showed that the lowest yield was obtained with POO1 (7.55 ± 0.57%), POO3 (9.62 ± 0.64%) and POO4 (10.72 ± 0.70%). Whereas, the highest yield was obtained with the POO7 (16.92 ± 0.57%), followed by POO6 (15.56 ± 0.54%), POO8 (14.86 ± 0.34%), POO5 (14.14 ± 0.19%) and POO2 (13.88 ± 0.13%). This observation was in agreement with Ferhat et al. (2014), who reported that the highest oil yield for pomace olive coming from three-phases and hydraulic press mill with the same mixture “Chloroform/Methanol 25-75%” were 15.91 ± 0.13% and 12.59 ± 0.16% respectively (g/100 g dry pomace olive). These results showed that solvents with different polarity were effective for oil extraction. It should be noted that, because of polarity differences between solvents, the solubility of the solute into the solvent was expected to be different. Petroleum ether, hexane, chloroform, acetone and methanol were arranged in the order of increasing polarity (Sadek, 2002).

Total phenolic and total flavonoid contents

Among the solvents used, the pomace olive oil extracted with pure acetone, mixtures of Chloroform/ Methanol and pure methanol lead to maximum TPC (POO1, POO5, POO6, POO7 and POO8), which showed significant differences between them (p<0.05). Further, Dermeche et al. (2013), reported a higher content of total phenolic in the Algerian pomace olive from two-phases and three-phases system which were 0.4-2.43% and 0.2-1.15%, respectively. these latter, were in agreement with TPC for Moroccan olive mill waste water reported by Leouifoudi et al., (2014), which ranged from 5.27 ± 0.17 to 10.1 ± 0.01 g GAE/L. However, the other oils extracted by hexane, petroleum ether and chloroform showed the lowest value of TPC (POO2, POO3 and POO4). These results were in agreement with those of some Italian pomace olive oil which ranged between 207.4 ± 10.5 and 210 ± 8.2 mg Oleuropein equivalent/Kg oil (Cioffi et al., 2010).

This remarkable difference of results could be explained by several factors such as geographic origin, oil extraction process and solvents, pomace storage conditions. Also, these results showed the TPC was mainly dependent on the solvent used for extracting pomace oil. Flavonoids are natural phenolic compounds and well know antioxidants. In various studies, antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids (Cakir et al., 2003). In the case of flavonoids, the highest content was observed in POO8 (27.66 ± 0.69 mg QE/100g oil), whereas POO4 has the lowest amount (1.05 ± 0.07 mg QE/100 g oil). These results were slightly lower than those for Tunisian pomace olive oil “Two-phases system” (8 ± 0.12 - 14.5 ± 0.19 mg Catechin/Kg) reported by Ammar et al., (2014). For the effectiveness of extracting technique, the results showed that “TFC” was better when pomace oil extraction was done with methanol (Sultana et al., 2009).

Antioxidant activity

The DPPH method is commonly used for
determination of free radical scavenging activity of antioxidant. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a very stable organic free radical and presents the ability of accepting an electron or hydrogen radical (Abozed et al., 2014). As expected, a higher percent of DPPH scavenging is correlated to a higher antioxidant activity (Liu et al., 2008).

The DPPH scavenging ability of the pomace olive oils was reported as the percent of DPPH scavenged (%DPPH scavenging). Significant (p<0.05) differences of DPPH scavenging capacities among oils were shown in Table 2.

Antioxidant capacity values of these oils ranged from 52.98 ± 0.15 to 71.40 ± 1.2 %. A maximum scavenging activity was offered by POO7, followed by POO8, POO4, POO3, POO6, POO2, POO1 and POO5. In addition, these results indicated that, the solvent systems used to extract oils affected DPPH scavenging percent, and suggested that the pomace oil, extracted with mixture “Chloroform/Methanol” (25/75) was given the highest scavenging activity on DPPH radicals.

In comparative title, our results were very close to the DPPH scavenging ability of some Italian extra virgin olive oil reported by Del Monaco et al. (2015), which ranged from 70 ± 1.03 to 87 ± 1.45%. However, Turkish olive oil (Halhali Varity) presented a lower DPPH scavenging capacity with 52 ± 0.01 to 58 ± 0.03% (Kesen et al., 2013). The DPPH radical scavenging activity of these oils was also expressed as standard equivalent antioxidant capacity (Table 3): gallic acid (0-80 mg/ml), BHA (0-200 mg/ml), quercetin (0-200 mg/ml) and vitamin C (0-400 mg/ml) were used as the standard for the calibration curve.

