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Optimization of Ultrasound-assisted Cold Brew Process to Develop Phenolics, Flavonoids, and Caffeine-rich Coffee Beverage

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ABSTRACT: Ultrasound-assisted cold brew (UACB) method emerged as a solution of a long brew traditional cold brew method in coffee. Apart from the particular taste and odor, cold brew coffee contains a number of phenolic (TPC), flavonoid compounds (TFC), and caffeine. The level of these compounds in the drinks is strongly influenced by various factors related to extraction process, such as extraction temperature (x_1: 4 and 25 °C), time (x_2: 5,15, and 25 min), UAE duty cycle (x_3: 20, 50, and 80 s^{-1}), and grind size (x_4: coarse and medium). Based on the TPC, TFC, and caffeine contents, the optimum condition was at 25 °C for 25 min with 80 s^{-1} UAE duty cycle and medium coffee grind size: with 2.40 ± 0.11 mg GAE/mL, 1.69 ± 0.05 mg RE/mL, and 1.10 ± 0.02 mg caffeine/mL, respectively. Compared with the traditional cold brew method, only the TPC and caffeine content were significantly lower than the UACB method ($p < 0.05$). Furthermore, the TPC and TFC were stable in 7-day refrigerator storage ($p > 0.05$). The IC_{50} values of UACB coffee were 7,487 mg/L for DPPH assay and 64,113 mg/L for ABTS assay.

Keywords: *cold brew coffee, ultrasound-assisted extraction, phenolic, caffeine, antioxidant activity*

INTRODUCTION

Coffee is the most consumed beverage in the world. Two well-known varieties of coffee, Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea robusta*), are grown easily in Indonesia. Arabica coffee in Indonesia has a unique taste and ranks among the best coffees in the world. This coffee has a higher demand because this type of coffee has unique flavor characteristics, which having a complex taste with a low bitterness than Robusta coffee (Gumulya and Helmi, 2017; Parliment, 2000). These specific tastes of coffee are contributed by the constituent components.

Coffee beverages contain volatile and non-volatile compounds with different functional properties. Phenolic and flavonoid compounds are polyphenol derivatives that proven has antioxidant properties (Cämmerer and Kroh, 2006; Górecki and Hallmann, 2020). Caffeine, an alkaloid compound, contributes to bitterness taste of coffee also serves as nerve stimulation, resulting in smooth muscle relaxation(Tazzeo et al., 2012).

Phenolic compounds are a thermolabile compounds which can be degraded in the range of 30-40 ºC processing temperature (Carrera et al., 2012). Furthermore, flavonoid and caffeine compounds in coffee could decrease in processing. Flavonoid compounds cannot withstand the temperatures of 50 °C within a certain extraction time. Under the combination of low temperature and short extraction time, the result of extracted flavonoid compounds will be low, while prolonged time with high temperature, resulting in the decomposition of flavonoid compounds (Górecki and Hallmann, 2020; Yuliantari et al., 2017).

Up to date, a wide variety of beverage products based on coffee is available in the market with particular serving methods and different varieties of coffee beans. The serving of coffee has evolved to present various serving methods ranging from using an espresso machine to using manual brewing methods such as the V60 method, French press, AeroPress, and different other serving methods. The development of coffee brewing methods has led to brewing coffee using cold water, commonly referred as cold brew (Mestdagh et al., 2017).

The hot brew method weakness can be prevented by a cold brew method. The cold brew method uses a room temperature water or cold water for a longer time, for at least eight hours. This method is intended to maximize the extraction process of chemical compounds in coffee powder without decreasing its polyphenol compounds (Rao and Fuller, 2018). This brewing method can reduce the acidity level in coffee drinks to be safe for sufferers of stomach acid symptoms. This method can also reduce the risk of heart attacks, increase insulin sensitivity, stabilize blood sugar, and lower blood pressure (Rodríguez-Artalejo and López-García, 2018). Grind size affects the size of the contact surface area between ground coffee and water. When the particle surface area larger, the liberation process of carbon dioxide runs rapidly, so are for reducing the diffusion distance for soluble substances during extraction and for the improved transfer of colloidal substances to the liquid phase (Cordoba et al., 2019).

