Subcritical water extraction of antioxidant phenolic compounds from XiLan olive fruit dreg

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Abstract Olive fruit dreg (OFD), waste from olive softdrink processing, has caused disposal problems. Nevertheless, OFD is a good source of functional ingredients, such as phenolic compounds. This study investigated the extraction conditions of phenolic compounds from OFD by using subcritical water (SCW) extraction method, antioxidant activity of SCW extracts, and components of phenolic compounds by LC-MS. SCW extraction experiments were performed in a batch stainless steel reactor at temperatures ranging from 100 to 180 °C at residence time of 5 to 60 min, and at solid-to-liquid ratio of 1:20 to 1:60. Higher recoveries of phenolic compounds [37.52 ± 0.87 mg gallic acid equivalents (GAE)/g, dry weight (DW)] were obtained at 160 °C, solid-to-liquid ratio of 1:50, and extract time of 30 min than at 2 h extraction with methanol (1.21 ± 0.16 mg GAE/g DW), ethanol (0.24 ± 0.07 mg GAE/g DW), and acetone (0.34 ± 0.01 mg GAE/g DW). The antioxidant activities of the SCW extracts were significantly stronger than those in methanol extracts at the same concentration of total phenolic contents. LC-MS analysis results indicated that SCW extracts contained higher amounts of phenolic compounds, such as chlorogenic acid, homovanillic acid, gallic acid, hydroxytyrosol, quercetin, and syringic acid. SCW at 160 °C, 30 min, and solid-to-liquid ratio of 1:50 may be a good substitute of organic solvents, such as methanol, ethanol, and acetone to recover phenolic compounds from OFD.

Keywords Subcritical water · Olive fruit dreg · Phenolic compounds · Antioxidant activity

Introduction

Antioxidants exhibit an important function in preventing deleterious oxidations. In food industries, synthetic antioxidants are widely used in production because these substances are effective, inexpensive, and stable under usual food processing and storage conditions (Kiritsakis et al. 2010). However, synthetic antioxidants may elicit possible toxicological effects (Gharavi et al. 2007). As such, researchers continuously investigate natural antioxidants that sufficiently replace synthetic antioxidants in food industries.

Studies have focused on phenolic compounds derived from fruits and vegetables as new natural antioxidants (D'Angelo et al. 2007; Druzynska et al. 2007; Atawodi et al. 2010; Moreno et al. 2006). For instance, XiLan olive (*Elaeocarpus serratus* L.), which originates in Sri Lanka, is one of the most important fruits cultivated widely in Hainan, China. In olive processing, a large amount of waste is generated in the form of dregs, causing handling problems. Nevertheless, OFD is considered as a good source of phenolic compounds exhibiting antioxidative properties that avoid food oxidation.

Phenolic compounds, extracted from olive pulp or dreg, can be used as natural antioxidants to improve the lipid stability of food (DeJong and Lanari 2009). Phenolic compounds are usually present in free and bound forms. The most common compounds in olive dreg are gallic acid, demethyloleuropein, tyrosol, caffeic acid, syringic acid, oleuropein, ligstroside aglycone, oleuropein aglycone, ferulic acid, hydroxytyrosol-1'- β -glucoside, luteolin-7-rutinoside, luteolin-7-glucoside, verbascoside, luteolin-7-rutinoside, luteolin-4-glucoside, 6'- β -glucopyranosyl-oleoside, 6'- β -

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rhamnopyranosyl-oleoside, caffeoyl-quinic acid, oleoside, rutin, hydroxytyrosol, and vanillic acid (Cioffi et al. 2010).

Previous studies showed that olive polyphenols have been extracted from olive oil, olive leaf, olive pulp, and olive mill wastewater by using common methods (Bouaziz et al. 2004; He and Xia 2007; Leonardis et al. 2009). These common methods include solvent extraction, hot water extraction, and microwave-assisted extraction. Bouaziz et al. (2004) used methanol and mixtures of methanol and water to extract phenols from an olive cultivar of Chemlali from Tunisia. He and Xia (2007) compared the extraction effects of solvent extraction, ultrasonic-assisted extraction, and microwave-assisted extraction of polyphenols from *Canarium album*. However, these techniques require long extraction time, exhibit low extraction efficiency, and result in low extraction yield.