With a 1.26 mg/g oil for total phenolic content, POO7 showed the highest level of total antioxidant capacity (71.40 ± 1.24%) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 11.56, 3.72, 8.84 and 33.72 mg/g oil respectively. However, with a 0.85 mg/g oil for total phenolic content, POO5 showed the lowest level of total antioxidant capacity (52.98 ± 0.15 %) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 8.71, 2.77, 6.31 and 25.61 mg/g oil respectively.

In the Beta-Carotene Bleaching (BCB) assay, the oxidation of linoleic acid produces free radicals due to the removing of hydrogen atom from diallylic methylene groups of linoleic acid (Dapkevicius et al., 1998). The highly unsaturated β-carotene then will be oxidized by the generated free radical. Degradation of the orange colored chromophore of β-carotene could be monitored spectrophotometrically. However, the presence of antioxidant constituents could prevent the bleaching of β-carotene because of their ability to neutralize the free radicals (Kulisic et al., 2004).

Using the β-carotene/linoleic acid method, pomace olive oil showed different patterns of antioxidant activities. As can be seen from Table 3, the most active oil was POO6 and POO5 which activity potentials were close together 84.05 ± 0.72 and 83.84 ± 0.80%. These values were followed by those of pomace oils, which were extracted with acetone and the mixture “Chloroform/Methanol”: POO4, POO1, POO7 and POO8 respectively (83.5 ± 0.47, 83.48 ± 0.82, 83.43 ± 0.66 and 82.13 ± 0.44). The weakest activity was exhibited by POO2 and POO3 (77.59 ± 1.31, 76.79 ± 0.7%).

Bleaching of β-carotene is a free-radical-mediated phenomenon resulting from the hydroperoxides formed by air oxidation. In the absence of antioxidants, the β-carotene molecules lose their double bonds by oxidation as well as the
characteristic orange color, which can be monitored spectrophotometrically. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the free radicals formed in the system (Azaizeh et al., 2012). These finding results were highest than the antioxidant activity for Spanish olive oils which ranged from 13.2 ± 1.1 to 40.4 ± 2.9% (Gorinstein et al., 2003). With a 1.06 mg/g oil for total phenolic content, POO6 showed the highest antioxidant activity with bleaching of β-carotene (84.05 ± 0.72%) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 4.45, 72.96, 5.18 and 55.40 µg/g oil respectively. However, with a 0.19 mg/g oil for total phenolic content, POO3 showed the lowest antioxidant activity with bleaching of β-carotene (76.79 ± 0.74%) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 3.89, 66.66, 4.67 and 50.76 µg/g oil respectively.

In the Ferric Reducing Antioxidant Power (FRAP) assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe³⁺/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl’s Prussian blue at 700 nm can monitor the Fe²⁺ concentration (Ferriera et al., 2007). The reducing power (Table 4) of these oils varied significantly (p<0.05) and considerably from 0.172 to 0.352 g GAE/100 g oil. These results were lowest than the ferric reducing antioxidant power for Spanish extra virgin olive oil which was 5.75 ± 0.11 % and also, Spanish extra virgin argan oil which ranged from 13.32 ± 2.49 to 20.20 ± 4.51% (Seiquer et al., 2015).

With a 1.37 mg/g oil for total phenolic content, POO8 showed the better level ferric reducing antioxidant power with a BHA, gallic acid, quercetin and vitamin C equivalents radical reducer values of 0.908, 0.352, 0.686 and 1.897g/100 g oil respectively. Even so, with a 0.19 mg/g oil for total phenolic content, POO3 showed the lowest level of ferric reducing antioxidant power with the same standard 0.458, 0.172, 0.349 and 0.998 g/100g oil respectively. Except FRAP test, the results of the present study showed that some oils had higher antioxidant capacity than the others. However, the total phenolic and flavonoid contents of the oils with the lowest antioxidant capacity were higher than the oils with best antioxidant capacity. This suggests

Table 3. Beta-Carotene Bleaching expressed as gallic acid, BHA, quercetin and vitamin C equivalent antioxidant capacity (mg/g Oil) of pomace olive oils