The 24 hours extraction time coupled with 4°C extraction temperature, and direct contact with air during extraction under traditional cold brew method increase the risk of uncontrolled microbes growth such as *Listeria monocytogenes (Ceylan, 2019)*. The risk needs to be minimized by speeding up the cold brew extraction process using an Ultrasound-assisted Extraction (UAE). The UAE emits ultrasonic waves that help solvent to penetrate the

coffee bean tissue and extract the target compounds. Thus, the extraction process time can be reduced significantly (Chemat et al., 2017). The conventional cold brew coffee has known for the TPC with 3.21 – 3.93 mg GAE/mL, TFC with 1.839 – 1.986 mg RE/mL(Castañeda-Rodríguez et al., 2020), and caffeine with 1.17 – 1.93 mg caffeine/mL (Portela et al., 2021). However, the bioactive compounds in the ultrasound assisted cold brew coffee have not been known.

Therefore, the aim of the present study was to evaluate the effect of water temperature (4 and 25 °C), coffee grind size (coarse and medium), UAE duty cycle (20, 50, and 80 s^{-1}), and extraction time (5, 15, and 25 min) in cold brew coffee extraction process using the UAE. These various determining factors needed to be optimized to obtain the best condition for making ultrasound-assisted cold brew coffee by maintaining the high numbers of phenolic, flavonoid, and caffeine compounds. In addition, the stability test of these compounds at the optimum condition was needed to uncover the ultrasound-assisted cold brew coffee storage in market for 7 days. To understand the radical scavenging capacity, the analysis of total antioxidant activities was measured using DPPH and ABTS assay in the traditional and ultrasound-assisted cold brew coffee.

MATERIALS AND METHODS

Chemicals and Reagents

The standard compounds of gallic acid, ascorbic acid, caffeine, quercetin-3-O-rutinoside (rutin), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$), and reagents were purchased from Sigma Aldrich Chemical Co. St. Louis, MO, USA.

Coffee Sample

Green coffee bean was purchased from local coffee beans producer in Gunung Halu, Bandung, Indonesia. The coffee beans were an Arabica varietal grown at an altitude of 1,400 m and processed by the full-washed method. The roasting process was performed by Bikonikal Coffee and Roasters, Yogyakarta, under a specific condition (222 ºC, 13 min) until reaching a dark roast profile (Appendix 1). The roasted beans were allowed to quickly cool to room temperature before grounding. Samples were grounded in a coffee grinder (Latina Coffee Grinder 600N, Taiwan, China) prior to analysis.

Ultrasound-assisted Extraction

The treatment of ultrasound-assisted extraction was performed by Hielscher UP-200 Ultrasonic Probe (Hielscher Ultrasonic GmbH, Teltow, Germany). The sample was weighed at 20 g and added with 200 mL of mineral water (1:10) in a beaker glass placed in the glass equipped with a Circulated Water Bath Cooler (LabTek SL-2060, Mahape, Navi Mumbai, India). The coffee was extracted by placing the probe in the middle of the solution under a specific condition due to the experiment. The temperature of coffee was measured using alcohol thermometer. After extraction, the coffee was filtered using a French press (Bialetti French Press Omino 350 mL, China), then followed with filter paper to separate the solids.

Traditional Cold Brew Extraction

The treatment of traditional cold brew extraction was intended as control of ultrasound-assisted cold brew coffee samples. The traditional cold brew coffee was made according to Rao and Fuller (2018) with a few modification. The traditional cold brew coffee was made by soaking the medium grind size of roasted coffee beans in water with the 1:10 coffee to water ratio. The extraction was done in 18 hours with room temperature condition (28-32°C) without ultrasound.