Subcritical water (SCW) has been considered as a potential alternative to extract phenolic compounds (Soto Ayala and Luque de Castro 2001). SCW is hot water subjected to pressure that sufficiently retains the liquid state (critical point of water, 22.4 MPa and 374 °C). SCW exhibits high density, high reactivity, and good solubility for a series of organic compounds with relatively low molecular weights; this method can also be used to hydrolyze ester and ether bonds in polymer chains. In contrast to common methods, SCW is an environmentally friendly technique; SCW also entails lower costs of extracting agents, produces higher extract quality, and reduces extraction time. Studies have also shown that SCW is an effective method used to extract polyphenols from plants (Budrat and Shotipruk 2009; Seo and Lee 2010; Kim et al. 2009; Rodríguez-Meizoso et al. 2006). Budrat and Shotipruk (2009) showed that the total phenolic contents obtained from bitter melon by SCW at 200 °C is seven times as high as those produced by MeOH extraction.

To the authors' knowledge, few studies have been conducted on the SCW extraction of olive polyphenols from OFD. Therefore, the main objectives of this study are listed as follows: to determine the effect of temperature, solid-to-liquid ratio, and extraction time on the removal of phenolic compounds from OFD by SCW; to investigate the antioxidant activities of SCW extracts and the components of the phenolic compounds; and to compare SCW with conventional solvent extraction.

Materials and methods

Materials

Olive fruit (cv. XiLan) samples were collected at ripe stages from a local orchard in Daxin (Hainan, China). Organic solvents, such as methanol, ethanol, and acetone were obtained

from Fisher (Suwanee, GA, USA). Other chemicals were of analytical grade.

Methods

Sample preparation

Olive dreg powder: After extraction was performed twice with hot water (100 °C, 30 min) in beverage production, the remaining dregs obtained from olive pulp was lyophilized for 2 days. The dried sample was then pulverized into fine homogeneous powder and stored at −18 °C.

SCW extraction

SCW extraction was performed in a batch type reactor (Fig. 1). OFD sample (2 g) and distilled water were placed in the reactor. The reactor was tightly closed and placed in a heating mantle. Index on the total phenol content, the optimum parameters of SCW extraction from OFD were investigated by using the single-factor test. Different temperatures (100, 110, 120, 130, 140, 150, 160, 170, 180 °C), different solid-to-liquid ratios (1:20, 1:30, 1:40, 1:50, 1:60), and different time (5, 10, 20, 30, 60 min) were discussed respectively. The reactor was removed from the heating mantle and cooled at room temperature. SCW extracts were filtered using a qualitative filter paper and the filtrate was stored at 4 °C for further experiments.

Solvent extraction

2 g of samples (olive dreg powder) was repeatedly extracted thrice by solvent extraction (methanol, ethanol, acetone), at room temperature and solid-to-liquid ratio of 1:15 for 2 h. The extracts were then filtered using a qualitative filter paper and the filtrate was stored at 4 °C for further experiments (He and Xia 2006).

Determination of total phenolic content

The total phenolic content of OFD extracts was determined using Folin-Ciocalteu reagent as described by He and Xia (2007). The SCW extracts were diluted fourfold with water because of excessive total phenolic contents. The solvent extracts were then directly analyzed without dilution. 2 mL of extract was mixed with 20 mL of distilled water, 3 mL of Folin-Ciocalteu reagent, and 12 mL of 10 % Na₂CO₃. The mixture was subsequently diluted with water to obtain a final volume of 50 mL. After 2 h of incubation at 30 °C in the dark, the absorbance of the mixture was measured using a UV-visible spectrophotometer (Evolution 300, Thermo Scientific, USA) at 765 nm. The total phenolic content was expressed as gallic acid equivalents in milligrams per gram dry weight.

Fig. 1 Schematic of SCW extraction. 1. Reactor; 2. Heating mantle; 3. External thermometer; 4. High pressure valve; 5. Piezometer; 6. Thermostat; 7. Wires; 8. Temperature probe

Reducing power (RP)

The RP of the OFD extracts was determined according to Shon et al. (2004) with slight modifications. In brief, OFD extracts obtained by SCW or methanol extraction were diluted to produce different concentrations of polyphenols (0.01, 0.02, 0.04, 0.06, and 0.08 mg/mL). 2.5 mL of diluted extract, 2.5 mL of phosphate buffer (pH 6.6), and 2.5 mL of potassium ferricyanide solution were mixed and incubated at 50 °C for 20 min. The mixture was added to 2.5 mL of 10 % trichloroacetic acid solution and centrifuged at $13,400 \times g$ for 5 min. 5 mL of supernatant was mixed with 5 mL of distilled water and 5 mL of 0.1 % ferric chloride. The resulting mixture was then allowed to stand for 10 min and the absorbance of the mixture was read at 700 nm. The samples and positive control L-ascorbic acid (L-AA) were determined at the same range of sample concentrations.

