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Microwave-Assisted Extraction of Phenolic Compounds from Olive By-Products

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In recent years, olive cultivation has spread globally because of increasing popularity of the health benefits of olive oil. Accordingly, large amounts of olive by-products, such as leaves, branches, and pomace, are being produced by the olive industry. The discharge of this agro-industrial waste into the environment results in ecological repercussions. However, these by-products are potential sources of phenolic compounds as antioxidants for food and cosmetic applications. Their extraction can result in the production of high value-added natural ingredients and a reduction in their harmful environmental impact. Microwave-assisted extraction method has attracted growing interest for extracting these phenolic compounds from plant materials. The purpose of this study was to extract phenolic compounds from olive by-products by maceration and microwave-assisted extraction methods and to compare the phenolic yield obtained with these two methods. The results showed that microwave-assisted extraction could extract a higher yield of phenolic compounds in a much shorter time compared with maceration extraction (10 min instead of 1 h). In addition, the amount of phenolic compounds obtained by microwave-assisted extraction increased with increasing microwave power. Indeed, the extracts obtained were shown to exhibit high antioxidant activities. These collective results indicate that microwave-assisted extraction has a higher extraction efficiency of phenolic compounds from olive by-products compared with the maceration method.

1. Introduction

Although olive (*Olea europaea* L.) is an ancient traditional plant native to the Mediterranean Basin, olive cultivation has spread globally owing to the purported health benefits of olive oil. The global olive oil production has tripled in the last 60 years, reaching 3.2 Mt in the 2019−2020 crop season (International Olive Council, 2020). The health benefits of olive oil (produced by mechanically pressing olive fruits without chemical treatment) are attributed to the presence of abundant monounsaturated fatty acids such as oleic acid and phenolic compounds such as antioxidants, all of which may prevent the development and progression of diseases such as coronary artery disease (Servili et al., 2009). In addition to its health benefits, olive oil is considered to have cosmetic benefits, such as moisturising and photoprotective effects on the skin (Kaur and Saraf, 2010; Kishimoto, 2020). Therefore, olive oil has also been used as a cosmetic product.

However, owing to this widespread growth of olive oil production, large quantities of by-products are produced by the olive industry. Olive by-products, such as pomace, leaves, and branches, are generated during oil extraction, olive tree pruning, maintenance, harvesting, and cleaning of olive fruits before oil extraction. These by-products are discharged into the environment and cause ecological issues due to their toxic organic loads and low pH (Dermeche et al., 2013). However, in recent years, based on the principles of green chemistry (Anastas and Warner, 1998), studies on the valorisation of olive by-products have been conducted for food and cosmetic applications (Rodrigues et al., 2015; Gullón et al., 2020). The advantages of agro-industrial waste, including olive by-products, are that they are effective, economical, and sustainable. This waste contains high value-added components, making it a potential source of phenolic compounds, such as antioxidants. The global market for food and cosmetics has been shifting towards sustainable and natural products to reduce adverse

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effects on the environment and health. As olive by-products emerge from farming, it has been speculated that their extracts could be significant sources of green ingredients.

With a growing interest in green technology, microwave-assisted extraction technique features among the most promising modern methods for extracting valuable compounds from plant materials (Destandau et al., 2013; Ibrahim and Zaini, 2017), allowing faster treatment with better energy efficiency and reduced loss of quality attributes. The advantages of this technique are that less solvent is required, extraction time is drastically reduced, and extraction is performed at a relatively lower temperature compared with other techniques (Danlami et al., 2014). However, the efficiency of this technique depends on the operating conditions. The selection of the solvent is the most important factor affecting the yield of bioactive compounds in microwave-assisted extraction (Veggi et al., 2013). The use of water as a solvent has numerous benefits in green extraction methods, because water is not only inexpensive and environmentally friendly, but also non-flammable and nontoxic compared with organic solvents. In addition to its environmental advantages, water has physical features suitable for microwave-assisted extraction (Destandau et al., 2013). It has a high dipole moment and strongly absorbs the microwave energy, hence facilitating the heating process, increasing the polarity of the extracting solvent and/or allowing the sample to be heated. Highly localised heating close to the boiling point of water leads to the expansion and rupture of cell walls of plant materials, which is followed by the liberation of target compounds from the materials to the surrounding solvent.