Total Phenolic Content Analysis

The extracted coffee solution was analyzed for total phenolic content (TPC) using GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States) according to the method from Lamuela-Raventós (2017). Briefly, 0.2 mL of the coffee and 50 mL of distilled water were added with 5 mL of Folin–Ciocalteu reagent, followed by 20 mL of sodium carbonate solution (anhydrous sodium carbonate, Na_2CO_3, made up into a 20 % w/v solution) and a few amounts of distilled water introduced into a 100 mL volumetric flask. The solution was allowed to react at room temperature for 30 min. The absorbance at 750 nm was then read through a cuvette using blank prepared with distilled water. The result of absorbance in the sample was compared with the result of absorbance in the standard curve of gallic acid (R2 = 0.9934). The range of standard concentration used for gallic acid are from 125 to 750 µg/mL. The total phenolic content of the samples was expressed as milligram of gallic acid equivalent per mL of coffee extract (mg GAE/mL).

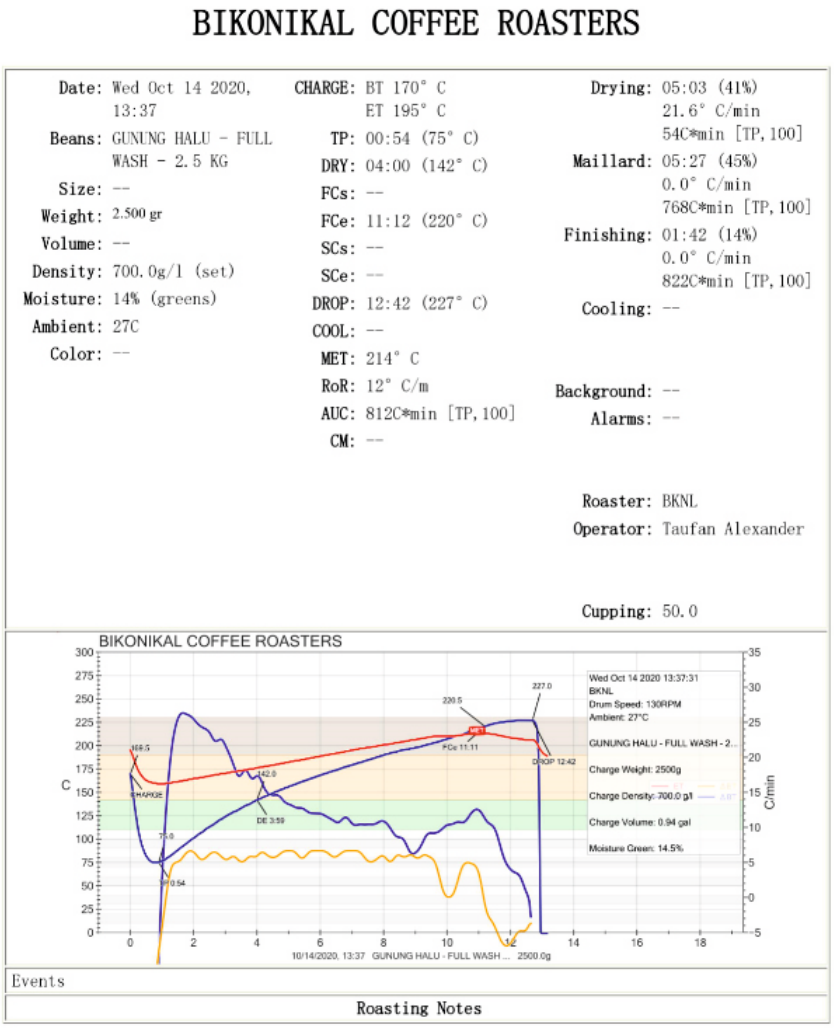
Total Flavonoid Content Analysis

The total flavonoid content (TFC) of the extracts was carried out using GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States) according to the colorimetric assay of Messyasz et al. (2018). Firstly, 200 µL of diluted coffee extract (1:10 v/v) was added to 1 mL distilled water, followed by 60 µL sodium nitrite solution (5%). The mixture was incubated for 5 minutes at room temperature, then 60 µL of aluminum chloride (10%) and 400 µL of sodium hydroxide 1N were added. The mixture was then incubated for 6 minutes. The volume of the reaction mixture was made to 2 mL with distilled water and mixed thoroughly. The absorbance was measured at 510 nm. The result of absorbance in the sample was compared with the result of absorbance in standard curve of rutin (R2 = 0.9911). The range of standard concentration used for rutin are from 300 to 900 µg/mL. The total flavonoid content of the samples was expressed as milligram of rutin equivalent per mL of coffee extract (mg RE/ mL).

Total Flavonoid Content Analysis

The total caffeine of the extracts was performed using GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States) according to Hainil et al. (2019). The liquid-liquid extraction (LLE) was used to separate the caffeine in coffee extract using $CaCO_3$, and extracting the caffein using chloroform in separating funnel. The extraction step was done 6 times until the color of coffee extract was clear. The chloroform solution was evaporated until the solution dried, followed by addition of 50 mL distilled water. An exact, 1 mL of solution was taken and