DPPH radical scavenging activity

The DPPH radical scavenging effect of the OFD extracts was evaluated according to a previously described method (Tang et al. 2009). The OFD extracts were diluted to obtain different polyphenol concentrations (0.001, 0.005, 0.01, 0.02 and 0.04 mg/mL). Precise 2 mL of extract with varying sample concentrations was added to 2 mL of DPPH ethanol solution (1 mmol/L). The prepared solution was blended and allowed to stand for 30 min at 30 °C. The absorbance of the solution was determined at 517 nm. DPPH radical scavenging activity was expressed as percentage according to the following equation: DPPH radical scavenging activity (%) = $[1 - (A_1 - A_2)/A_0] \times 100$, where A_1 is the absorbance of the sample added to 2 mL of DPPH ethanol solution, A_2 is the absorbance of sample mixed with 2 mL of ethanol, and A_0 is the absorbance of ultra-pure water added to 2 mL of DPPH ethanol solution. L-AA was used as positive control.

ABTS radical scavenging activity

The ABTS radical scavenging activity of the OFD extracts was determined according to Re et al. (1999) with slight modifications. ABTS radical cation was obtained by reacting 5 mL of ABTS stock solution (7 mmol/L) with 88 μ L of potassium persulfate (14 mmol/L) and by allowing the mixture to stand in the dark at room temperature for 12 to 16 h for further experiments. Before use, the mixture was diluted with phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm by using a spectrophotometer.

OFD extracts were diluted to obtain different concentrations of polyphenols (0.01, 0.02, 0.04, 0.06, and 0.08 mg/mL). Precise 0.1 mL of diluted extract was mixed with 3.9 mL of prepared ABTS solution and incubated at 30 °C in the dark for 10 min. Immediately, the absorbance of the solution was obtained at 734 nm. The ABTS radical scavenging activity in percent was calculated using the following equation: ABTS radical scavenging activity (%) = $[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$, where A_{control} is the absorbance of the control reaction (ultrapure water added to ABTS solution) and A_{sample} is the absorbance of the test compound. L-AA was used as a positive control sample.

LC-MS analysis

Two samples (OFD extracts obtained by SCW; OFD extracts obtained by methanol extraction) were analyzed using LCMS-IT-TOF (Shimadzu, Japan). Chromatographic separation was performed in a C_{18} column (250 mm $\times$ 4.6 mm; Waters, USA) using the solvent system of methanol (A) and formic acid-water [1:19; (B)] starting at 5 % A and a gradient to obtain the following: at 3 min, 15 % A; 13 min, 25 % A; 25 min, 30 % A; 35 min, 35 % A; 39 min, 40 % A; 42 min, 45 % A; 45 min, 45 % A; 50 min, 47 % A; 60 min, 48 % A; 64 min, 50 % A; and 66 min, 100 % A. An injection volume of 80 μ L and a flow rate of 0.9 mL/min were used. Chromatographic data were accumulated at 240 to 600 nm (Vinha et al. 2005).

MS analysis was performed by electrospray ionization (ESI) operating at positive and negative modes under the following conditions: electrospray voltage, 3,500 V; and heated capillary and curved desolvation line (CDL) temperatures adjusted to 200 °C. Nitrogen was used as a nebulizing gas at a pressure of 65 psi and the flow rate was set to 1.5 L/min. Full-scan mass spectra were obtained from m/z 100 to m/z 800.

Statistical analysis

The experiments were performed in triplicate. Data were analyzed using SPSS statistical software (v. 17.0). Duncan's multiple range tests were performed to determine significant differences between the mean values of the treatments ($P < 0.05$).

