Oxidative Stability of Soybean Oil Enriched with Ethyl Acetate Extract of Olive By-Products

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ABSTRACT

In this study, oxidative stability of soybean oil (SBO) enriched with ethyl acetate extracts of olive by-products was investigated. Total phenolic contents, phenolic profiles and antioxidant activities of olive wastewater (OMWW) and olive pomace (OP) extracts were also determined. Total phenolic contents of extracts obtained from OMWW and OP were 134.45 and 281.43 mg gallic acid equivalent (GAE)/g extract, respectively. While antioxidant activities of OMWW extracts in the linoleic acid emulsion were in the range of 85.79 % and 88.54 %, OP extracts had 83.30 % and 90.09 % at different concentrations (0.5, 1, 2 and 3 mg/mL) after incubation at 37°C. β-carotene bleaching activities of the extracts at 50°C were found as 26.80-66.63% in OMWW extracts and 18.76-53.32% in OP extracts, respectively. 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of OP extracts were higher than those of OMWW extracts and ranged from 30.6 % to 87.7 % in OP extracts and 16.6 % to 54.1 % in OMWW extracts at these concentrations. Both the antioxidant and antiradical activities of extracts significantly increased with increased concentration. K232 values of SBO containing ethyl acetate extracts were lower than control during the oxidation test at 60°C. OP extracts were more effective than OMWW extracts at 1 mg/g. Results showed that ethyl acetate extracts of olive wastes could be a source of antioxidants for the stabilization of SBO.

Introduction

Pressing, centrifugation and sinolea methods are the main olive oil production methods. Two by-products named olive mill wastewater (OMWW) and olive pomace (OP) are obtained along with olive oil. Both of them are rich in phenolic compounds, organic acids, sugars, nitrogenous compounds, pectins and oil (Lafka et al., 2011; Chowdhury et al., 2014; Goula and Lazarides, 2015). Olive mill by-products are the perfect sources of natural antioxidants since OMWW and OP have a considerable amount of phenolic compounds. 98% of the phenolic compounds found in olives remain in these by-products (Alu’datt et al., 2010; Lafka et al., 2011; De Marco et al., 2007; Mulinacci et al., 2001; Sánchez de Medina et al., 2011). Their high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) because of their phenolic content makes them hazardous by-products to nature (Chatzistathis and Koutsos, 2017). There are some researches to minimize their hazardous effects (Mulinacci et al., 2001; Alu’datt et al., 2010; Lafka et al., 2011; Goula and Lazarides, 2015; Chowdhury et al., 2014; Paini et al., 2015; Agalias et al., 2007; Dermeche et al., 2013). And antioxidant capacities of these by-products are investigated (Farag et al., 2007; Dejong and Lanari, 2009; Cioffi et al., 2010; Orozco-Solano et al., 2011; Di Mauro et al., 2017).

Generally, synthetic antioxidants are used for increasing the oxidative stability of oils. Due to health concerns, consumers have recently preferred natural antioxidants instead of synthetic antioxidants (Balasundram et al., 2006).

This study aims to investigate the effects of OMWW and OP extracts on the oxidative stability of soybean oil (SBO) and determine their antioxidant and antiradical activities. In this study, phenolic compounds were extracted with ethyl acetate from OMWW and OP. Total phenolic contents, phenolic profiles, antioxidant and antiradical activities of extracts were determined. Schaal oven test was applied at 60°C to evaluate the effects of extracts on the oxidative stability of soybean oil.
Material and Methods

Materials
OMWW and OP were obtained from an olive oil plant (Laleli Tayieli Olive and Olive Oil Plant, Burhaniye, Balikesir), which was produced by a two-phase centrifuge system. OMWW and OP were stored at -18°C until their extraction was prepared.

Linoleic acid (99%), β-carotene (99%), butylated hydroxytoluene (BHT), hydroxytyrosol, tyrosol and oleuropein were taken from Sigma-Aldrich (St Louis, USA). 2,2′-dipyridyl (99%) and iron (III) chloride hexahydrate were obtained from Acros Organics (New Jersey, USA). Other reagents were obtained from Merck (Darmstadt, Germany).

