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Extraction of Coffee Silverskin to Convert Waste into a Source of Antioxidant

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Abstract. Coffee silverskin (CS) is a thin layer of coffee bean, and is regarded as a waste during coffee roasting process. In this work, coffee silverskin was extracted by three types of method: conventional extraction (CE) with agitation, conventional extraction (CE) without agitation and ultrasound-assisted extraction (UAE). The total phenolic content, the total flavonoid content and the antioxidant activity of the extract were analyzed. It was found that the type of extraction method, the extraction time and the extraction temperature strongly influenced the total phenolic content, the total flavonoid content and the antioxidant activity of the extract. Comparison between conventional extraction (CE) and ultrasound-assisted extraction (UAE) were statistically analyzed using 3-way ANOVA test. The optimum extraction time and temperature for each method were analyzed using 2-way ANOVA test. It was found that the optimum condition to obtain a high antioxidant activity of 68.9% was by using CE with agitation with the extraction time and temperature of 60 minutes and 60˚C, respectively.

Keywords: Coffee Silverskin, Ultrasound-assisted extraction, Conventional extraction, Antioxidant Activity.

INTRODUCTION

Coffee is the second most commonly traded commodities after crude oil and their derivatives (Njoroge, et al 2005). According to the Indonesian Ministry of Agriculture, the amount of coffee production in Indonesia in 2014 was 675.881 tons. In the coffee production, the coffee cherries are harvested from the plantation when they have ripened. Then, the pulp and the parchment of the beans are removed by using either dry or wet method. The final product of this process is green bean, which is commonly still wrapped by a thin outer layer of the coffee beans, called coffee silverskin. Figure 1 shows the structure of a coffee cherry. During the roasting process, the coffee bean is exposed at a high temperature, causing the water contained in the beans to evaporate rapidly, and the silverskin detaches from the beans. In Indonesian coffee roasting industries, this coffee silverskin is considered as a waste. In some countries, the coffee silverskin is mostly used as fertilizers (Saenger, et al., 2001).

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Coffee Silverskin (CS) represents about 4.2% (w/w) of coffee beans (Ballesteros, Teixeira, & Mussatto, 2014). Some researchers have investigated the potential of coffee silverskin, and reported that coffee silverskin can be used as a natural source of prebiotic ingredients, fibre and antioxidants (Borrelli, R.C. *et al.,* 2004). Recent studies have indicated that phenolic compounds have antioxidant (Stratil, P. et al., 2006) antiviral (Chiang, L.C. et al., 2002), anti-inflammatory (Paulino, N. et al., 2008) antitumor (Sawa, T. et al., 1999), and immunomodulatory effects (Park, J.H. et al., 2004)

In this work, in order to utilize coffee silverskin as a source of antioxidant, the coffee silverskin was extracted by three types of method: conventional extraction (CE) with agitation, conventional extraction (CE) without agitation and ultrasound-assisted extraction (UAE). The objective of this research is to study the effect of the extraction method, the extraction time and the extraction temperature on the total phenolic content, total flavonoid content and antioxidant activity of the extract.

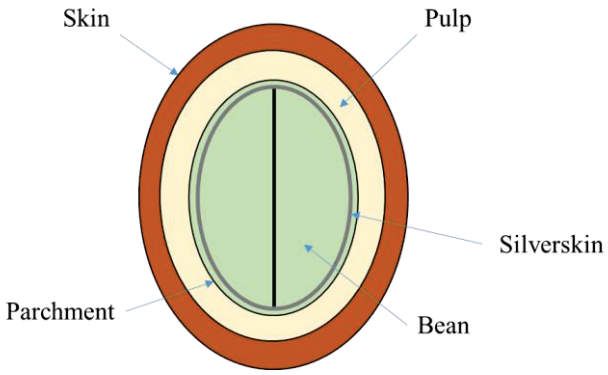

FIGURE 1. Coffee cherry structure

EXPERIMENTAL

Materials

Coffee silverskin was obtained from Arabica coffee roasting process and provided by Morph Coffee Roasting (PT Kopi Tiga Selaras), Jakarta, Indonesia. Folin-Ciocalteu phenol reagent, Gallic acid and 2,2-Diphenyl-1-picryhlhydrazyl (DPPH) were obtained from Sigma-Aldrich, Germany. Sodium carbonate, aluminum chloride and potassium acetate were used and obtained from Merck, Germany.