Results and discussion

Comparison of SCW extraction with solvent extraction

Figure 2 shows that different extraction conditions (temperature, time contact, solid-to-liquid rate) significantly affected the total phenolic content of OFD extracts obtained by SCW. Temperature was observed as the most important factor affecting extraction efficiency ($p < 0.01$). The phenolic contents of SCW extracts remarkably increased as temperature increased from 100 to 160 °C; at >160 °C, the phenolic contents decreased. Studies have observed that high temperature and long duration of SCW extraction can reduce the extraction rate and antioxidant activities of phenolics; this result is possibly due to further degradation at high temperatures (Ong and Len 2003; Kim et al. 2009). Fig. 2 also shows that the maximum phenolic content of the OFD extracts (37.52 ± 0.87 mg/g) was obtained at solid-to-liquid ratio of 1:50 at 160 °C for 30 min. SCW extraction was also conducted to extract phenolic compounds of coffee silverskin and bitter melon. The result showed that a high phenolic content was obtained from coffee silverskin (36 mg/g, at 210 °C) and bitter melon (52.63 mg/g, at 200 °C) by SCW (Narita and Inouye 2012; Budrat and Shotipruk 2009). Meanwhile, the minimum phenolic content of the OFD extracts (1.52 ± 0.31 mg/g) was obtained at 100 °C. Because the dregs was the residues of olive pulps,

extracted twice with hot water (100 °C, 30 min) in beverage production. When temperature of the SCW extraction was set at 100 °C, the efficiency of extracting phenolic compounds was poor.

Fig. 2 Effects of SCW extraction conditions (temperature, time contact, solid-to-liquid rate) on the total phenolic content of the SCW extracts. **a** Effects of different extraction temperatures were discussed on the total phenolic content of the SCW extracts at solid-to-liquid ratio of 1:50 for 30 min; **b** Effects of different extraction time were discussed on the total phenolic content of the SCW extracts at solid-to-liquid ratio of 1:50 at 160 °C; **c** Effects of solid-to-liquid ratios were discussed on the total phenolic content of the SCW extracts at 160 °C for 30 min. Values are given as mean $\pm$ SD ($n=3$)

The total phenol content of OFD extracts obtained by solvent extraction (at room temperature and solid-to-liquid of 1:15 for 2 h, repeated three times) was determined. Table 1 shows that methanol extracts contained a higher amount of phenolic compounds than acetone and ethanol extracts. A significant difference was observed in SCW extracts and methanol extracts in terms of total phenol content. The effect of SCW extraction was evidently superior to solvent extraction. This result possibly occurred because the dielectric constant of water decreases, hydrogen bonding weakens, and the density and polarity of water decrease as temperature increases during SCW extraction. As a result, phenolic dissolution increases. A decrease in surface tension and viscosity of water caused by increased water temperature also increases the extraction rate of phenolics. Furthermore, heating processes in SCW extraction can effectively release polyphenolic compounds in OFD by the decomposing polysaccharide-lignin network of the cell wall matrix (Baek et al. 2008). In solvent extraction, pure organic solvent may be insufficient to destroy the bonding of polyphenols and proteins or other substances. As a result, low extraction efficiency is obtained. These results indicated that SCW extraction was more effective in the extraction of phenolic compounds from OFD than solvent extraction.

Antioxidant activity

Phenolic compounds exhibit antioxidant and free-radical scavenging activities. The amount of phenolic compounds in extracts is an important factor affecting antioxidant activities. In this study, the antioxidant activities of SCW extracts were determined by RP assay, DPPH radical scavenging assay, and ABTS radical scavenging assay to evaluate antioxidant activities of SCW extracts from OFD. The results were then compared with methanol extracts at a concentration range from 0.001–0.08 mg/mL.

The RP of OFD extracts was determined by determining the reduction of ferric ion (Fe^{3+}) as a form of ferricyanide complex to ferrous ion (Fe^{2+}) (Kim et al. 2009). The RPs of SCW extracts, methanol extracts, and L-AA (positive control) are shown in Table 2. These extracts exhibited almost the same trend for RP, in which RP increased as phenolic contents increased. At 0.01 to 0.02 mg/mL, no significant difference

was observed between the RP of SCW extracts and methanol extracts at the same concentrations ($p>0.05$). At 0.02 to 0.08 mg/mL, the RP of SCW extracts was significantly stronger than that of methanol extracts at the same concentration ($p<0.05$). At 0.08 mg/mL, the RP of methanol extracts (0.896 ± 0.061) was slightly higher than that of L-AA (0.676 ± 0.039). The RP of SCW extracts (1.564 ± 0.078) was two to three times as strong as that of L-AA. These results showed that SCW is a promising solvent to extract the antioxidant components from OFD at specific RP.

