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FULL PAPER

Effects of domestic cooking on the bioactive compounds of pohpohan leaves (*Pilea trinervia* L)

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Abstract

This study aimed to analyze the influence of domestic cooking methods on the total phenolic content (TPC), antioxidant activity, and bioactive compounds of pohpohan leaves. This is the first study to examine the effect of domestic processing on the bioactive compounds of pohpohan leaves. The cooking methods included boiling for 5 and 15 mins at 100°C, boiling for 5 and 15 mins at 70°C, steaming for 5 and 15 mins, and heating by microwave for 1 and 3 mins. The TPC was determined by using the Folin-Ciocalteu method, while the antioxidant activity analysis was conducted by 2,2-diphenyl-1-picrylhydrazyl assay. Moreover, the bioactive components (caffeic and ferulic acids) were analyzed by a reversed phase-ultra high-performance liquid chromatography. The results showed that domestic cooking significantly decreased the TPC and antioxidant activity of pohpohan leaves, with the lowest changes recorded for steaming and microwave heating. The highest TPC was observed in raw leaves extract, measuring about 84.43±3.43 mg GAE/100 g wet basis, followed by microwave heating, steaming, and boiling, respectively. The highest antioxidant activity was measured in raw leaves extract, followed by steaming and boiling. Additionally, microwave heating significantly enhanced the antioxidant activity of pohpohan leaves and preserved the ferulic and caffeic acid content. In summary, domestic processing methods have different effects on the preservation of TPC, antioxidant activity, and bioactive compounds and these results are implied in the functional properties of pohpohan leaves.

1. Introduction

Pohpohan (*Pilea trinervia* L.) is one of the indigenous vegetables that are commonly consumed by Indonesians. Pohpohan is widely consumed in West Java as fresh vegetable (*lalapan*). It is a wild plant that usually grows at the edge of forests, fields, and gardens, far from the residential areas. Its productivity is concentrated in several locations in West Java, such as Ciapus with a wide area of 8.42 kg/ha, Cipanas with 4.95 kg/ha, and Tasikmalaya with 1.43 kg/ha (Prawati, 2011).

Pohpohan leaves contain several phenolic and flavonoid compounds. These compounds are important substances that act as antioxidants by breaking free radical reactions (Amalia, 2006). Raw pohpohan leaf extracts contain phenolic compounds such as luteolin, quercetin, and kaempferol (Batari, 2007). Additionally, several previous studies have shown that pohpohan extract could act as an anti-diabetic agent in male mice (Rahayuningsih and Shinta, 2015) and exhibits antibacterial (Khudry, 2014) and antioxidant activity (Andarwulan *et al*., 2010). Based on the findings of Kurniasih (2010), pohpohan leaves contain 87.68% of water, 25.17% of protein, 5.12 mg/100 g of total carotenoid samples, 90.24 mg/100 g of ascorbic acid samples, 0.75 mg/100 g of anthocyanin samples, 1.48 mg/100 g of beta-carotene samples, and 27% of fatty acid (Kurniasih, 2010; Prawati, 2011).

Nevertheless, pohpohan leaves are also commonly consumed in cooked form such as soup (Asdhiana, 2014). Several studies have been conducted to determine the effects of the types of household processing on the antioxidant content and capacity of vegetables. Vegetable processing such as boiling, steaming, and frying could reduce the nutritional quality and affect the physicochemical properties of plants (Miglio *et al*., 2008). In another study, it was reported that among processing methods such as boiling, steaming, microwave heating, and frying, processing by steaming

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was considered the best method to minimize the decrease in polyphenols and antioxidant activity in sweet potato leaves (Sun *et al*., 2014).

Cooking with heat can change the levels and activity of bioactive components in vegetables. In fact, boiled cabbage leaves contained lower total phenolic content (TPC) and antioxidant activity than raw cabbage (Girgin and El, 2015). A subsequent study showed a significant reduction in the crude fat content due to processing compared to raw conditions (Sun *et al*., 2014). Thus, it is necessary to research the effect of processing on the phenolic content and antioxidant activity in pohpohan leaves. This is the first study to measure the effect of domestic processing on the bioactive compound of pohpohan leaves. The purpose of this study was to examine the effect of household-scale processing (boiling, steaming, and microwave heating) on the phenolic content, antioxidant activity, and bioactive components contained in pohpohan leaves. This study will help identify the most appropriate method to process pohpohan leaves.