<table>
<thead>
<tr>
<th>Beta-Carotene Bleaching (%)</th>
<th>GAEC</th>
<th>BHAEC</th>
<th>QEAC</th>
<th>VtCEAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>POO1</td>
<td>83.48 ± 0.82 ±c</td>
<td>72.47 ± 7.86</td>
<td>04.40 ± 0.65</td>
<td>05.14 ± 0.10</td>
</tr>
<tr>
<td>POO2</td>
<td>77.59 ± 1.31 ±c</td>
<td>67.35 ± 7.35</td>
<td>03.86 ± 0.47</td>
<td>04.73 ± 0.09</td>
</tr>
<tr>
<td>POO3</td>
<td>76.79 ± 0.74 ±e</td>
<td>66.66 ± 7.26</td>
<td>03.89 ± 0.46</td>
<td>04.67 ± 0.09</td>
</tr>
<tr>
<td>POO4</td>
<td>83.51 ± 0.47 ±c</td>
<td>72.49 ± 7.87</td>
<td>04.41 ± 0.65</td>
<td>05.14 ± 0.10</td>
</tr>
<tr>
<td>POO5</td>
<td>83.84 ± 0.80 ±e</td>
<td>72.49 ± 7.87</td>
<td>04.43 ± 0.66</td>
<td>05.10 ± 0.10</td>
</tr>
<tr>
<td>POO6</td>
<td>84.05 ± 0.72 ±e</td>
<td>72.96 ± 7.92</td>
<td>04.45 ± 0.66</td>
<td>05.10 ± 0.11</td>
</tr>
<tr>
<td>POO7</td>
<td>80.43 ± 0.05 ±c</td>
<td>72.42 ± 7.86</td>
<td>04.40 ± 0.66</td>
<td>05.13 ± 0.10</td>
</tr>
<tr>
<td>POO8</td>
<td>82.13 ± 0.44 ±c</td>
<td>71.39 ± 7.74</td>
<td>04.30 ± 0.64</td>
<td>05.04 ± 0.10</td>
</tr>
</tbody>
</table>

Note: The data are expressed as mean ± SD (n=3). Values with different letters in the same column are significant different at p<0.05.

Table 4. Ferric Reducing Antioxidant Power expressed as gallic acid, BHA, quercetin and vitamin C equivalent antioxidant capacity (g/100 g Oil) of different pomace olive oils

<table>
<thead>
<tr>
<th>Ferric Reducing Antioxidant Power expressed as</th>
<th>GA (g/100 g Oil)</th>
<th>BHA (g/100 g Oil)</th>
<th>Q (g/100 g Oil)</th>
<th>Vit C (g/100 g Oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POO1</td>
<td>0.301 ± 0.001 ±e</td>
<td>0.770 ± 0.003 ±e</td>
<td>0.590 ± 0.002 ±e</td>
<td>1.641 ± 0.005 ±e</td>
</tr>
<tr>
<td>POO2</td>
<td>0.188 ± 0.001 ±b</td>
<td>0.403 ± 0.003 ±b</td>
<td>0.375 ± 0.002 ±b</td>
<td>1.068 ± 0.006 ±b</td>
</tr>
<tr>
<td>POO3</td>
<td>0.172 ± 0.001 ±a</td>
<td>0.458 ± 0.002 ±a</td>
<td>0.349 ± 0.002 ±a</td>
<td>0.998 ± 0.004 ±a</td>
</tr>
<tr>
<td>POO4</td>
<td>0.212 ± 0.001 ±a</td>
<td>0.557 ± 0.002 ±a</td>
<td>0.423 ± 0.001 ±a</td>
<td>1.196 ± 0.003 ±a</td>
</tr>
<tr>
<td>POO5</td>
<td>0.258 ± 0.001 ±d</td>
<td>0.669 ± 0.003 ±d</td>
<td>0.506 ± 0.002 ±d</td>
<td>1.419 ± 0.006 ±d</td>
</tr>
<tr>
<td>POO6</td>
<td>0.301 ± 0.001 ±e</td>
<td>0.779 ± 0.003 ±e</td>
<td>0.589 ± 0.002 ±e</td>
<td>1.640 ± 0.004 ±e</td>
</tr>
<tr>
<td>POO7</td>
<td>0.337 ± 0.001 ±f</td>
<td>0.870 ± 0.003 ±f</td>
<td>0.658 ± 0.002 ±f</td>
<td>1.822 ± 0.006 ±f</td>
</tr>
<tr>
<td>POO8</td>
<td>0.352 ± 0.001 ±g</td>
<td>0.908 ± 0.003 ±g</td>
<td>0.686 ± 0.002 ±g</td>
<td>1.807 ± 0.006 ±g</td>
</tr>
</tbody>
</table>

Note: The data are expressed as mean ± SD (n=3). Values with different letters in the same column are significant different at p<0.05.
that the antioxidant activity depends probably on the quality of the existing phenolics or there are some other active components in the oils with best antioxidant capacity, other than phenolic compounds, which may contribute to their antioxidant capacity.