DPPH is a stable free radical that can accept an electron and hydrogen radical to form a stable diamagnetic molecule. DPPH radical scavenging assay has been frequently conducted to determine the antioxidant activity of various samples (Amarowicz et al. 2004). The DPPH radical scavenging effects of SCW extracts, methanol extracts, and L-AA (positive control) are summarized in Table 3. The DPPH radical scavenging activity of the extracts was positively correlated with phenolic contents. The maximum DPPH radical scavenging activity (95 %) was obtained when the phenolic content of SCW extracts was 0.005 mg/mL and the phenolic content of methanol extracts was 0.02 mg/mL. At the same phenolic content (0.005 mg/mL), the DPPH radical scavenging activity of SCW extracts (95.36 ± 2.03) was significantly stronger than that of methanol extracts (50.42 ± 3.78 ; $p<0.01$). Our results suggested that antioxidant compounds can be effectively extracted from OFD by SCW extraction compared with other solvent extraction methods.

ABTS radicals are scavenged by antioxidants by donating hydrogen ions and assessed by determining the decrease in absorption at 405 nm (Seo and Lee 2010). The ABTS radical scavenging activities of SCW extracts, methanol extracts, and L-AA (positive control) are shown in Table 4. The ABTS radical scavenging activities of SCW and methanol extracts increased as phenolic content increased, showing a good linear relationship. The ABTS radical scavenging activity of SCW extracts was significantly higher than that of methanol extracts and L-AA ($p<0.05$) at the same phenolic content. Furthermore, the ABTS radical scavenging activity of SCW extracts was 100 % at 0.06 mg/mL. This result was higher than that of methanol extract (91.59 %) at 0.08 mg/mL. The

Table 1 Total phenolic content obtained by different extraction methods

Extraction methods	Methanol	Ethanol	Acetone	SCW
Total phenolic content (mg/g)(DW)	1.21 ± 0.16^B	0.24 ± 0.07^C	0.34 ± 0.01^C	37.52 ± 0.87^A

Methanol: methanol extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; Ethanol: ethanol extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; Acetone: acetone extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; SCW: SCW extracts obtained at 160 °C, solid-to-liquid of 1:50 for 30 min. ^{A–C}: Different letters within a row indicate significant difference ($P<0.01$), $n=3$. Values are given as mean $\pm$ SD ($n=3$)

Table 2 Effects of extracts obtained by SCW and methanol extraction on reducing power

Sample	$A_{700\text{nm}}$				
	Concentration (mg/mL)				
	0.01	0.02	0.04	0.06	0.08
SCW	0.226±0.021 ^{cy}	0.434±0.056 ^{dy}	1.116±0.032 ^{cz}	1.377±0.067 ^{bz}	1.564±0.078 ^{az}
Methanol	0.191±0.033 ^{cy}	0.373±0.023 ^{dy}	0.573±0.060 ^{cy}	0.721±0.050 ^{by}	0.896±0.061 ^{ay}
L-AA	0.101±0.023 ^{cx}	0.155±0.020 ^{dx}	0.300±0.028 ^{cx}	0.487±0.047 ^{bx}	0.676±0.039 ^{ax}

SCW: SCW extracts obtained at 160 °C, solid-to-liquid of 1:50 for 30 min; Methanol: methanol extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; L-AA: L-ascorbic acid solution. ^{a-c}: Different letters within a row indicate significant difference ($P<0.05$), $n=3$; ^{x-z}: Different letters within a column indicate significant difference ($P<0.05$), $n=3$. Values are given as mean ± SD ($n=3$)

results of ABTS radical scavenging activity analyses indicated that SCW can be effectively used to increase the antioxidant activities of OFD extracts.

