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Optimization of the extraction process of coffee pulp as a source of antioxidant

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Abstract. Coffee pulp is a by-product of coffee cherry processing during the production of coffee beans, and is usually disposed of as waste or used as compost. The valorization of coffee pulp is very important since a large quantity of coffee pulp is produced during coffee processing. In this study, the coffee pulp was extracted using various solvents at different temperatures and times to maximize the total phenolic content and the antioxidant activity of the extract. The total phenolic content was analyzed using Folin-Ciocalteu method, whereas the antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. It was found that the total phenolic content and the antioxidant activity of the coffee pulp extract were strongly influenced by the extraction condition. The coffee pulp extract in powder form produced using a spray dryer also showed a high total phenolic content and a high antioxidant activity. The findings of this study showed that coffee pulp extract has great potential to be used as a source of antioxidants for various products such as foods, beverages, and herbals.

1. Introduction

Coffee is one of the most popular beverages in the world. The coffee industries have become an important key player in the world market due to the increasing supply and demand for coffee [1]. Top coffee producer countries such as Brazil, Vietnam, Columbia, and Indonesia have been focusing on how they can increase the production of coffee beans and fulfill the demand of the world market. Coffee beans are produced from coffee cherries through the wet method or dry method. During the de-pulping and the de-hulling process of the coffee cherries in the coffee processing industries, the coffee pulp is produced as a byproduct. The coffee pulp is usually disposed of as waste or used as compost for the plantation. Since coffee bean has been known to have an antioxidant property [2,3], the study on the bioactive compounds in the coffee byproduct is important with the aim to give an economical added value of this byproduct [4–6]. Our previous research on coffee silverskin - a byproduct from the coffee roasting process - showed that coffee silverskin contains phenolic compounds such as chlorogenic acids which show a high antioxidant activity [7,8]. Coffee silverskin is a thin layer covering the coffee bean, and this layer is usually detached from the coffee bean by the heat during the roasting process. The outer thin layer covering the silverskin is called parchment, while the most outer layer called coffee pulp. Since about 40% of the weight of fresh coffee cherries is coffee pulp [9], an abundant amount of coffee pulp is produced as a byproduct. One method for the valorization of this abundant byproduct is the extraction of the coffee pulp to obtain an extract that can be used as a source of antioxidant. In this work, the optimization of the coffee pulp extraction process was studied with the aim to maximize the



concentration of the phenolic compounds of the extract and thus the antioxidant activity of the extract. Further, coffee pulp extract in the form of powder was produced and was analyzed for its antioxidant activity after the drying process.

2. Experimental

2.1. Materials and equipment

Coffee pulp sample was obtained from a coffee plantation located in West Java, Indonesia. Technical grade ethanol (96% v/v) was purchased from PT Sumber Abadi, a local chemical supplier in Indonesia. Sodium carbonate (BDH, England), Folin-Ciocalteu reagent (Merck, Germany), gallic acid powder (Aktin Chemical, China), aluminum chloride (Merck, Germany), potassium acetate (BDH, England), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich), and analytical grade ethanol (Smart Lab, Indonesia) were used. Distilled water was used.

The equipment used in this work includes Erlenmeyer flasks (Iwaki pyrex), hot plate (Cimarec, USA), beaker glasses (Iwaki pyrex), magnetic stirrer, thermometer, volumetric glass (Pyrex), water bath shaker (Mettler, Germany), analytical balance (Ohaus PA214, USA), filter instrument, Whatmann filter paper 1001 125 (GE, UK), micropipette (Eppendorf, Germany), micropipette tips, cuvette (Brand MBH, Germany), vortex (Vortex-Genie 2, USA), UV-Vis Spectrophotometer (Genesys 10-S, USA), rotary vacuum evaporator (Ika HB 10, China), spray dryer (Buchi mini spray dryer B-290, Switzerland).