The microwave-assisted extraction of phenolic compounds from olive leaves has previously been studied using different solvents, including water, methanol, ethanol, and acetone (Rafíee et al., 2011). However, the effects of power levels on the extraction of compounds from olive by-products, such as olive branches and pomace, but not leaves, and the functional properties of the obtained extracts, have not been examined. The present study aimed to evaluate the effectiveness of microwave-assisted extraction of phenolic compounds from olive by-products (leaves, branches, and pomace) under microwave irradiation at several microwave power levels compared with the conventional extraction method by maceration. The findings were discussed based on the phenolic yield and antioxidant activities of the extracts obtained using these two methods.

2. Materials and Methods

2.1 Materials

Olive by-products such as leaves, branches, and pomace of the Mission cultivar were collected from Shodoshima Island (Kagawa, Japan). These by-products were shade-dried for one week and crushed (16 mesh). Gallic acid was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

2.2 Maceration extraction

Two grams of each dried material was blended with 100 mL water for different periods (0.5, 1, 2, 3, 4, 5, and 6 h) with agitation at 95 ˚C.

2.3 Microwave-assisted extraction

A household microwave oven (Panasonic NE-EH229, Osaka, Japan) was operated at different powers (150, 500, and 700 W). Two grams of each dried material was mixed with 100 mL water and then irradiated in the microwave (15 s power on, 15 s power off to avoid boiling) (Rafíee et al., 2011). The standard temperature of 95 ˚C was not maintained in this extraction method. Irradiation was performed for different durations (2, 4, 6, 8, 10, 12, and 15 min).

2.4 Determination of total phenolic content

The extracts obtained from both methods were filtered through a paper filter (ADVANTEC, No. 131). The total phenolic content (TPC) of the extract samples was determined using the Folin-Ciocalteu assay (Singleton et al., 1999) with some modifications. Briefly, 0.5 mL of the extract solution appropriately diluted with water was mixed with 0.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), followed by the addition of 0.5 mL Na_2CO_3 solution (10%) after 1 min. Subsequently, the mixture was incubated at 35 ˚C for 30 min, and its absorbance was measured at 700 nm. Gallic acid was used as the standard for the calibration curve. TPC was expressed as mg of gallic acid equivalent (GE)/g of dry weight (DW).

2.5 Determination of antioxidant activities

The free radical-scavenging activity of the extracts was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) Antioxidant Assay Kit (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions (Shimamura et al., 2014). DPPH radical-scavenging activity was expressed as μmol Trolox equivalent (TE)/g of DW using the standard Trolox curve.

The levels of superoxide dismutase (SOD)-like activity in the extracts were measured using the SOD Assay Kit-WST (Dojindo Laboratories), according to the manufacturer's instructions. Briefly, in a 96-well plate, 20 µL of sample solution was added to the sample and blank 2 well, and 20 µL of water was added to blank 1 and blank 3 wells. Then, 200 µL of WST working solution was added to each well. After mixing, 20 µL of dilution buffer was added to blank 2 and blank 3 wells, and 20 µL of enzyme working solution (15 µL of enzyme mixed with 2.5 mL dilution buffer) was added to the sample and blank 1 well. The plate was incubated at 37 ˚C for 20 min, and the absorbance was determined at 450 nm using a microplate reader (SH-9000Lab; Corona Electronic, Co., Ltd., Japan). The SOD-like activity was calculated using the following equation:

$$\text{SOD-like activity (inhibition rate \%)} = \{[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})] / (A_{blank1} - A_{blank3})\} \times 100$$

where A_{blank1}, A_{blank2}, A_{blank3}, and A_{sample} are the absorbance values of blank1, blank2, blank3, and the sample, respectively.

2.6 Statistical analysis

Data are presented as the mean ± standard deviation (SD) from three replicates. The statistical significance of differences between the two groups was analysed using the Student's *t*-test in Microsoft Excel. The Tukey-Kramer test in Microsoft Excel was used to identify significant differences among the means of multiple groups. The data were analysed by one-way analysis of variance, followed by the Tukey-Kramer test in Microsoft Excel. Statistical significance was set at $p < 0.05$. Pearson's correlation coefficient in Microsoft Excel was used to determine a significant linear relationship between TPC and antioxidant activity.