The results of RP assay, DPPH radical scavenging assay, and ABTS radical scavenging assay showed that SCW extraction could be used as an effective method to extract antioxidants from OFD. In the three assays, the antioxidant activities of OFD extracts were mainly caused by the amounts of phenolic compounds. Other reasons may also affect the antioxidant activity of OFD extracts. For instance, the difference in the antioxidant activities of SCW extracts and methanol extracts at the same phenolic content could be attributed to the structure and components of phenolic compounds. In SCW extraction, the polarity, density, surface tension, and viscosity of water decrease as water temperature increases. These properties indicate that SCW is a polar solvent with a different polarity from methanol. Hence, high amounts of different polar phenolic antioxidants from OFD, which contain the majority of phenolics, dissolve in SCW. Furthermore, heat treatment in SCW extraction converts insoluble phenolic compounds to soluble phenolics, thereby affecting the structure, constitution, and antioxidant activity of phenolic compounds (Baek et al. 2008). In addition to phenolic compounds, other antioxidative compounds, such as caramelization and

Maillard reaction products, can be extracted by SCW extraction (Rodríguez-Meizoso et al. 2010).

LC-MS qualitative analysis

In SCW extraction, impurities (e.g., polysaccharides), which may influence the analysis results of the extracts, could be extracted. Before LC-ESI-MS was conducted, SCW extracts were purified by macroreticular resin AB-8 absorption. SCW extracts were concentrated under vacuum conditions and then filtered using a microporous membrane (0.22 µm). The two samples (OFD extracts obtained by SCW extraction; OFD extracts obtained by methanol extraction) were examined by LC-MS with ESI in positive and negative ion modes to generate total ion current (TIC) chromatograms.

The components of phenolic compounds are shown in Table 5. The identified phenolic compounds are listed in an elution order. The structural assignment was based on a systematic search for pseudomolecular ion peaks and major fragment ion peaks by using and comparing the extracted ion mass chromatograms with the data of phenolic compounds and fragmentation patterns presented in previous studies (Sheng and Tang 2008). For example, the ion mass chromatogram of the peak at a retention time of 8.933 min showed

Table 3 Scavenging activity of the extracts obtained by SCW and methanol extraction against DPPH radical

Sample	Scavenging activity (%)				
	Concentration (mg/mL)				
	0.001	0.005	0.01	0.02	0.04
SCW	82.92±3.92 ^{bz}	95.36±2.03 ^{az}	93.67±1.89 ^{az}	93.86±2.51 ^{ay}	94.03±1.43 ^{ay}
Methanol	15.09±4.45 ^{dy}	50.42±3.78 ^{cy}	74.96±6.36 ^{by}	94.36±2.09 ^{ay}	94.2±1.65 ^{ay}
L-AA	4.98±1.83 ^{bx}	5.47±2.02 ^{bx}	4.8±1.94 ^{bx}	7.96±1.56 ^{ax}	10.95±2.60 ^{ax}

SCW: SCW extracts obtained at 160 °C, solid-to-liquid of 1:50 for 30 min; Methanol: methanol extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; L-AA: L-ascorbic acid solution. ^{a-c}: Different letters within a row indicate significant difference ($P<0.05$), $n=3$; ^{x-z}: Different letters within a column indicate significant difference ($P<0.05$), $n=3$. Values are given as mean ± SD ($n=3$)

Table 4 Scavenging activity of extracts obtained by SCW and methanol extraction against ABTS radical

Sample	Scavenging activity (%)				
	Concentration (mg/mL)				
	0.01	0.02	0.04	0.06	0.08
SCW	50.18±1.00 ^{dz}	54.28±2.52 ^{cz}	96.53±1.25 ^{bz}	100.00 ^{az}	100.00 ^{az}
Methanol	12.77±0.29 ^{cy}	28.06±1.74 ^{dy}	49.56±1.77 ^{cy}	72.33±1.97 ^{by}	91.59±1.95 ^{ay}
L-AA	1.80±0.18 ^{dx}	4.17±0.72 ^{cx}	10.68±0.52 ^{bx}	11.30±0.72 ^{bx}	17.98±2.32 ^{ax}

SCW: SCW extracts obtained at 160 °C, solid-to-liquid of 1:50 for 30 min; Methanol: methanol extracts obtained at room temperature and solid-to-liquid of 1:15 for 2 h; L-AA: L-ascorbic acid solution. ^{a-c}: Different letters within a row indicate significant difference ($P<0.05$), $n=3$; ^{x-z}: Different letters within a column indicate significant difference ($P<0.05$), $n=3$. Values are given as mean ± SD ($n=3$)

a major pseudomolecular ion peak $[M-H]^-$ at m/z 153.000 in the negative mode and a major fragment ion peaks at m/z 127.0308 in the positive mode. On the basis of the cleavage rule of phenolic compounds, the ion fragment at m/z 127.0308 may be due to the loss of CO $[M + H - CO]^+$ in the positive mode. Therefore, the peak was identified as hydroxytyrosol (molecular mass=154).