2.2. Extraction of coffee pulp

Coffee pulp sample was washed with water and dried in an oven at 40°C. The dried sample was milled using a mixer grinder to reduce the size. The coffee pulp was then washed using n-hexane to remove carotenoids, waxes, and other lipophilic compounds. After drying at room temperature, the dried coffee pulp was extracted using a solvent with a weight ratio of the coffee pulp and the solvent of 1:40. The extraction was carried out in an Erlenmeyer flask put on a hot plate equipped with a magnetic stirrer at a speed of 350 rpm. The solvent for the extraction was pure water or a mixture of water and ethanol with a weight ratio of water and ethanol varied at 25:75, 50:50, and 75:25. The extraction temperature was varied at 40, 50, 60 and 70°C, while the extraction time was varied at 20, 40, 60, 90 and 120 min. The flask was covered with aluminum foil to prevent loss of solvent due to evaporation during heating. After the extraction, the extract solution was filtered using a Whatmann filter paper to remove the undissolved solid materials, and the extract solution was stored in a refrigerator prior to analysis.

2.3. Spray drying of the extract

The coffee pulp extract solution was dried using a spray dryer with an inlet temperature of 175°C and an outlet temperature of 125°C with a feed flow rate of 16.7 mL/min. The dried extract in powder form was then stored in a refrigerator prior to analysis.

2.4. Analysis of total phenolic content and antioxidant activity

The coffee pulp extract solution was then analyzed for its total phenolic content and antioxidant activity. The total phenolic content was determined by using Folin-Ciocalteu method using a gallic acid standard calibration curve according to Costa *et al* [6] with some modifications. The absorbance of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm, and the total phenol content (TPC) was then calculated according to by using the following equation:

$$\text{Total Phenolic Content (mg GAE/L)} = \frac{\text{Abs} \times \text{DF}}{m} \quad (1)$$

where Abs is the absorbance, m is the gradient of the gallic acid standard curve, and DF is the dilution factor. The total phenolic content was expressed in mg of gallic acid equivalent per liter extract solution (mg GAE/L), and then converted to mg GAE/g dry coffee pulp (mg GAE/g dry CP).

The antioxidant activity of the coffee pulp extract was determined using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The antioxidant activity was expressed as an inhibition percentage and calculated by using the following equation:

$$\text{Antioxidant Activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100\% \quad (2)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The antioxidant activity (the free radical scavenging activity) obtained by this method was expressed as IC_{50} (in ppm), which means the concentration of the sample needed to inhibit 50% of the DPPH as the free radical.

2.5. Statistical analysis

The experimental data were analyzed by using IBM SPSS analytical statistics software (IBM SPSS Statistics 24) for One-Way analysis of variance (ANOVA) with Tukey's post hoc test which was used to determine the significant differences between the means at 5% level. When the probability or significance is less than 0.05, the comparison is concluded to be significantly different. The estimated probability gives evidence to say that the statistical comparison only has a 5% probability falsely rejects the null hypothesis.

3. Results and discussion

Figure 1 shows the effect of the weight ratio of ethanol-water as a solvent on the total phenolic content of the coffee pulp extract solution. Based on the statistical analysis, the weight ratio of ethanol-water had a significant effect ($p < 0.05$) on the total phenolic content. The extraction using pure water exhibited a total phenolic content of 26.12 ± 2.5 mg GAE/g dry CP, and interestingly the total phenolic content increased when a mixture of ethanol-water was used as the solvent for the extraction. This result indicated that the phenolic compounds in the coffee pulp could be better extracted in a semi-polar solvent such as ethanol-water mixture than in pure water solvent. A maximum value of the total phenolic content of 40.63 ± 3.77 was reached using a water-ethanol solvent with a weight ratio of 50:50. However, a further increase of ethanol concentration in the solvent with a weight ratio of ethanol-water of 75:25 did not result in an increase in the total phenolic content, indicating that the phenolic compounds were not completely extracted in a too polar solvent. The use of solvent at this weight ratio even decreased the total phenolic content to 29.69 ± 3.16 mg GAE/g dry CP. Therefore, the ethanol-water weight ratio of 50:50 was concluded as the optimum ratio of the solvent to obtain a maximum of the total phenolic content of the coffee pulp extract solution.

The antioxidant activity of the coffee pulp extract solution was expressed by using IC_{50} value, which means the concentration of the sample (in ppm) needed to inhibit 50% of the DPPH as the free radical. A low IC_{50} value indicates a high antioxidant activity. Figure 2 shows the effect of the weight ratio of ethanol-water as a solvent on the value of IC_{50} of the extract solution. As can be seen, the IC_{50} value was strongly affected by the weight ratio of ethanol-water as a solvent. This was confirmed by the statistical analysis that showed that the weight ratio of ethanol-water had a significant effect ($p < 0.05$) on the IC_{50} value. The use of water-ethanol solvent with a weight ratio of 50:50 resulted in a minimum of the IC_{50} value of 487.96 ± 5.94 ppm, which means a maximum of the antioxidant activity. This result is in accordance with the analysis of the total phenolic content as described above. Since the phenolic compounds are the bioactive compounds in the coffee pulp which have the antioxidant property, the maximum value of the total phenolic content led to a minimum value of the IC_{50} or a maximum value of the antioxidant activity.