Determination of Some Properties of OMWW and OP
pH, oxidation/reduction potential, electrical conductivity and/or dissolved oxygen concentration of OMWW and OP were determined by Thermo Orion Star multi parameter (Thermo Scientific, USA). The dry matter contents of OMWW and OP were determined according to AOAC Official Method of 925.10 (AOAC, 1990). The oil contents of olive by-products were determined according to Troncosco et al. (2009).

Preparation of Extracts
5 g of OMWW and 15 g of OP were weighted in a flask. 150 mL methanol was added. Then, flasks were shaken at 100 rpm using a shaking water bath for 4 h. After waiting overnight at 20±2°C, all samples were filtered through a filter paper. Methanol in flasks was evaporated by a rotary evaporator. 100 mL acetone: water (%50 v/v) was added to the residue in the flasks. All flasks were washed with 50 mL hexane, 50 mL chloroform and ethyl acetate, respectively and each step was repeated 3 times. The ethyl acetate phase was collected. Then, ethyl acetate was removed under vacuum at 50°C using a rotary evaporator. Extracts were transferred into a colored bottle and nitrogen gas was given for 20 min to remove the ethyl acetate. The extraction yields were calculated as g/100 g (Chiou et al., 2004).

OMWW and OP extract solutions were prepared at 0.5, 1, 2 and 3 mg/mL concentrations. Extract solutions were used to determine DPPH antiradical and antioxidant activities using conjugated diene method in the linoleic acid system and β-carotene bleaching (BCB) test.

Preparation of SBO Samples
Ethyl acetate extracts of OMWW and OP were added into SBO at 1 mg/g concentration after dissolving in propanediol. Oil samples were called as SBO+OMWW and SBO+BHT, respectively. SBO+BHT were prepared with BHT at 0.2 mg/g concentration. All samples were vortexed thoroughly and were kept at 40°C for 5 minutes in an ultrasonic water bath to increase the amount of dissolved extract. SBO samples were used for the accelerated oxidation test at 60°C.

Total Phenolic Content (TPC)
TPC of OMWW, OP, and their extracts were determined by the Folin-Ciocalteu method according to Iqbal et al. (2008). 0.002 g of extracts was weighted and dissolved in 1 mL ethyl acetate. The analysis was done by taking 0.2 mL of this solution. Total phenolic content was determined as mg gallic acid equivalent (GAE) per gram of extracts. The analyses were done in triplicate.

Phenolic Profile of Extracts
Tyrosol, hydroxytyrosol and oleuropein contents of the ethyl acetate extracts of OMWW and OP were determined according to Cioffi et al. (2010) with some modification. Shimadzu Prominence UFLC (Shimadzu, Japan) was used equipped with DAD detector and COL-Analysis C18 column (5 µm, 250 × 4.6 mm, Perkin Elmer, USA). The mobile phase consisted of acidified water (0.1%) (solvent A) and methanol (solvent B). The flow rate of the mobile phase was 0.8 mL/min. The wavelength was set at 278 nm. The gradient used during analysis was as follows: 0 min, 100% A; 2 min, 95% A; 8 min 75% A; 10 min, 60% A; 10-30 min 100% B, 30-35 min 40% B; and 35-45 min 5% B. Pure standards of tyrosol, hydroxytyrosol and oleuropein (Sigma-Aldrich, USA) at different concentrations were used.

Antioxidant Activity of Extracts
DPPH Radical Scavenging Activity of Extracts
0.1 mL of OMWW or OP extract solution was mixed with 4 mL of DPPH reactive solution. Absorbance was measured against methanol at 517 nm after waiting 20 minutes in the dark using a UV-visible spectrophotometer. The same procedure was carried out in the control experiment but, 0.1 mL of ethyl acetate was used instead of the extract solution. Analyses were done in triplicate. Radical scavenging activity (RSA) was calculated according to the following formula as percentage inhibition (Kulisic et al., 2010; Nor et al., 2008).

\[
RSA(\%)=\left(\frac{A_{\text{control}}-A_{\text{sample}}}{A_{\text{control}}}\right)\times 100
\]

\[
A_{\text{control}}: \text{Absorbance of control at } t=0 \text{ min}
\]

\[
A_{\text{sample}}: \text{Absorbance of OMW or OP extracts at } t=20 \text{ min}
\]