The identified phenolic compounds (Table 5) were synthetically analyzed. The results revealed that SCW and methanol extracts contain significantly different phenolic compounds

that were obtained from OFD. SCW extracts and methanol extracts also contain similar compounds, including oleuropein, genkwanin, and caffeic acid. Other phenolic compounds, such as chlorogenic acid, homovanillic acid, gallic acid, hydroxytyrosol, quercetin, and syringic acid, are further obtained in SCW extracts but not in methanol extracts (Ryan et al. 1999; Damak et al. 2008; Ryan et al. 2002; Antolovich et al. 2004; Bouaziz et al. 2005; Kong et al. 2011; Jemai et al. 2009; Kontogianni and Gerothanassis 2012). Olive dreg extracts and olive pulp extracts obtained using SCW (data not

Table 5 Identification of phenolic compounds from olive fruit dreg by LC-MS-IT-TOF

No.	Compound	RT	Molecular mass	Major peak			
				A		B	
				ESI+	ESI−	ESI+	ESI−
1	Chlorogenic acid	4.213	354			n.d.	353.0241
2	Unknown	5.213	192	381.0787	190.9939	193.0319	190.9949
3	Homovanillic acid	5.453	181			182.0785	n.d.
4	Gallic acid	6.933	170			n.d.	168.9919
5	Hydroxytyrosol	8.933	154			127.0380	153.0000
6	Unknown	10.920	304			305.0651	303.0078
7	Quercetin	11.787	302			n.d.	300.9942
8	Unknown	12.627	228	n.d.	112.9722	201.1140	226.9890
				259.0189			
9	Unknown	13.640	220		219.0211		
				201.0189			
10	Unknown	16.000	320			321.1267	316.9857
							318.8660
11	Syringic acid	26.547	198			n.d.	197.0246
12	Oleuropein	34.107	540	541.2616	n.d.	541.2549	n.d.
13	Hyperoside	41.360	464	465.1029	463.0260		
14	Unknown	69.080	226	227.1614	225.1191	227.1605	225.1810
15	Unknown	70.693	209	209.1090	n.d.	209.1000	n.d.
16	Genkwanin	77.387	284	n.d.	283.2261	n.d.	283.2231
17	Caffeic acid	78.013	180	181.0487	n.d.	181.0476	n.d.

RT Retention time; n.d not detected. A: OFD extracts obtained by methanol extraction. B: OFD extracts obtained by SCW

shown) slightly differed in terms of the composition of phenolic compounds because some phenolic compounds are lost in hot water extraction during beverage production. Other compounds could only be identified partially. Such compounds may be the extracted impurities, together with phenolic compounds. The comparison of phenolic compounds is also shown in Table 5. The results confirmed our suppositions that the composition of phenolic compounds in different extracts could affect antioxidant activities to some extent. Therefore, the antioxidant activity of SCW extracts was higher than that in methanol extracts at the same phenolic content. This difference in antioxidant activities could be correlated with the composition of phenolic compounds, although the contribution of other compounds to the antioxidant activity of the SCW extracts could not be ruled out.

Conclusion

In summary, antioxidative phenolic compounds from XiLan olive dregs were successfully extracted using SCW. Under appropriate extraction conditions, the effect of SCW extraction (37.52 ± 0.87 mg/g) was evidently superior to solvent extraction. The results of RP assay, DPPH radical scavenging assay, and ABTS radical scavenging assay further showed that the antioxidant activities of SCW and methanol extracts were strongly and positively correlated with the amounts of phenolic compounds. At the same phenolic content, the antioxidant activities of SCW extracts were stronger than those of methanol extracts. SCW at 160 °C, solid-to-liquid of 1:50 and 30 min might be a good substitute to organic such as methanol to obtain phenolic compounds from OFD. LC-MS qualitative analysis results indicated that SCW extracts exhibit strong antioxidant activities that could be attributed to the presence of higher amounts of phenolics than methanol extracts.

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