Equipments

The equipments used in this experiment were hot plate Cimarex (Barnsteadthermolyne, USA), magnetic stirrer, thermometer, Whatmann filter paper 1001 125 (GE, UK), Sonicator Bath Sonorex Super 10P (Bandelin , Germany), Genesys 10 UV-Vis Spectrophotometer (Thermo Electron Corporation, USA), Digital Balance ED 6202S-CW (Sartorius, Germany) and Micropipette (Dumo, Indonesia).

Extraction Experiment

For the conventional extraction (CE), first 100 ml of water-ethanol mixture with a concentration of 50% was filled into a 200 ml beaker and was heated using a hot plate. The temperature was varied at 30, 40, 50 and 60˚C. The temperature was monitored using a thermometer and when the temperature is reached, 2 grams of coffee silverskin were poured into the beaker. The beaker was covered with aluminum foil to prevent heat loss and evaporation of the solvent. The extraction time was varied at 5, 10, 20, 30, 40, and 60 minutes. The conventional extraction was carried out with and without agitation. For CE with agitation, a magnetic stirrer was put in the middle of the beaker, and the stirring was set at a level of 6 (350 rpm). The extract was then collected and filtered using filter paper (Whatmann 1001 125, GE, UK).

For the ultrasound-assisted extraction (UAE), Sonicator Bath Sonorex Super 10P (Bandelin , Germany) was used. First, the ultrasonic bath was filled with water and the temperature was varied at 30, 40, 50 and 60˚C. Then 100 ml of solvent (water-ethanol 50%) was filled into a 200 ml beaker and placed in the ultrasonic bath. When the temperature is reached, 2 grams of coffee silverskin was poured into beaker. The beaker was sealed with an aluminum foil to prevent water entering during the process. The extraction temperature was varied at 30, 40, 50 and 60˚C, and the extraction time was varied at 5, 10, 20, 30, 40, 60 minute. Then the beaker was put inside the ultrasonic bath. Top cover of ultrasound was closed and the ultrasonic bath was started at afrequency of 35 kHz. The coffee silverskin (CS) extract was then collected and filtered using filter paper.

Analysis

The total phenolic content (TPC) of the samples was measured based on the modified method of Alves et al. (2010). Firstly, the coffee silverskin extract was diluted with 1:10 ratio and was mixed with 2.5 ml of Folin-Ciocalteu phenol reagent and 2 ml of sodium carbonate solution. The mixture was then vortex and incubated in dark at room temperature for 1 hour. After the incubation, the mixture was poured into cuvette and was directly checked by the spectrophotometer at 765 nm. The absorbance was then noted and checked to the equation of gallic acid standard calibration curve. The phenolic content was expressed as mg of gallic acid equivalents (GAE)/l of extract.

The total flavonoid content (TFC) of the silverskin extract was determined by the modified method of Alves et al. (2010). Briefly, 500 µL of coffee silverskin extract was mixed with 1.5 mL of methanol and was then brought to a vortex. For blank sample, 500 µL sample was substituted with 500 µL of distilled water. Each test tube were poured with 100 µL of aluminium chloride 10% and vortex and then 100 µL of 1 M potassium acetate, addition of 2.8 mL of distilled water were altogether mixed and vortex. The mixture were incubated at room temperature for 30 minutes and

then measured at 415 nm using a UV-Vis spectrophotometer. The flavonoid content was expressed as mg of quercetin equivalents (QE)/l of extract.

The DPPH radical scavenging activity / antioxidant activity (AA) towards coffee silverskin extract was determined by 50 µl of coffee silverskin extract mixed with 800 µl of ethanol (96%) and 150 µl of DPPH stock solution. The mixture was vortex and then sealed with aluminum foil and incubated in a dark room at room temperature for 30 minutes. After incubation, the mixture was poured into cuvettes and then measured at 515 nm using a spectrophotometer. The blank sample from this analysis was 1 ml distilled water and the control was a mixture of 50 µl of distilled water and 800 µl of ethanol (96%) and 150 µl of DPPH stock solution. The DPPH radical scavenging activity was calculated using the equation below, where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the coffee silverskin extract.

$$Antioxidant\ Activity\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}}\ X\ 100$$

RESULTS AND DISCUSSION

In the first step of this study, three extraction methods (conventional extraction without agitation, conventional extraction with agitation and ultrasound-assisted extraction) were used to extract phenolic compounds from coffee silverskin. The total phenolic content, the flavonoid content and the antioxidant activity of the extracts were analyzed and compared. Table 1 shows the result of the extraction using different extraction methods.