Antioxidant Activity (AA) in Linoleic Acid Emulsion by Conjugated Diene Method
Conjugated diene method in the linoleic acid emulsion was performed according to Iqbal et al. (2008) and Mau et al. (2004). 0.5 mL of extract solutions at different concentrations (0.5, 1, 2 and 3 mg/mL) were added to linoleic acid emulsion and incubated at 37°C. The antioxidant activities of the extracts were calculated according to the change in absorbance values before and after oxidation. Analyses were done in triplicate.

\[
AA(\%)=\left(\frac{\Delta A_{\text{control}}-\Delta A_{\text{sample}}}{\Delta A_{\text{control}}}\right)\times 100
\]

\[
\Delta A_{\text{control}}: \text{Change in absorbance of control before and after incubation}
\]

\[
\Delta A_{\text{sample}}: \text{Change in absorbance of the sample before and after incubation}
\]
Antioxidant Activity by β-carotene Bleaching (BCB) Method

The antioxidant activity of OMWW and OP solution was determined according to β-carotene bleaching (BCB) method described by Gündüz and Turan (2018). About 0.3 mL of OMWW and OP extracts at different concentrations (0.5, 1, 2, and 3 mg/mL) were added to linoleic acid emulsion containing β-carotene and tween 80 and allowed to stand at 50°C in a shaking water bath. Absorbance of emulsions was determined with 15 minutes intervals for 150 minutes. BCB activity (%) was calculated according to the following formula using the absorbance values of the sample and control. Analyses were done in duplicate.

\[ \text{AA} = (\frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}}) \times 100 \]

\[ R = \ln(a/b)/t \]

\[ a = \text{Absorbance at } t=0 \text{ min} \]

\[ b = \text{Absorbance at } t=150 \text{ min} \]

Accelerated Oxidation Test at 60°C

SBO samples were allowed to oxidize at 60°C for 21 days according to the Schaal oven test (Gündüz and Turan, 2018). \( K_{232} \) and \( K_{270} \) values of the oil samples taken at the beginning of the oxidation and 3 days intervals (3, 6, 9, 12, 15, 18, 21 days) were determined according to IUPAC methods (Paquot and Hautfenne, 1987).

Statistical Analyses

The statistical analyses were performed with the SPSS package software, version 18.0 (SPSS Inc., Chicago, IL). Results were presented as means ± standard deviation of the two or three replicates of each experiment. Analysis of variance was performed. Significant differences among the means (P<0.05) were determined by Duncan’s multiple tests.

Results and Discussion

Characterization of OMWW and OP

Table 1 shows some properties of OMWW, OP and their extracts. pH, O/R potential, electrical conductivity, dissolved oxygen content, total dry matter content, oil content and total phenolic content of OMWW were determined as 4.97, 114.5 mV, 5.83 mS/cm, 5.95 mg/L, 5.3% (w/w), 0.10% and 0.83 g/kg, respectively. El Abbassi et al. (2012) have specified that OMWW and its ethyl acetate extract were 12 g/L and 2 mg GAE/L total suspended solids, 9.82 and 6.11 g/L total phenolic content as tyrosol equivalents. In another study, Goula and Lazarides (2015) found that OMWW from a three-phase olive mill consisted of 15.8% total solids and 7% oils. Similar results were found that OMWW from the traditional discontinuous system had 5.4 pH and 70 g/L total solids and total phenolic content of OMWW were 12 g GAE/L (Daâssi et al., 2014).

\[ \text{pH}, \text{O/R potential}, \text{total dry matter content}, \text{oil content} \]

and total phenolic content of OP pomace were found as 4.93, 117.2 mV, 34.4% (w/w), 4.6%, 1.65 g/kg, respectively. Uribe et al. (2014) found higher TPC for the fresh OP (4226.23 mg GAE/100 g d.m.) than our values.

Extraction Yields and Total Phenolic Content (TPC) of Extracts

Extraction yields of OMWW and OP extracts were determined as 1.90 % and 2.53 %, respectively (Table 1). Lafka et al. (2001) stated that ethyl acetate extract of olive by-products from the two-phase system had the lowest extraction yield among many solvents (methanol, ethanol, isopropanol) with 33.3%. Also, the extraction yield of OMWW extract was determined as 0.05% of OMWW by De Leonards et al. (2007). Lesage-Meessen et al. (2001) emphasized that ethyl acetate extracted 9.6% and 14.2% dry residue from OMWW obtained using the two-phase and three-phase system, respectively.