diluted until 10 mL distilled water. The absorbance was scanned between 200-800 nm to obtain the highest wavelength. The highest wavelength was at 206 nm. The absorbance of sample was compared with the standard curve of caffeine (R2 = 0.9941). The range of standard concentration used for caffeine are from 10 to 60 µg/mL. The caffein of the samples was expressed as milligram of caffeine equivalent per mL of coffee extract (mg caffeine/mL).



Appendix 1. Roasting Process of Beans

Shelf-Life Analysis

The shelf-life investigation was conducted using 100 mL of coffee extract in an Erlenmeyer covered with aluminum foil, stored in a 4°C refrigerator for 7 days according to the method from Sopelana et al. (2013). The measurement was done in day 0 and day 7 to analyze the difference between both TPC, TFC, and caffeine.

Evaluation of Antioxidant Capacity

The antioxidant capacities of coffee extract were determined by three methods: DPPH• free radical scavenging assay, and $ABTS^{•+}$ radical cation decolorization assay. Ascorbic acid was used as standard antioxidants for DPPH• free radical scavenging assay, while Trolox was used as the control for $ABTS^{•+}$ radical cation decolorization assay. All antioxidant capacity values were calculated using IC^{50} value.

DPPH• free radical scavenging assay

To determine the free radical scavenging activity of coffee extract, DPPH• free radical scavenging assay was done according to the method from Jung et al. (2017). The coffee extract (0.5 mL) was added with 0.5 mL of 0.1 mM DPPH• in methanol. The mixture was shaken on vortex for a half minute and kept at room temperature for 30 min in the dark. The absorbance of each sample solution was measured at 515 nm using GENESYS™ 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States). Ascorbic acid was used as standard. The percentage inhibition of DPPH• free radical is calculated using the equation below:

$$\% \text{ inhibitions of DPPH} = \frac{Ab - As}{Ab} \times 100\%$$

Ab is the absorbance of the control, and As is the absorbance of the extract. The IC^{50} values were calculated using linear regression analysis between %inhibition and reference standard concentration (mg/L).

$ABTS^{•+}$ radical cation decolorization assay

The ABTS analysis was done according to the method from Re et al. (1999). $ABTS^{•+}$ reagent was prepared with the follows; 5 mL of 7 mmol/liter of ABTS solution (ABTS powder purchased from Merck and Sigma Aldrich Singapore) and 88

µL of 140 mmol/liter potassium persulfate solution were mixed and saved in the darkroom for 12-16 hours, then diluted with Phosphate Buffer Saline (PBS 5mM (pH 7.4)) until the absorbance value of reagent was Abs. 734 nm = 0.70 ± 0.02, this absorbance value was taken as blank absorbance value.