TABLE 1. Result of the extraction of coffee silverskin using different extraction methods

Method	Total Phenolic Content (TPC) (GAE mg/L)	Total Flavonoid Content (TFC) (QE mg/L)	Antioxidant Activity (AA) (%)
CE without agitation	347.37 (c)	5.57 (c)	39.74 (c)
CE with agitation	459.21 (a)	14.00 (a)	59.94 (a)
UAE	395.39 (b)	9.35 (b)	51.23 (b)

Based on the result in Table 1, the highest amount of all of the three analysis (TPC, TFC, and AA) was achieved by using CE with agitation. CE with agitation is able to form a vortex mixing when 350 rpm is set. In UAE setup, there is ultrasound vibrations which helps in mixing the extract. The TPC, TFC and AA using UAE was higher than using CE without agitation, however they were lower than that using CE with agitation. CE without agitation has no vortex or even vibrations which explains why the amount of the three analysis is the lowest. The sound waves of UAE were able to release more phenolic compounds on the cells of coffee silverskin, however possibly polyphenol oxidase (PPO) was also released to the extract. This PPO enzyme which is present in plant cells are able to change the structure

of monophenol into a diphenol and into quinone (Espin et al., 2000), resulting in a lower total phenolic content, flavonoid content and antioxidant activity.

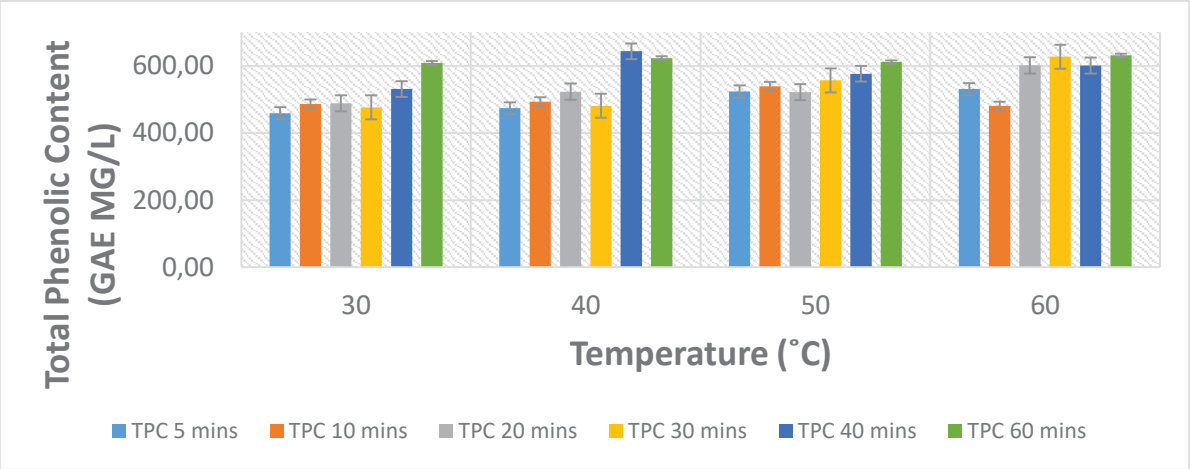

FIGURE 2. Effect of time and temperature on total phenolic content for CE with agitation.

Figure 2 shows the effect of extraction time and extraction temperature on the total phenolic content for conventional extraction with agitation. As seen in Figure 2, the time of extraction correlates dependently towards the total phenolic content. The longer the extraction time resulted in a higher amount of total phenolic content. The increasing temperature resulted in a slight increase in the total phenolic content. The highest amount of total phenolic content of 643 GAE mg/l was obtained at an extraction time of 40 minutes and an extraction temperature of 40˚C. This result is in accordance with the statistical analysis which shows that the optimum extraction condition is at 40 minutes and 40˚C.