It was reported that ethyl acetate was selective for low or medium molecular weight phenolic compounds (De Marco et al., 2007; Lafka et al., 2011; Visioli et al., 1999) and could not solve high molecular weight phenols especially soluble in water (Visioli et al., 1999). In a similar study, ethyl acetate was reported to be more effective in extracting phenols than n-propanol, chloroform and hexane (Pérez et al., 1992). It has clarified that ethyl acetate extracted a large number of monomeric phenols such as hydroxytyrosol in OMWW (El-Abbassi et al., 2012; Fki et al., 2005; Khoufi et al., 2008).

TPC of OMWW and OP extracts were determined as 134.45 and 281.43 mg GAE/g extract, respectively. Daâssi et al. (2014) have specified that TPC in OMWW and its ethyl acetate extract were 12 g/L and 28 mg GAE/mg, respectively. It was stated that TPC of ethyl acetate extract of olive mill waste was 0.43% (w/w) caffeic acid equivalent by Lafka et al. (2011). El-Abbassi et al. (2012) also stated that TPC of OMWW was 9.82 and 6.11 g tyrosol equivalent/L for the semi-modern and modern three-phase system, respectively. TPC of these extracts consisted mostly of flavonoids (66.8% and 44.3%). Suárez et al. (2009) showed that OMWW extracts contained mainly phenolic alcohol, phenolic acids, and secoiridoid derivatives.

| Method                                      | OMWW          | OP            |
|---------------------------------------------|---------------|---------------|
| pH                                          | 4.97±0.01     | 4.93±0.02     |
| O/R potential (mV)                          | 114.5±0.1     | 117.2±0.1     |
| Conductivity (mS/cm)                        | 5.83±0.00     | -             |
| Dissolved oxygen (mg/L)                     | 5.95±0.01     | -             |
| Total dry matter (%)                        | 5.3±0.0      | 34.4±34.4    |
| Oil content (%)                             | 0.10±0.03     | 4.6±0.24     |
| Total Phenolic Content (g/kg)               | 0.83±0.10     | 1.65±0.03     |
| Extraction Yield (%)                        | 1.90±0.01     | 2.58±0.08     |
| Total Phenolic Content of Extracts (mg GAE/g) | 134.45±8.6   | 281.43±24.2  |

Analyses were done in duplicate or triplicate. OMWW, olive mill wastewater; OP, olive pomace.
Phenolic profile of OMWW and OP extracts

Hydroxytyrosol, tyrosol and oleuropein of the OMWW and OP extracts are shown in Figure 1. Ethyl acetate extracts of OMWW and OP had 2543.2 and 1478.6 mg/kg hydroxytyrosol; 2949.7 and 4462.4 mg/kg tyrosol; and 4957.4 and 5648.4 mg/kg oleuropein, respectively.

Dejong and Lanari (2009) have remarked that wastewater extract of OP from the two phase system contained 70.6% hydroxytyrosol, 17.5% tyrosol, 9.5% caffeic acid, 1.9% p-coumaric acid, and 0.3% vanillic acid. De Leonards et al. (2007) have investigated that OMWW consists of 66.7% hydroxytyrosol, 16.7% tyrosol, 8.3% caffeic acid, %8.3 ferulic acid. Additionally, it has been reported that pomace methanol extracts consisted of oleuropein, lingostroside aglycone, oleuropein aglycone, gallic acid, hydroxytyrosol, tyrosol, caffeic acid (Cioffi et al., 2010).

DPPH Radical Scavenging Activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time (Cheung et al., 2003). DPPH radical scavenging activity (%) of extracts are given in Figure 2.

OP extracts had higher DPPH radical scavenging activity than OMWW extracts. Radical scavenging activities of extracts significantly increased with the increased concentration (P<0.05). Radical scavenging activities ranged from 30.6% to 87.7% in OP extracts and from 16.6% to 54.1% in OMWW extracts. In a similar study, BHA, BHT and α-tocopherol at 0.2 mg/mL had 49.96%, 12.81%, and 33.20% radical scavenging activity, respectively (Günal and Turan, 2018). OP extracts had higher values at 1-3 mg/mL concentrations compared to these values and OMWW extracts at 2 and 3 mg/mL concentrations had higher values than BHT and α-tocopherol. Additionally, radical scavenging activities of methanol and ethanol extracts of OMWW and OP reported in the range of 14.67%- 63.52% and 10.64%- 54.85%, respectively (Günal and Turan, 2018). Compared to the methanol and ethanol extracts, ethyl acetate extract of OP had higher values, although OMWW had similar values.