2. Materials and methods

2.1 Materials

The raw pohpohan leaves were obtained from Citayam Market (Depok), West Java, Indonesia. The materials used for the analysis were obtained from Merck, Darmstadt, Germany, and were HPLC grade methanol, Folin-Ciocalteu reagent, Na_2CO_3, and ascorbic acid. Additionally, gallic acid (Acros Organics, New Jersey, USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO), and standard trans-ferulic acid and caffeic acid (Sigma-Aldrich, St. Louis, MO) were used.

2.2 Sample preparation

A total of 5.0 kg of samples was immediately cleaned and sorted to separate dirty and damaged leaves. The sorted leaves were then weighed at 6.25 g for each treatment such as boiling, steaming, and microwave heating. Each treatment and replication had two apical buds and several large leaves. Raw samples that had been directly extracted with methanol were used as the control.

2.3 Processing methods

The processing methods used were boiling, steaming, and microwave heating. For boiling at 100°C, 6.25 g of the sample was cooked using a stainless-steel pan with 500 mL of distilled water. The sample was cooked for 5 and 15 mins after the water reached boiling point. For boiling at 70°C, 6.25 g of the sample was cooked using a 500 mL beaker in a water bath shaker with 500 mL of distilled water. After the water temperature stabilized at 70°C, the sample was cooked for 5 and 15 mins. For steaming, 6.25 g of the sample was placed in a vessel and steamed for 5 and 15 mins after the temperature reached 100°C (boiling). For microwave heating, 6.25 g of the sample was placed in a glass container and heated for 1 and 3 mins in a 450 W commercial microwave.

2.4 Sample extraction

The extraction method used in this study was based on the modified Mediani *et al*. (2013) method. The dried pohpohan leaves were added to 25 mL of 100% methanol (HPLC grade). Then, the sample was sonicated in a water bath sonicator at 30°C for 1 hr. Subsequently, the extract was centrifuged at 10,000 rpm at 4°C for 20 mins. Afterwards, it was separated from the precipitate using filter paper and stored in an amber bottle at 4°C until analysis was carried out.

2.5 Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) was analyzed using the Folin-Ciocalteu method (Yoshimoto *et al*., 2002). The analysis of antioxidant activity on the cosmos leaves was carried out using the modified DPPH method (Ahmad *et al*., 2009). The modification of the method used was applied to the volume of the extract, and milli-Q and DPPH were used. The decrease in the absorbance of DPPH free radicals was read at a wavelength of 517 nm. Additionally, 100% methanol was used as the control.

2.6 Determination of bioactive compounds

The analysis of caffeic and ferulic acid levels in the pohpohan leaves was carried out based on the Sun *et al*. (2014) method with reversed phase-ultra high-performance liquid chromatography (RP-UHPLC). Detection and quantification were carried out with a photodiode array detector at a wavelength of 300 nm, and separation was carried out with a C-18 column with a length of 10 cm. The mobile phase consisted of acetonitrile (eluent A) and 3% formic acid in milli-Q (eluent B) using a gradient system (Table 1). The eluent

Table 1. The eluent gradient experiment

Time (mins)	Eluent A (%)	Eluent B (%)
0-2	10	90
2-7	30	70
7-12	40	60
12-17	60	40
17-22	80	20
22-25	100	0

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flow rate was measured at 0.3 mL/min. The amount of caffeic and ferulic acids were expressed in units of mg/100 g WB of pohpohan leaves.

2.7 Data analysis

The resulting data were analyzed using analysis of variance with the SPSS 20 program, with α = 0.05. If the difference was significant, Duncan's difference test was conducted on it. A correlation analysis (r) was conducted to determine the relationship between the TSF content and the antioxidant activity using the SPSS 20 program.