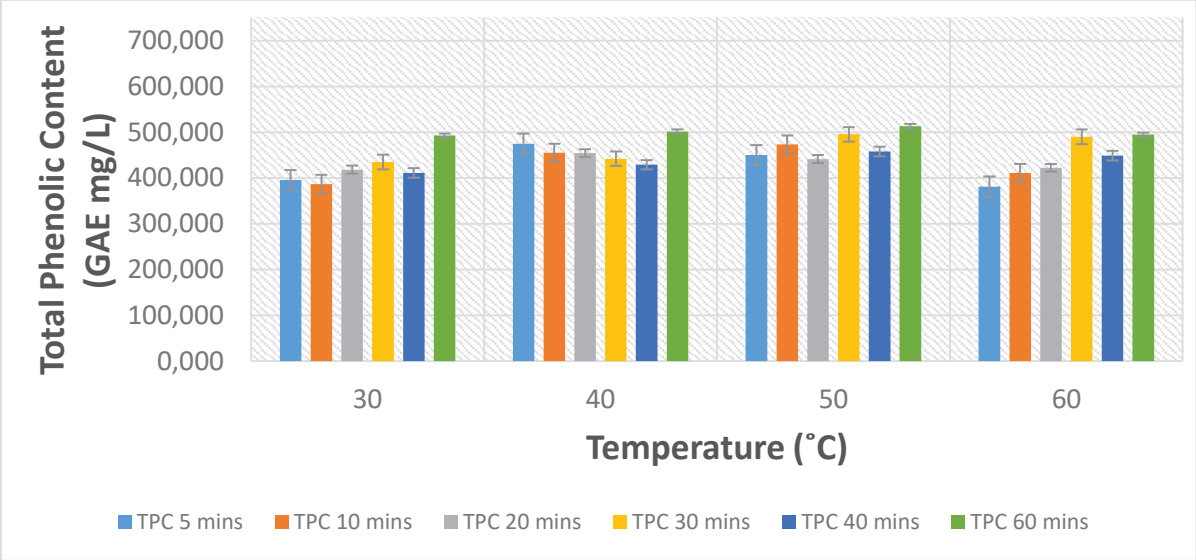

FIGURE 3. Effect of time and temperature on total phenolic content for UAE.

Figure 3 shows the effect of extraction time and extraction temperature on the total phenolic content for ultrasound-assisted extraction. In general, an increase in the extraction time resulted in a higher amount of total phenolic content, however at 40°C a decrease in total phenolic content with time was observed, possibly due to the release of polyphenol oxidase (PPO) as explained previously. The effect of increasing temperature from 30˚C to 50˚C causes a slight increase in the total phenolic content, but when the temperature reached 60˚C the amount of total phenolic content slightly decreases. According to the statistical analysis, the optimum extraction time and temperature are 30 minutes and 50˚C, respectively.

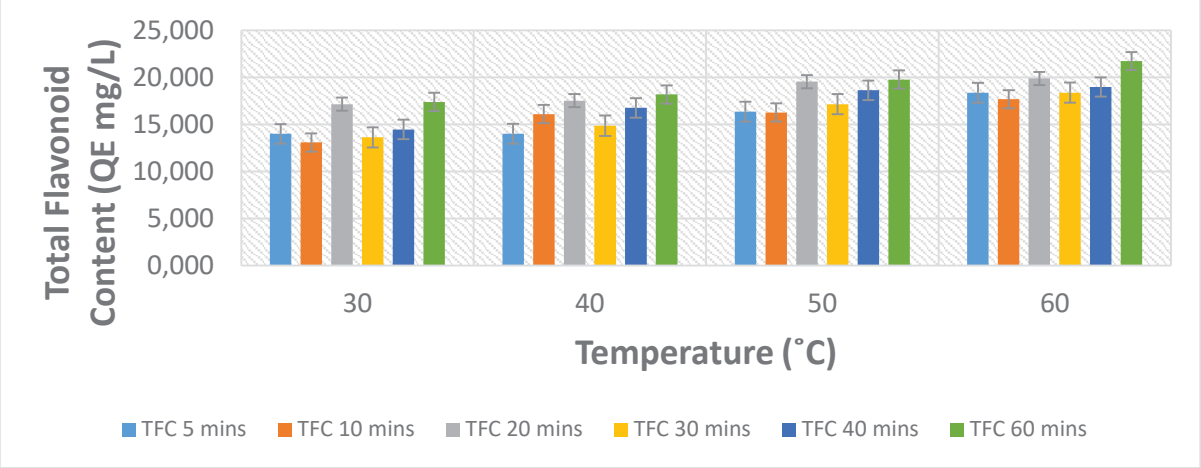

FIGURE 4. Effect of time and temperature on total flavonoid content for CE with agitation.