Lafka et al. (2011) reported that ethyl acetate extract of olive by-products had 34.2 % inhibition. In the same study, ethyl acetate extract had the lowest inhibition compared to methanol, ethanol, n-propanol and isopropanol extracts. Ethyl acetate extraction was not effective in the extraction of phenols among polar solvents having good water solubility (Moure et al., 2001).

Antioxidant Activity in Linoleic Acid Emulsion

During incubation of linoleic acid emulsion at 37°C, linoleic acid was oxidized by means of heat and oxygen. Antioxidants present in extract solutions inhibit linoleic acid oxidation to a certain extent.

Antioxidant activities of extracts in linoleic acid emulsion are shown in Figure 3. As the concentration increased, there was a slight increase in the antioxidant activities of the extracts in the linoleic acid emulsion. This increase was significant (P<0.05) for OP extract. However, the change in the antioxidant activities of OMWW extract
in linoleic acid emulsion was found insignificant (P>0.05). Besides, the obtained values were close to each other for the same concentrations of OMWW and OP extracts. While antioxidant activities of OMWW in the linoleic acid emulsion were in the range of 85.79% and 88.54%, antioxidant activities of OP were 83.30% and 90.09%. Aissa et al. (2017) clarified that ethyl acetate extract of OMWW had 256.57 µg/mL IC₅₀ value in linoleic acid emulsion.

**Antioxidant Activity by BCB Method**

Yellow color of the β-carotene diminishes because of the active radicals generated during linoleic acid oxidation in this method. Antioxidants in extracts slowed down the bleaching rate of β-carotene (Kulisic et al., 2004).

Antioxidant activities (%) of the extracts determined by BCB method increased with increasing concentration (P<0.05) (Figure 4). The highest decline in absorbance was observed in the control sample during the incubation period of 150 minutes. The absorbances of samples containing OMWW and OP extracts decreased more slowly than the control sample. This means that phenolic compounds in samples inhibit radical formation via oxidation of linoleic acid, thus the bleaching of β-carotene. According to the BCB method, antioxidant activities of OMWW and OP extracts were in the range of 26.89-66.63% and 18.76-53.32% at the studied concentrations (0.5–3 mg/mL), respectively. Contrary to total phenolic contents of the extracts, OMWW extracts had higher antioxidant activities than OP extracts according to BCB method. In our another study, the antioxidant activities of BHA, BHT and α-tocopherol at 0.2 mg/mL concentration in BCB method increased with increasing concentration (P<0.05) (Günal and Turan, 2018). Ethyl acetate extracts had lower antioxidant activities than OP extracts according to BCB method. In our another study, the antioxidant activities of methanol and ethanol extracts of OMWW and OP ranged from 32.19% to 84.06% in our previous study (Günal and Turan, 2018). These values were higher than the values of OMWW and OP ethyl acetate extracts in this study.

**Accelerated Oxidation Test**

The change in K₂₅₂ and K₂₇₀ values during the oxidation of SBO samples at 60°C is shown in Table 2. Primary oxidation products (hydroperoxides, conjugated dienes) and secondary oxidation products (aldehydes, ketones, short-chain fatty acids, alcohols, conjugated trienes) cause an increase in absorbance in 232-234 nm and 268-270 nm, respectively (Suja et al., 2004). Therefore, it is possible to obtain information about oil oxidation by measuring the absorbance values at these wavelengths.

As the primary oxidation products increased gradually over time under accelerated oxidation conditions, K₂₅₂ values of all samples increased significantly (P<0.05). K₂₅₂ value of SBO increased from 6.06 to 20.53 until the end of the 21st day. Addition of extracts to SBO increased slightly the resistance of the oil to oxidation. K₂₅₂ values of SBO+OMWW and SBO+OP increased to 17.41 and 17.52 at the end of the 21st day, respectively. These values were lower than SBO and SBO+BHT. The change in K₂₅₂ values of oil groups was found insignificant (P>0.05) between 3rd-15th days. At 18th and 21st days of the oxidation, significant changes (P<0.05) were observed in K₂₇₀ values of oil groups.