The standard curve was taken from the absorbance value of the various concentration of 6-minute incubated mixed solution of 3 mL ascorbic acid's standard solution (0.1-1 mmol/liter) with 3 mL of $ABTS^{\bullet+}$ reagents. The sample absorbance value was plotted in regression graphic with the %RSA as y-axis and the standard's concentration as x-axis to form y = ax+b equation.

$$\%RSA = \frac{Blank\ Absorbance - Sample\ Absorbance}{Blank\ Absorbance\ Value} \times 100\%$$

The sample was done by the same method as the standard curve by replacing the ascorbic acid solution with the sample solutions.

Statistical Design of Experiment

Multilevel factorial design with four independent factors: including temperature (x1: 4 and 25 °C), time (x2: 5, 15, and 25 min), UAE duty cycle (x3: 20, 50, and 80 s^{-1}), and grind size (x4: coarse [particle size: ±600 µm] and medium [particle size: ±480 µm]); where the levels for each factor are 2×3×3×2 respectively. The design consisted of 36 units, replicated two times each, thus giving 72 runs. The level of independent factors is listed in Table 1, and the design with a total of 36 units in randomized order is presented in Table 2. Three responses were measured, including total phenolic content, total flavonoid content, and caffeine.

The data obtained from the analysis were carried out in triplicate. The data were analyzed using Microsoft Excel 365 and software STATGRAPHIC Centurion XVIII (Statpoint Technologies, Inc., USA). The effect of temperature, time, UAE duty cycle, and grind size on TPC, TFC, and caffeine concentration were analyzed by factorial ANOVA (p = 0.05). Post hoc analysis was used to compare and determine the optimum condition on each factor. The data were analyzed by Least Significant Different test (LSD, p = 0.05) to determine the significance of means between analyses. The data of shelf-life investigation was analyzed using a sample paired T-test.

Table 1. The level of independent factors

Factors	Level			Unit
	-1	0	1	
Temperature (x_1)	4		25	°C
Time (x_2)	5	15	25	min
UAE duty cycle (x_3)	20	50	80	s^{-1}
Grind size (x_4)	Coarse (±600)		Medium (±480)	µm