Figure 4 shows the effect of extraction time and extraction temperature on the total flavonoid content for conventional extraction with agitation. The total flavonoid content does not show a similar pattern with the total phenolic content graph. The effect of extraction time is at its peak at the extraction time of 20 min and 60 min. The temperature increase resulted in the increase in the amount of flavonoid extracted. The highest amount of flavonoid extracted was 21.7 QE mg/l at 60 minutes and 60˚C. The optimum time and temperature according to the statistical analysis are 20 minutes and 60˚C.

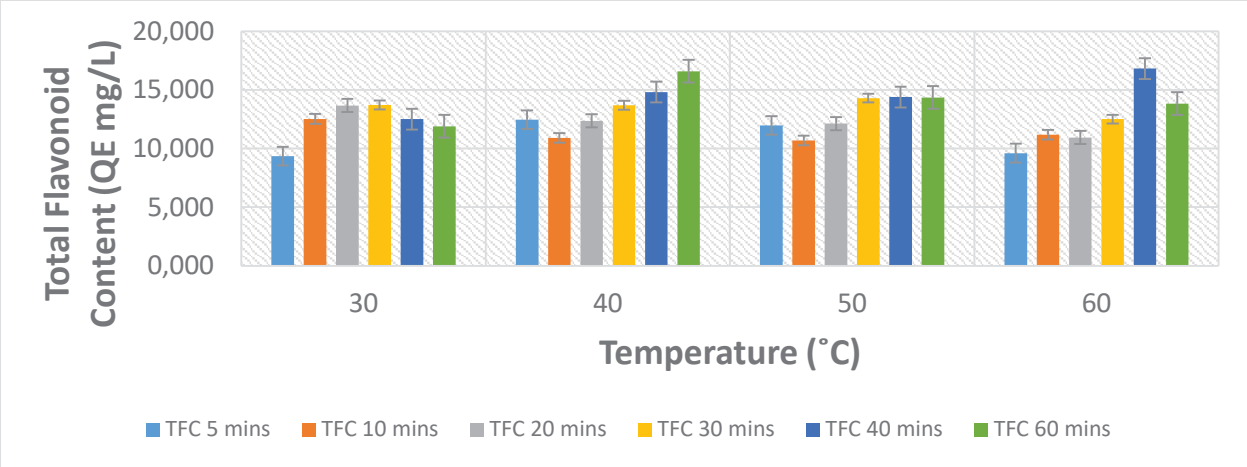

FIGURE 5. Effect of time and temperature on total flavonoid content for UAE.

The effect of extraction time and extraction temperature on the total flavonoid content for UAE method is shown in Figure 5. As can be seen, the extraction time and the extraction temperature strongly influenced the total flavonoid content. According to the statistical analysis, the optimum condition for the extraction time and extraction temperature is 30 minutes and 30˚C, respectively.

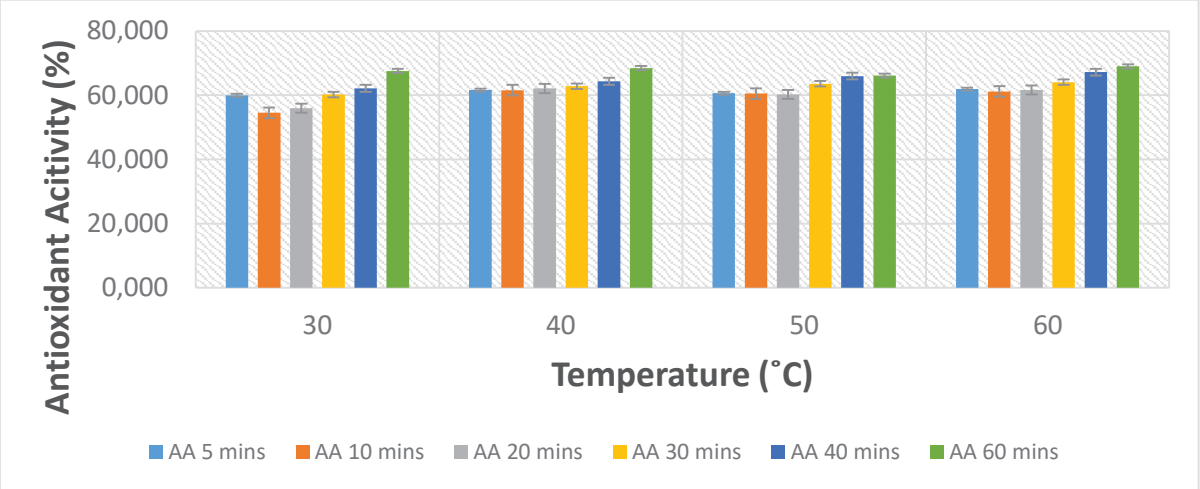

FIGURE 6. Effect of time and temperature on antioxidant activity for CE with agitation.