3. Results and Discussion

3.1 Maceration extraction of phenolic compounds from olive by-products

The total concentrations of phenolic compounds in different extracts of olive by-products (leaves, branches, and pomace) obtained through maceration extraction were compared over a 6 h period in order to evaluate the efficiency of extraction (Table 1). The total phenolic content (TPC) was significantly different among the extracts, or even in the same type of extract obtained through different extraction durations. The olive leaf extract exhibited the highest TPC, and the olive pomace extract had the lowest. The TPC values of all extracts reached a plateau at 1 h after extraction.

Table 1: TPCs (mg GE/g DW) of extracts prepared by maceration extraction.

Time (h)	Olive leaf extract	Olive branch extract	Olive pomace extract
0.5	31.8 ± 0.5 ^{aA}	4.0 ± 0.4 ^{bA}	2.5 ± 0.1 ^{cA}
1	35.3 ± 0.5 ^{aAB}	6.0 ± 0.6 ^{bB}	2.8 ± 0.1 ^{cB}
2	35.5 ± 0.5 ^{aB}	6.6 ± 0.3 ^{bB}	2.9 ± 0.1 ^{cB}
3	38.7 ± 3.3 ^{aB}	6.8 ± 0.4 ^{bB}	2.9 ± 0.1 ^{cB}
4	37.2 ± 2.0 ^{aB}	6.9 ± 0.3 ^{bB}	2.9 ± 0.1 ^{cB}
5	38.0 ± 2.1 ^{aB}	7.0 ± 0.6 ^{bB}	2.8 ± 0.1 ^{cB}
6	38.0 ± 1.8 ^{aB}	7.1 ± 0.4 ^{bB}	2.9 ± 0.1 ^{cB}

$^{a-c}$Values are significantly different at the same heating time ($p < 0.05$).
^{A,B}Values are significantly different in the same extract ($p < 0.05$).

3.2 Microwave-assisted extraction of phenolic compounds from olive by-products

The total concentrations of phenolic compounds extracted from olive by-products under microwave irradiation at different power levels (150, 500, and 700 W) for 15 min were evaluated (Table 2−4) and then compared to those extracted by maceration. The TPC values of these extracts increased with increasing microwave power. The TPC values increased with increasing extraction time as well, but reached a plateau at 10 min after extraction, regardless of the microwave power level used. These phenolic compound extraction rates were similar to those reported in a previous study (Rafíee et al., 2011). The total amount of phenolic compounds extracted from the olive leaves under microwave irradiation at 150 W was similar to the amount extracted in a previous study (Rafíee et al., 2011). The TPC value of the extracts prepared under microwave irradiation at 500 and 700 W for 10 min were 1.7−2.0 and 2.1−2.7 times that at 150 W for 10 min, respectively.

Table 2: TPCs (mg GE/g DW) of olive leaf extracts prepared by microwave-assisted extraction.

Time (min)	Microwave power levels		
	150 W	500 W	700 W
2	19.0 ± 0.8 ^{aA}	23.2 ± 2.2 ^{bA}	33.1 ± 1.3 ^{cA}
4	26.6 ± 1.2 ^{aB}	39.9 ± 2.4 ^{bB}	48.8 ± 4.2 ^{cB}
6	29.8 ± 1.6 ^{aBC}	47.2 ± 3.1 ^{bB}	58.8 ± 4.9 ^{cC}
8	35.2 ± 1.2 ^{aC}	57.0 ± 1.9 ^{bC}	70.1 ± 4.5 ^{cD}
10	40.4 ± 2.3 ^{aCD}	68.1 ± 2.6 ^{bD}	83.3 ± 2.0 ^{cE}
12	44.2 ± 5.1 ^{aD}	74.4 ± 3.0 ^{bD}	91.4 ± 1.5 ^{cE}
15	43.3 ± 3.3 ^{aD}	72.6 ± 4.3 ^{bD}	90.6 ± 2.8 ^{cE}

$^{a–c}$Values are significantly different for the same treatment duration ($p < 0.05$).
$^{A–E}$Values are significantly different at the same power level ($p < 0.05$).