3. Results and discussion

3.1 The physical appearance of pohpohan leaves

The processing methods were divided into three, boiling, steaming, and microwave heating. The boiled leaves became very wilted, and the water used turned into a yellowish-green colour. This was presumably because of the presence of chlorophyll dissolved in the water. The steamed leaves were a little wet but not as wilted as the boiled leaves, while the microwave-heated leaves became dry as the heating time increased. The reason behind this is the evaporation of the water contained in the leaves. The results of the processing of pohpohan leaves can be seen in Figure 1.

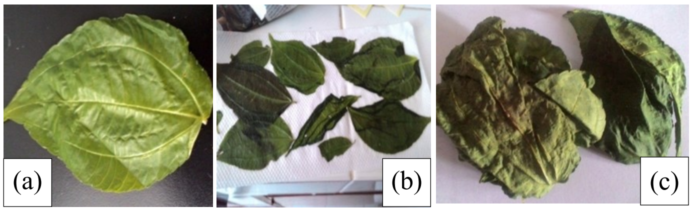

Figure 1. Pohpohan leaves: a) raw leaves, b) boiled leaves, and c) microwaved leaves

3.2 Total phenolic content

The total phenolic content (TPC) in the pohpohan leaves ranged from 20.24 to 84.43 mg of GAE/100 g WB (Table 2). The raw pohpohan leaves had the highest TPC (84.43 mg of GAE/100 g WB). The TPC in this study was greater than that in the previous study by 70.11 mg of GAE/100 g WB (Batari, 2017). However, the difference was probably due to the differences in pohpohan leaves samples and extraction methods. The lowest TPC was found in the leaves boiled for 15 mins at 100°C (20.24±0.91 mg of GAE/100 g WB).

Boiling the pohpohan leaves at 100°C for 5 and 15 mins reduced ($p<0.05$) its TPC by 75.14% and 76.03% compared to the TPC of raw leaves (Table 2). TPC tended to decrease with greater boiling time. The TPC of leaves boiled for 5 mins at 100°C (20.99 mg of GAE/100 g BS) was slightly higher than those boiled for 15 mins at the same temperature (20.24 mg of GAE/100 g BS). This is the result of long contact between the leaves with water and heat. Hence, the longer the contact, the more phenolic compounds were lost. The phenolic compounds in vegetables are generally polar, as they are bound to sugars (Yulia, 2007). Flavonoid, one of the phenolic components, is polar, as it is bound to sugars. Therefore, the flavonoid is a polar compound soluble in polar solvents such as ethanol, methanol, acetone, water, and others (Melodita, 2011). In the presence of water, the loss of phenolic compounds due to boiling would be higher than steaming, as the boiled leaves were in direct contact with the water. This is based on the principle that polar solvents dissolve polar substances, while non-polar solvents dissolve non-polar substances (Zumdahl and Zumdahl, 2010).

Table 2. Total phenolic content of pohpohan leaves

Processing	TPC (mg GAE/100 g WB)	Change** (%)
Raw	84.43±3.43^{a}	-
Boiling (100°C; 5 mins)	20.99±0.23^{b}	-75.14
Boiling (100°C; 15 mins)	20.24±0.91^{b}	-76.03
Boiling (70°C; 5 mins)	24.67±1.58^{c}	-70.79
Boiling (70°C; 15 mins)	24.45±0.85^{c}	-70.71
Steaming (5 mins)	43.84±1.83^{d}	-48.07
Steaming (15 mins)	49.90±2.37^{e}	-40.9
Microwave (1 min)	61.31±2.66^{f}	-27.39
Microwave (3 mins)	69.70±1.76^{g}	-17.44

Values are presented as mean±SD, n = 4. Values with different superscript within the same column are significantly different at $p<0.05$. WB, wet basis.
**% Change = (TPC Pohpohan leaves processing – TPC raw pohpohan)/TPC Pohpohan leaves processing × 100%

In contrast to the boiling method, the longer steaming and microwave heating times can increase ($p<0.05$) the TPC in pohpohan leaves because heat damages the food matrix. The phenolic compounds in the matrix can be extracted better despite the degradation of phenolic compounds due to heat (Oboh and Rocha, 2007). Additionally, the absence of water, which could dissolve these phenolic compounds, reduced the loss of phenolic compounds in the steamed and microwaved extracts. In comparison to the TPC in raw leaves, steaming for 5 and 15 mins reduced ($p<0.05$) the TPC by 48.07% and 40.90%, while microwave heating for 1 and 3 mins reduced the TPC by 27.39% and 17.44%, respectively. Processing could free flavonol (kaempferol glycosides) but remove flavanol (monomeric and condensed tannins) (Zhang *et al.*, 2014). Furthermore, processing could produce free ferulic acid bound to the matrix (Boz, 2015).