The antioxidant activity correlates with an increase of extraction time as shown on Figure 6. The longer extraction time resulted in an increase in the antioxidant activity of the extract. The antioxidant activity slightly increased with increasing extraction temperature. The highest antioxidant activity was achieved at 60 minutes of extraction time and 60˚C of extraction temperature as obtained by the statistical analysis.

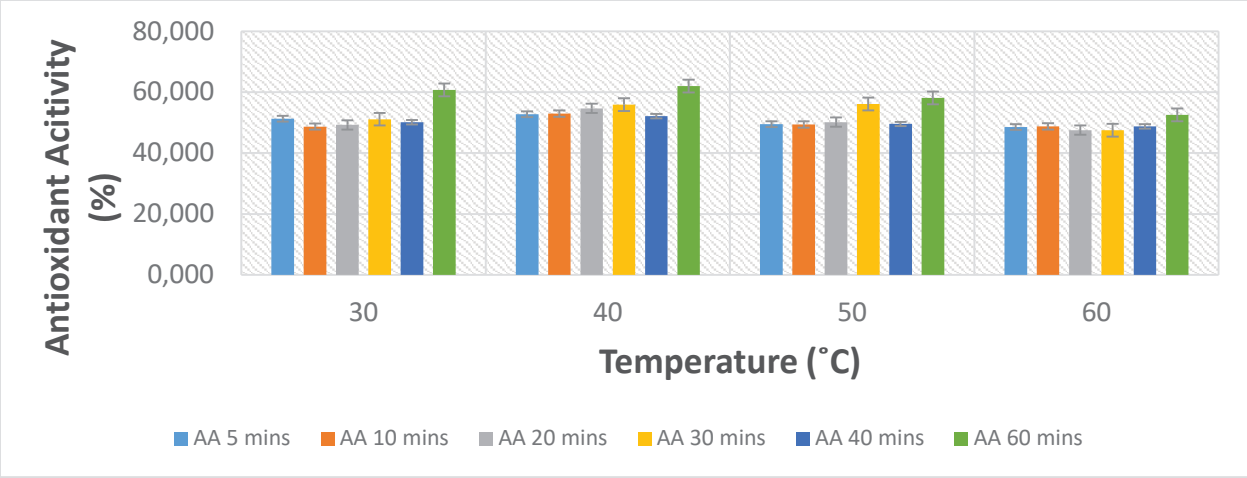

FIGURE 7. Effect of time and temperature on antioxidant activity for UAE.

Using the UAE method, as shown in Figure 7, the pattern of the antioxidant activity towards the extraction time is similar to Figure 6. In general, an increase of extraction time resulted in an increase in the antioxidant activity of the coffee silverskin extract, however a low antioxidant activity was observed at an extraction time of 40 min, since the total phenolic content at 40 min are low as shown in Figure 3. Similar to the pattern in Figure 3, the increase in the temperature does not result in the significant increase in the antioxidant activity of the coffee silverskin extract. According to the statistical analysis, the optimum time and temperature for UAE are 60 minutes and 40˚C, respectively.

CONCLUSION

Coffee silverskin, which is regarded as waste in coffee roasting industries, is very potential to be used as a source of antioxidant since it contains a high amount of phenolic compounds. This research showed that the types of extraction methods, the extraction temperature and the exctraction time strongly influenced the total phenolic content, the total flavonoid content and the antioxidant activity of the extract. Conventional extraction (CE) with agitation shows the highest amount of total phenolic content, total flavonoid content and antioxidant activity. The highest antioxidant activity of 68.9% was achieved using CE with agitation at an optimum extraction time and temperature of 60 minutes and 60˚C, respectively. It was observed that ultrasound-assisted extraction (UAE) is also a potential extraction method to extract the coffee silverskin. UAE resulted in a higher amount of total phenolic content, total flavonoid content and antioxidant activity, compared with CE without agitation.

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