When K₂₇₀ values were taken into consideration, there were fluctuations in these values during oxidation of all samples. The change in K₂₇₀ values of oil samples taken at different days was found significant (P<0.05) except for in those of SBO+OMWW samples. The K₂₇₀ value of SBO increased slightly from 1.93 to 2.65 after 21 days. SBO had slightly higher K₂₇₀ value than those of SBO+OMWW, SBO+OP and SBO+BHT. However, K₂₇₀ values of oil groups were found insignificant (P>0.05) for all storage days.

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**Table 2.** K₂₃₂ and K₂₇₀ values of SBO samples at 60°C

| Days | SBO (6.06±0.03) | SBO+OMWW (5.95±0.24) | SBO+OP (5.74±0.06) | SBO+BHT (6.78±0.05) |
|------|----------------|----------------------|-------------------|---------------------|
| 0    | 6.00±0.03     | 5.95±0.24            | 6.78±0.05         |
| 3    | 7.48±0.01     | 7.97±0.06            | 9.29±1.70         |
| 6    | 9.30±0.26     | 8.72±0.05            | 10.06±0.24        |
| 9    | 10.52±0.03    | 9.85±0.65            | 10.68±0.74        |
| 12   | 13.62±0.62    | 12.63±0.18           | 13.90±0.06        |
| 15   | 15.34±1.51    | 13.77±0.24           | 15.93±2.02        |
| 18   | 17.73±0.87    | 16.39±0.29           | 18.66±0.51        |
| 21   | 20.53±1.04    | 17.52±0.17           | 19.16±0.50        |

| Days | SBO (1.93±0.02) | SBO+OMWW (1.91±0.08) | SBO+OP (1.90±0.02) | SBO+BHT (1.90±0.02) |
|------|----------------|----------------------|-------------------|---------------------|
| 0    | 1.92±0.00     | 1.91±0.08            | 1.90±0.02         |
| 3    | 1.95±0.08     | 2.04±0.03            | 2.09±0.24         |
| 6    | 1.96±0.06     | 1.89±0.02            | 2.00±0.04         |
| 9    | 1.92±0.01     | 1.90±0.14            | 1.88±0.99         |
| 12   | 2.29±0.21     | 2.28±0.11            | 2.25±0.04         |
| 15   | 2.31±0.11     | 2.26±0.16            | 2.47±0.39         |
| 18   | 2.63±0.44     | 2.53±0.03            | 2.71±0.34         |
| 21   | 2.65±0.26     | 2.34±0.02            | 2.39±0.13         |

Analyses were done in duplicate. SBO, soybean oil; OMWW, olive mill wastewater at 1 mg/g concentration; OP, olive pomace at 1 mg/g concentration; BHT, butylated hydroxytoluene at 0.2 mg/g. ** Shows the difference between days in the same oil (P<0.05). * Shows the difference between oils in the same day (P<0.05)
Under accelerated oxidation conditions, PO and OMWW extracts were more effective than BHT. The extracts were found to be more effective in inhibiting the formation of primary and secondary oxidation products compared to SBO+BHT.

Conclusion

When the total phenolic content and antioxidant activities were taken into consideration, ethyl acetate extracts of OMWW and OP were considered as a highly effective antioxidant source. Furthermore, when the accelerated oxidation test was evaluated, it was seen that OMWW and OP extracts were more effective than BHT, which is a synthetic antioxidant. Ethyl acetate can be selected for extraction phenolic compounds from olive by-products due to high antioxidant activities. In later studies, the dominant phenolic compounds contained in these extracts can be purified and the effects of these phenolic compounds on oxidation can be examined. Besides, the effects of these extracts at different temperatures can be examined by applying thermal oxidation tests or frying at higher temperatures.