Pearson correlation analysis

It was important to examine the correlation between the oil yield, the content of the main antioxidant compounds (TPC) and the total antioxidant capacity of the studied oils (Table 5). With some exception, a high positive correlation was found between oil yield and TPC. On the other hand, the TFC are negatively correlated with oil yield. Some authors claim that there is no correlation between the TPC and the radical scavenging capacity (Yu et al., 2002), which was in accordance with our results (r=0.122). But, a very high significant correlation was found between antioxidant capacity determined by β-carotene (r=0.725, p < 0.01) and FRAP (r=0.994, p < 0.01). However, no significant relationship was observed between TFC and antioxidant activity as determinate by DPPH (r= -0.046). High significant positive correlation was found between TPC and antioxidant activity (olive oil case). These results were in agreement with those reported by Vielioglu et al. (1998).

Conclusion

This study, presented the valorization of olive pomace through the extraction with different solvents of oil and phenolics from pomace olive oil. All results obtained showed that the yield of oil as well as phenolics compounds and antioxidant activity are strongly influenced by the solvent extraction. Special attention should be given to the high extraction yield obtained with Hexane, Methanol and Chloroform/Methanol mixture. It must also be pointed out that the combination of organic solvents may have a positive effect on oil extraction. Pomace olive oil contains bioactive compounds that contribute directly to antioxidative action. The results obtained indicated that the POO8 showed the highest phenol and flavonoid content. The methanolic extracts of eight pomace olive oil showed different antioxidant levels depending on the test used where POO8 exhibited the highest radical scavenging DPPH and ferric reducing antioxidant power (FRAP). Whereas, POO4, POO5, POO6 and POO7 exhibited the best activity with β-carotene bleaching. Based on these findings, we suggest that the pomace olive oil could be studied as a potential natural antioxidant. Also, its antioxidant properties require further investigation and it is necessary to focus the attention on the other bioactive compounds.

Acknowledgement

The authors thank the oil factory’s owner of the “Ain Touta” area (Batna, Algeria) for supplying pomace oil used in this study.

References


Table 5. Correlation matrix between oil yield, antioxidant activity and antioxidants

<table>
<thead>
<tr>
<th></th>
<th>TPC</th>
<th>TFC</th>
<th>DPPH</th>
<th>β-Carotene</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>POO1</td>
<td>0.99*</td>
<td>0.771</td>
<td>-0.631</td>
<td>0.964</td>
<td>0.987</td>
</tr>
<tr>
<td>POO2</td>
<td>0.498</td>
<td>-0.514</td>
<td>0.056</td>
<td>0.957</td>
<td>0.489</td>
</tr>
<tr>
<td>POO3</td>
<td>-0.963</td>
<td>-0.99*</td>
<td>0.040</td>
<td>0.708</td>
<td>0.731</td>
</tr>
<tr>
<td>POO4</td>
<td>0.994</td>
<td>-0.146</td>
<td>0.694</td>
<td>-0.504</td>
<td>-0.057</td>
</tr>
<tr>
<td>POO5</td>
<td>0.539</td>
<td>-0.174</td>
<td>-0.691</td>
<td>0.390</td>
<td>0.960</td>
</tr>
<tr>
<td>POO6</td>
<td>0.588</td>
<td>-0.287</td>
<td>-0.833</td>
<td>0.709</td>
<td>-0.997*</td>
</tr>
<tr>
<td>POO7</td>
<td>-0.727</td>
<td>0.270</td>
<td>0.035</td>
<td>0.852</td>
<td>0.966</td>
</tr>
<tr>
<td>POO8</td>
<td>0.946</td>
<td>-0.046</td>
<td>0.709</td>
<td>0.330</td>
<td>0.431</td>
</tr>
</tbody>
</table>

Note: * significant at 0.05 level, ** significant at 0.01 level


Cioffi, G., Pesca, M. S., De Capraris, P., Braca, A., Severino, L. and De Tommasi, N. 2010. Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity. Food Chemistry 121: 105-111.


Comparison of extracts prepared from plant by-products using different solvents and extraction time. Journal of Food Engineering 71: 214-222.
Li, H., Qiu, N., Ding, H. and Yao, R. 2008. Polyphenols contents and antioxidant capacity of 68 Chinese herbsals suitable for medicinal or food uses. Food Research International 41: 363-370.
Meziane, S. 2012.