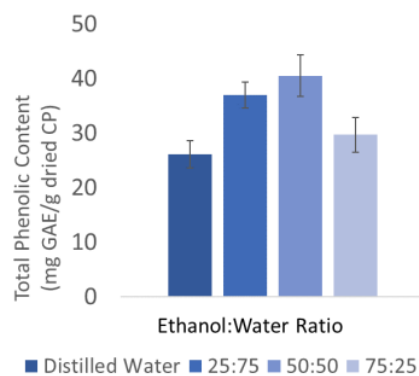


Figure 1. Effect the weight ratio of ethanol-water as a solvent on the total phenolic content of the coffee pulp extract solution.

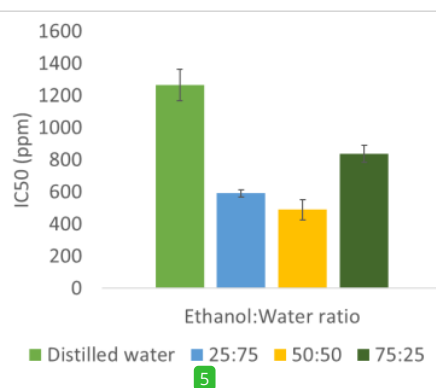


Figure 2. Effect the weight ratio of ethanol-water as a solvent on the IC₅₀ value of the coffee pulp extract solution.

Figure 3 shows the effect of the extraction temperature on the total phenolic content of the coffee pulp extract solution. The ethanol-water weight ratio of 50:50 was used for the extraction as this ratio was the most suitable solvent as described above, and the extraction time was kept constantly at 40 min. As can be seen, the increase in the temperature resulted in an increase in the total phenolic content. The statistical analysis showed that the effect of the extraction temperature had a significant effect ($p < 0.05$) on the total phenolic content. A maximum value of the total phenolic content of 46.72 ± 1.85 mg GAE/g dry CP was achieved at an extraction temperature of 70°C. This result indicated that heat could promote the release of the phenolic compounds from the coffee pulp cell wall and increase the solubility and the diffusion rate of the phenolic compounds from coffee pulp into the solvent. However, the extraction experiment at a temperature higher than 70°C was not conducted here as the temperature will be close to the boiling point of the solvent. Moreover, too high extraction temperature should be avoided for the industrial application as it will consume more energy. Further, the effect of the extraction temperature on the IC₅₀ value can be seen in figure 4. The lowest IC₅₀ value (or the high antioxidant activity) was achieved by increasing the extraction temperature due to the high total phenolic content in the extract solution. Since there was no significant change in the IC₅₀ value between the extraction temperature of

60°C and 70°C, the extraction temperature of 60°C was concluded as the optimum temperature to obtain a high antioxidant activity of the coffee pulp extract solution.

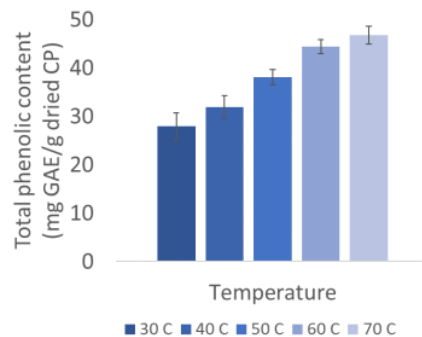


Figure 3. Effect of the extraction temperature on the total phenolic content of the coffee pulp extract solution.

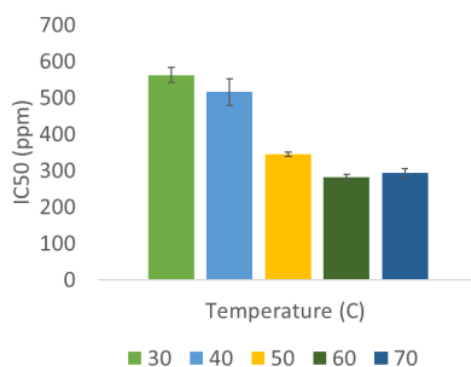


Figure 4. Effect of the extraction temperature on the IC₅₀ value of the coffee pulp extract solution.