Table 3: TPCs (mg GE/g DW) of olive branch extracts prepared by microwave-assisted extraction.

Time (min)	Microwave power levels		
	150 W	500 W	700 W
2	3.7 ± 0.1 ^{aA}	4.9 ± 0.1 ^{bA}	7.7 ± 0.2 ^{cA}
4	4.5 ± 0.1 ^{aB}	8.6 ± 0.3 ^{bB}	10.6 ± 0.3 ^{cB}
6	5.3 ± 0.1 ^{aC}	9.7 ± 0.1 ^{bC}	12.8 ± 0.4 ^{cC}
8	6.2 ± 0.1 ^{aD}	11.6 ± 0.2 ^{bD}	16.1 ± 0.2 ^{cD}
10	7.2 ± 0.3 ^{aE}	13.8 ± 0.4 ^{bE}	18.2 ± 0.4 ^{cE}
12	7.6 ± 0.1 ^{aE}	14.0 ± 0.3 ^{bE}	18.8 ± 0.3 ^{cE}
15	7.6 ± 0.2 ^{aE}	13.6 ± 0.8 ^{bE}	18.9 ± 0.2 ^{cE}

$^{a–c}$Values are significantly different for the same treatment duration ($p < 0.05$).
$^{A–E}$Values are significantly different at the same power level ($p < 0.05$).

Table 4: TPCs (mg GE/g DW) of olive pomace extracts prepared by microwave-assisted extraction.

Time (min)	Microwave power levels		
	150 W	500 W	700 W
2	0.8 ± 0.1 ^{aA}	1.5 ± 0.1 ^{bA}	2.2 ± 0.1 ^{cA}
4	1.3 ± 0.1 ^{aB}	2.8 ± 0.1 ^{bB}	3.6 ± 0.2 ^{cB}
6	1.7 ± 0.1 ^{aC}	3.6 ± 0.1 ^{bC}	5.0 ± 0.3 ^{cC}
8	2.2 ± 0.1 ^{aD}	4.6 ± 0.2 ^{bD}	6.9 ± 0.3 ^{cD}
10	2.9 ± 0.1 ^{aE}	5.7 ± 0.2 ^{bE}	7.7 ± 0.3 ^{cE}
12	3.1 ± 0.1 ^{aE}	5.9 ± 0.4 ^{bE}	7.9 ± 0.4 ^{cE}
15	3.1 ± 0.1 ^{aE}	6.0 ± 0.3 ^{bE}	7.9 ± 0.4 ^{cE}

$^{a–c}$Values are significantly different for the same treatment duration ($p < 0.05$).
$^{A–E}$Values are significantly different at the same power level ($p < 0.05$).

In comparison of these two extraction methods, the values of TPC in all extracts obtained by microwave-assisted extraction for 10 min were higher than those obtained by maceration extraction for 1 h. The TPC values of the olive leaf, branch, and pomace extracts prepared under microwave irradiation at 150, 500, and 700 W for 10 min were 1.0−1.2, 1.9−2.3 and 2.4−3.0 times those of the extracts prepared by maceration extraction for 1 h, respectively. This enhancing effect on phenolic extraction by the microwave-assisted method may be due to the destruction of plant material after microwave treatment (Zhang et al., 2008). Microwave treatment is thought to cause a rapid increase in temperature and pressure inside the plant material which promotes cell rupture and

the consequent release of phenolic compounds into the surrounding solvent. These results indicate that the microwave-assisted method has higher extraction efficiency with dramatically reduced extraction time, resulting in its consideration as an appropriate alternative for conventional maceration method, for the extraction of phenolic compounds from olive by-products.

3.3 Comparison of antioxidant capacity of the extracts prepared by maceration and microwave-assisted extraction methods

In general, many phenolic compounds have the potential to function as antioxidants by scavenging or stabilising free radicals involved in oxidative processes through hydrogenation or complexation with oxidising species (Rietjens et al., 2007). The antioxidant capacities of olive leaf, branch, and pomace extracts obtained by maceration extraction for 1 h, and microwave-assisted extraction at different microwave power levels for 10 min, were investigated. The extracts with different TPC levels exhibited DPPH radical-scavenging (Table 5) and SOD-like activities (Table 6). In addition, highly positive correlations were also found between the TPC value and the DPPH radical-scavenging activity or SOD-like activity in all extracts. These results suggest that the olive extracts obtained by these two extraction methods have potential bioactive properties with antioxidant capacities, which depend on the concentrations of total phenolic compounds in the extracts.