3.3 Antioxidant activity

The DPPH method was used to analyze the

antioxidant activity of pohpohan leaves. The percentage of inhibition in raw pohpohan leaves before and after domestic processing can be seen in Table 3.

Table 3. Radical scavenging activity (RSA) of pohpohan leaves

Processing	RSA	Change**(%)
Raw	65.74±2.59[a]	-
Boiling (100°C; 5 mins)	28.22±0.79[b]	-57.07
Boiling (100°C; 15 mins)	28.50±0.35[c]	-56.65
Boiling (70°C; 5 mins)	31.96±1.59[b]	-51.39
Boiling (70°C; 15 mins)	27.43±0.62[c]	-58.27
Steaming (5 mins)	54.46±2.31[d]	-17.16
Steaming (15 mins)	55.95±3.57[d]	-14.89
Microwave (1 min)	68.03±2.98[a,e]	+3.49
Microwave (3 mins)	71.40±3.49[e]	+8.61

Values are presented as mean±SD, n = 4. Values with different superscript within the same column are significantly different at p<0.05. WB, wet basis.
**% Change = (TPC Pohpohan leaves processing – TPC raw pohpohan)/TPC Pohpohan leaves processing × 100%

The percentage of radical inhibition in pohpohan leaves ranged from 27.43% to 71.40%. The raw pohpohan leaves had a higher percentage of radical inhibition ($p<0.05$) than the boiled and steamed leaves, as they contained a higher TPC. Different results were obtained when comparing the percentage of radical inhibition in raw leaves to those heated by microwave, as microwave heating reduced the TPC. However, the microwave-heated leaves had a higher percentage of radical inhibition than raw leaves.

The highest percentage of inhibition was observed in the leaves heated by microwave for 3 mins. This result was not directly proportional to the TPC, which can be seen in Table 2, as the high-heating process caused the release of glycosylated phenolic compounds, resulting in an increase in free phenolic compounds (Hayat *et al*., 2010). Glycosylated flavonoids tend to decrease antioxidant activity because of a decrease in the number of phenolic groups (Brewer, 2011). Some phenolic compounds have fairly good heat resistance, such as luteolin (Lin *et al*., 2008), quercetin-3,4'-O-diglucoside and quercetin-4'-O-monoglucoside (Sharma *et al*., 2015), 5-O-caffeoylquinic acid, and 3,5-di-O-caffeoylquinic acid (Takenaka *et al*., 2006).

A decrease in the percentage of radical inhibition was observed in the steamed and boiled pohpohan leaves (Table 3). Compared to the raw pohpohan leaves extract, boiling pohpohan leaves decreased the ability to inhibit radicals by more than 50%, while steaming reduced the radical inhibition by 14–18%. These results were supported by research conducted by Hayat *et al*. (2010) and Zhuang *et al*. (2011). This decrease in the ability of radical inhibition can be caused by the loss of antioxidant compounds contained in the materials (Rahim *et al*., 2010). Heat treatment affects plant cell membranes, causing the loss of phenolic compounds from vegetable tissue to the cooking media (Hayat *et al*., 2010; Zhuang *et al*., 2011).

The linear regression analysis demonstrated the relationship between the TPC and the per cent inhibition of pohpohan leaves is presented in Figure 2. There is a positive correlation between the TPC and the per cent inhibition with a correlation coefficient value of R^2 = 0.88. This data showed that the antioxidant activity of pohpohan leaves was affected by the TPC. Previous research has outlined the relationship of positive correlation between antioxidant activity and TPC. Lai and Lim (2011) reported a strong correlation between the total value of phenolic compounds and the percent inhibition in the fern plant extracts with a correlation coefficient value of R^2 = 0.95. Other studies have demonstrated that there is a positive relationship between the total antioxidant compounds and the antioxidant capacity, such as in asparagus and broccoli (Sun *et al*., 2007), lemongrass leaves (Sah *et al*., 2012), as well as leaf and flower vegetables (Mandarini, 2014).