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References

Agalias A, Magiatis P, Skaltounis AL, Mikros E, Tsarbopoulos A, Gikas E, Manios T. 2007. A New Process for the Management of Olive Oil Mill Waste and Recovery of Natural Antioxidants. J Agric Food Chem, 55: 2671-2676.
Aissa I, Kharrat N, Aloui F, Sellami M, Bouaziz M, Gargouri Y. 2017. Valorization of antioxidant extracts from olive mill wastewater. Biotechnol Applied Biochem, 64(4): 579-589.
Alu’datt M, Ali I, Ereifeja K, Alhamad M, Al-Tawaha A, Rababah T. 2010. Optimisation, characterisation and quantification of phenolic compounds. Food Chem, 123: 117-122.
AOAC. 1990. Method 925.10. In Official Methods of Analysis of the Association of Official Analytical Chemists, Association of Official Analytical Chemists. Washington, DC.
Araújo M, Pimentel F, Alves R, Oliveira M. 2015. Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. Trends Food Sci Technol, 45: 200-211.
Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem, 99: 191-203.
Chatzistathis T, Koutrou T. 2017. Olive mill wastewater as a source of organic matter, water and nutrients for restoration of degraded soils and for crops managed with sustainable systems. Agr Water Manage, 190: 55-64.
Chowdhury A, Michailides M, Akratos C, Tekerlekopoulou A, Pavlou S, Vayenas D. 2014. Composting of three phase olive mill solid waste using different. Int Biodeter Biodegr, 91: 66-73.
Mau JL, Chang CN, Huang SJ, Chen CC. 2004. Antioxidant properties of methanolic extracts from Grifola frondosa, Morchella esculenta and Termitomyces albuminosus mycelia. Food Chem, 87: 111-118.

Moure A, Cruz J, Franco D, Manuel Domínguez J, Sineiro J, Domínguez H, Núñez MJ, Carlos Parajó, J. 2001. Natural antioxidants from residual sources. Food Chem, 72: 145-171.

Mulinacci N, Romani A, Galardi C, Pinelli P, Giachetineri C, Vincieri F. 2001. Polyphenolic Content in Olive Oil Waste Waters and Related Olive. J Agric Food Chem, 49(8): 3509-3514.

Orozco-Solano M, Priego-Capote F, Luque de Castro M. 2011. Influence of Simulated Deep Frying on the Antioxidant Fraction of Vegetable Oils after Enrichment with Extracts from Olive Oil Pomace. J Agric Food Chem, 59: 9806-9814.

Paini, M, Aliakbarian B, Casazza A, Lagazzo A, Botter R, Prego P. 2015. Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. LWT - Food Sci Technol, 62: 177-186.

Paquot C, Hautfenne A. 1987. Standard methods for the analysis of oils, fats and derivatives. In C. Paquot, A. Hautfenne (Eds), International union of pure and applied chemistry (IUPAC). London: Blackwell Scientific Publications.

Pérez J, De La Rubia T, Moreno J, Martinez J. 1992. Phenolic content and antibacterial activity of olive oil waste waters. Environ Toxicol Chem, 11(4): 489-495.

Sánchez de Medina V, Priego-Capote F, Jiménez-Ot, C, Luque de Castro M. 2011. Quality and Stability of Edible Oils Enriched with Hydrophilic Antioxidants from the Olive Tree: The Role of Enrichment Extracts and Lipid Composition. J Agric Food Chem, 59: 11432-11441.

Suárez M, Romero MP, Ramo T, Maciá A, Motilva MJ. 2009. Methods for Preparing Phenolic Extracts from Olive Cake for Potential Application as Food Antioxidants. J Agric Food Chem, 57: 1463-1472.

Suja K, Abraham J, Thamizh S, Jayalekshmy A, Arumughan C. 2004. Antioxidant efficacy of sesame cake extract in vegetable oil protection. Food Chem, 84: 393-400.

Troncoso E, Pedreschi F, Zúñiga R. 2009. Comparative study of physical and sensory properties of pre-treated potato slices during vacuum and atmospheric frying. LWT-Food Sci Technol, 42(1): 187-195.

Uribe E, Lemus-Mondaca R, Vega-Gálvez A, Zamorano M, Quispe-Fuentes I, Pasten A, Di Scala K. 2014. Influence of process temperature on drying kinetics, physicochemical properties and antioxidant capacity of the olive-waste cake. Food Chem, 147: 170-176.

Visioli F, Romani A, Mulinacci N, Zarini S, Conte D, Vincieri F, Galli C. 1999. Antioxidant and Other Biological Activities of Olive Mill Waste Waters. J Agric Food Chem, 47: 3397-3401.