Table 5: DPPH radical-scavenging activities (µmol TE/g DW) of extracts prepared by the different extraction methods.

Extraction method and coefficient of correlation (*r*)	Olive leaf extract	Olive branch extract	Olive pomace extract
Maceration	285.8 ± 19.0 ^{aA}	66.7 ± 2.5 ^{bA}	23.7 ± 2.0 ^{cA}
Microwave at 150 W	330.1 ± 13.4 ^{aA}	71.3 ± 8.8 ^{bA}	25.4 ± 1.3 ^{cA}
Microwave at 500 W	645.8 ± 14.3 ^{aB}	100.7 ± 14.1 ^{bB}	42.8 ± 3.0 ^{cB}
Microwave at 700 W	721.7 ± 39.1 ^{aC}	170.7 ± 10.3 ^{bC}	63.6 ± 6.0 ^{cC}
r	0.987*	0.971*	0.989*

$^{a–c}$Values are significantly different for the same treatment duration ($p < 0.05$).

$^{A–C}$Values are significantly different for the same extract ($p < 0.05$).

*Pearson's correlation coefficient between DPPH radical-scavenging activity and TPC ($p < 0.05$).

Table 6: SOD-like activities (%) of extracts prepared by the different extraction methods.

Extraction method and coefficient of correlation (*r*)	Olive leaf extract	Olive branch extract	Olive pomace extract
Maceration	63.0 ± 1.2 ^{aA}	18.4 ± 3.5 ^{bA}	9.8 ± 0.9 ^{cA}
Microwave at 150 W	66.3 ± 2.2 ^{aA}	25.1 ± 1.5 ^{bA}	11.5 ± 0.8 ^{cA}
Microwave at 500 W	76.0 ± 0.8 ^{aB}	43.9 ± 4.1 ^{bB}	17.3 ± 2.0 ^{cB}
Microwave at 700 W	99.0 ± 0.6 ^{aC}	55.3 ± 2.4 ^{bC}	32.0 ± 1.3 ^{cC}
r	0.951*	0.986*	0.952*

$^{a–c}$Values are significantly different for the same treatment duration ($p < 0.05$).

$^{A–C}$Values are significantly different for the same extract ($p < 0.05$).

*Pearson's correlation coefficient between SOD-like activity and TPC ($p < 0.05$).

4. Conclusions

The results of this study demonstrated that microwave-assisted extraction was more effective than maceration extraction for the extraction of phenolic compounds from olive by-products (leaves, branches, and pomace). The phenolic yields were higher via microwave-assisted extraction than via maceration extraction, and this further increased with increasing microwave power. The phenolic compound content in the extracts obtained by microwave-assisted extraction at 500 and 700 W was over twice of that in the extracts obtained by maceration extraction. Moreover, the phenolic yields in microwave-assisted and maceration extractions reached a plateau after 10 min and 1 h, respectively, indicating that microwave-assisted extraction was able to extract phenolic

compounds in a much shorter time period, when compared with maceration extraction. Indeed, the extracts possessed antioxidant activities, such as DPPH radical-scavenging and SOD-like activities, in proportion to the content of phenolic compounds. Thus, the microwave-assisted technique has advantages over the conventional maceration technique owing to its reduced extraction time and higher extraction efficiency, which makes it a favourable method for the extraction of phenolic compounds from olive by-products. Therefore, the microwave-assisted extraction method may be a sustainable and environmentally friendly technique to extract high value-added natural ingredients for applications in foods and cosmetics. In future studies, microwave extraction techniques may be combined with other advanced technologies, such as ultrasonic, high-pressure, and vacuum, to further enhance the extraction efficiency of phenolic compounds from olive by-products. A scale-up method for the extraction of phenolic compounds can also be developed.

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