The result of the study on the effect of the extraction time on the total phenolic content of the coffee pulp extract solution is shown in figure 5. The ethanol-water weight ratio of 50:50 was used for the extraction as this ratio was the most suitable solvent as described above, and the extraction temperature was kept constantly at 60°C. As can be seen in figure 5, the total phenolic content increased by increasing the extraction time from 20 min until 60 min. After 60 min, the total phenolic content did not change significantly, indicating that the phenolic compounds were completely extracted from the coffee pulp at 60 min. As a result, a low IC₅₀ value of the coffee pulp extract solution was obtained after 60 min of extraction time as can be seen in figure 6. The extraction time is considered a crucial factor during the extraction process as it will determine the energy and consequently the operational cost of the industrial scale extraction process. Thus, the extraction time of 60 min was concluded as the optimum time for the extraction of the coffee pulp to obtain a maximum antioxidant activity of the extract.

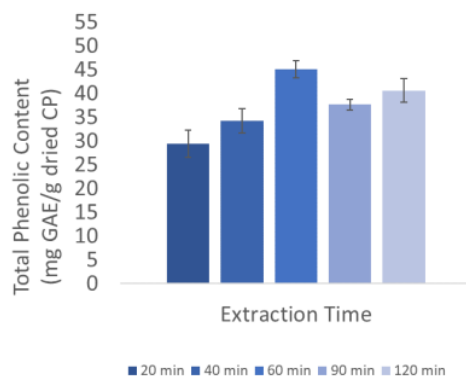


Figure 5. Effect of the extraction time on the total phenolic content of the coffee pulp extract solution.

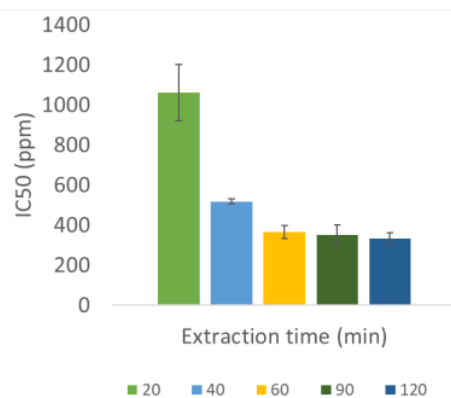


Figure 6. Effect of the extraction time on the IC₅₀ value of the coffee pulp extract solution.

The coffee pulp extract in powder form was produced by drying the coffee pulp extract solution using a spray dryer with an inlet temperature of 175°C and an outlet temperature of 125°C. Table 1 depicts the total phenolic content and the IC₅₀ value of the coffee pulp extract powder in comparison with those of the coffee pulp extract solution. Compared with the extract solution, the coffee pulp extract in powder form showed a lower total phenolic content and consequently a higher IC₅₀ value. The degradation of the phenolic compounds is reasonable since the extract was treated at a high temperature during the spray drying process [10]. However, a difference about 14% is considered as acceptable, since the IC₅₀ value of the extract powder of 383.54 ppm still indicates a high antioxidant activity of the extract powder. This result showed that the antioxidant compounds in the coffee pulp are quite stable against heat treatment.

Table 1. Comparison of the total phenolic content and the IC₅₀ of the coffee pulp extract before and after spray drying.

	Coffee pulp extract		Difference (%)
	before drying	after drying	
Total phenolic content (mg GAE/g solid extract)	96.35	83.62	13.2
IC ₅₀ (ppm)	331.93	383.54	14.5

4. Conclusion

The ethanol-water weight ratio of the solvent, the extraction temperature and the extraction time are important factors affecting the total phenolic content and the antioxidant activity of the coffee pulp extract. The optimization of the extraction condition is important to maximize the total phenolic content and the antioxidant activity of the extract. Ethanol-water with a weight ratio of 50:50 was concluded as the most suitable solvent to extract the phenolic compounds in the coffee pulp. The optimum extraction temperature and time to maximize the total phenolic content and the antioxidant activity were 60°C and 60 min. The result of this work showed that coffee pulp extract has great potential to be used as a source of antioxidants for various products such as additive for foods, beverages, herbals, and cosmetic products.

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