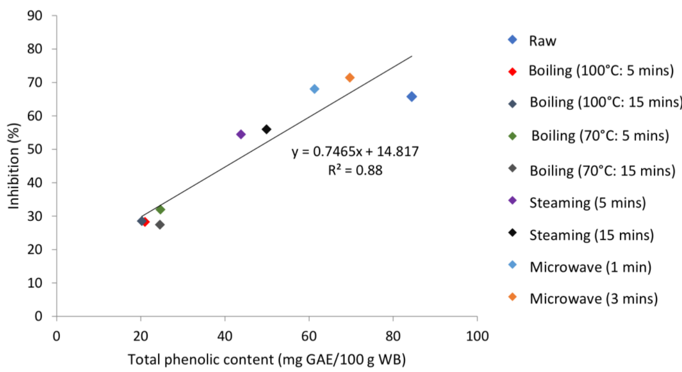

Figure 2. Correlation between TPC and RSA (%)

3.4 Bioactive compounds of pohpohan leaves

The measurement of two active compounds (ferulic acid and caffeic acid) contained in the pohpohan leaves was carried out using RP-UHPLC (Table 4). The ferulic acid content of pohpohan leaves ranged from 1.48 to 6.85 mg/100 g WB, with the highest content recorded in leaves processed using microwave heating treatment. The presence of ferulic acid release in the matrix upon heating (Boz, 2015) results in high levels of ferulic acid after the microwave heating process. Consequently, this increase in ferulic acid causes a rise in radical inhibition by 8.61% (Table 2) in leaves heated by microwave for 3 mins. The microwave heating process can increase the TPC and antioxidant activity in plants compared to raw leaves (Nusrat *et al*., 2019).

Processing by boiling did not lead to detectable ferulic acid content, possibly due to the degradation and loss of ferulic acid in the boiling media. However, the content of caffeic acid in the pohpohan leaves ranged from 1.28 to 6.55 mg/100 g WB, while the highest content was observed in leaves processed using microwave treatment. Ferulic acid and caffeic acid belong to the phenolic acid group (Giada, 2013). Furthermore, the phenolic acid can be divided into two groups, namely, benzoic and cinnamic acid derivatives, which can be characterized by the presence of a benzene ring, a carboxyl group, and one or more hydroxyl and/or methoxyl groups. The phenolic acids are bound with sugars or organic acids and are usually components of complex structures such as lignin and tannin (Lattanzio, 2013).

Table 4. Bioactive compound content of pohpohan leaves

Processing	Ferulic acid (mg/100 g WB)	Caffeic acid (mg/100 g WB)
Raw	1.48	1.33
Boiling (100°C; 5 mins)	nd	1.28
Boiling (100°C; 15 mins)	nd	nd
Boiling (70°C; 5 mins)	nd	1.06
Boiling (70°C; 15 mins)	nd	2.39
Steaming (5 mins)	3.09	2.73
Steaming (15 mins)	1.52	3.59
Microwave (1 min)	2.45	1.98
Microwave (3 mins)	6.85	6.55

WB: Wet Basis, nd: no detection

4. Conclusion

The highest TPC was observed in the methanol extract of raw pohpohan leaves, measuring 84.43±3.43 mg of GAE/100 g WB, followed by the methanol extract of leaves processed using microwave heating, steaming, and boiling. Regarding antioxidant activity, boiling and steaming treatments significantly reduced the percentage of radical inhibition, while microwave heating for 3 mins significantly increased the radical inhibition of pohpohan leaves. Additionally, boiling and steaming treatment decreased the antioxidant capacity in comparison to the raw leaves. Furthermore, microwave processing for 3 mins could increase the content of ferulic and caffeic acids in the pohpohan leaves.

Conflict of interest

The authors declares that there is no conflict of interest.

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