Table 2. Design of experiment with their observed

Runs	x_1	x_2	x_3	x_4	TPC (mg GAE/mL)	TFC (mg RE/mL)	Caffeine (mg caffeine/mL)
1	-1	1	-1	-1	0.74 ± 0.11	0.83 ± 0.14	0.75 ± 0.10
2	1	0	0	1	1.77 ± 0.14	1.21 ± 0.10	0.88 ± 0.01
3	1	0	0	-1	1.20 ± 0.09	0.74 ± 0.03	0.65 ± 0.02
4	-1	-1	-1	-1	0.86 ± 0.05	0.78 ± 0.10	0.65 ± 0.01
5	-1	-1	1	-1	1.29 ± 0.11	0.78 ± 0.08	0.72 ± 0.09
6	1	-1	0	1	1.41 ± 0.11	0.98 ± 0.04	0.76 ± 0.09
7	-1	-1	0	-1	0.99 ± 0.22	0.74 ± 0.04	0.57 ± 0.27
8	-1	1	1	-1	1.38 ± 0.17	1.18 ± 0.02	0.88 ± 0.18
9	1	1	-1	1	1.10 ± 0.03	1.15 ± 0.24	0.82 ± 0.19
10	1	-1	1	1	1.82 ± 0.18	1.17 ± 0.09	1.02 ± 0.23
11	1	1	1	1	2.40 ± 0.11	1.69 ± 0.05	1.10 ± 0.02
12	1	0	-1	1	1.49 ± 0.15	0.91 ± 0.06	0.76 ± 0.02
13	1	-1	-1	1	1.12 ± 0.03	1.01 ± 0.36	0.69 ± 0.23
14	-1	0	1	1	1.77 ± 0.07	1.31 ± 0.16	0.90 ± 0.02
15	-1	-1	-1	1	1.21 ± 0.03	1.08 ± 0.24	0.66 ± 0.16
16	1	0	1	1	2.04 ± 0.07	1.48 ± 0.06	1.13 ± 0.02
17	1	0	1	-1	1.53 ± 0.08	1.21 ± 0.05	0.90 ± 0.05
18	-1	1	1	1	1.52 ± 0.11	1.47 ± 0.14	0.79 ± 0.07
19	-1	-1	1	1	1.54 ± 0.18	1.09 ± 0.06	0.77 ± 0.09
20	-1	0	0	-1	1.09 ± 0.09	0.86 ± 0.06	0.72 ± 0.03
21	-1	1	0	-1	1.19 ± 0.06	1.02 ± 0.46	0.63 ± 0.08
22	-1	0	1	-1	1.48 ± 0.08	0.93 ± 0.03	0.73 ± 0.02
23	1	1	-1	-1	1.25 ± 0.11	0.78 ± 0.04	0.63 ± 0.09
24	-1	0	-1	1	1.46 ± 0.15	1.12 ± 0.05	0.72 ± 0.12
25	-1	1	0	1	1.25 ± 0.17	1.18 ± 0.11	1.01 ± 0.01
26	1	-1	1	-1	1.09 ± 0.11	0.86 ± 0.04	0.74 ± 0.12
27	-1	0	0	1	1.31 ± 0.14	1.14 ± 0.12	0.85 ± 0.01
28	1	-1	0	-1	1.012 ± 0.22	0.86 ± 0.08	0.66 ± 0.06
29	1	1	0	-1	1.75 ± 0.06	1.00 ± 0.01	0.94 ± 0.04
30	1	1	1	-1	1.96 ± 0.17	1.28 ± 0.01	1.05 ± 0.02
31	1	0	-1	-1	1.19 ± 0.10	0.79 ± 0.03	0.71 ± 0.09
32	1	-1	-1	-1	0.78 ± 0.05	0.75 ± 0.12	0.69 ± 0.12
33	-1	-1	0	1	1.31 ± 0.11	0.93 ± 0.02	0.82 ± 0.06
34	-1	1	-1	1	1.29 ± 0.03	1.17 ± 0.30	0.60 ± 0.05
35	1	1	0	1	1.76 ± 0.17	1.26 ± 0.11	0.93 ± 0.09
36	-1	0	-1	-1	1.25 ± 0.10	0.82 ± 0.04	0.63 ± 0.02

x_1 = Temperature (-1 = 4 °C; 1 = 25 °C)

x_2 = Time (-1 = 5 min; 0 = 15 min; 1 = 25 min)

x_3 = UAE duty cycle (-1 = 20 s^{-1}; 0 = 50 s^{-1}; 1 = 80 s^{-1})

x_4 = Grind Size (-1 = Coarse [particle size: ±600 µm]; 1 = Medium [particle size: ±480 µm])

Average in a column with different colors are significantly different. In each TPC, TFC, and caffeine column contains 4 variables: x_1, x_2, x_3, and x_4, respectively. (LSD test; $p < 0.05$)

RESULT AND DISCUSSION

Optimum Condition of Extraction

The total of 72 randomized samples were run to obtain the optimum condition of extracting total phenolic, total flavonoid, and caffeine. Four factors (temperature, time, UAE duty cycle, and grind size) showed a significant different ($p < 0.05$) on the TPC, TFC, and caffeine content, except for the temperature on the TFC, as seen in the Table 2. The optimum condition of extraction process to reach the highest TPC and TFC was at 25 °C for 25 min with 80 s^{-1} UAE duty cycle and medium coffee grind size, with 2.40 ± 0.11 mg GAE/mL and 1.69 ± 0.05 mg RE/mL, respectively. Phenolic compounds exhibit their antioxidant activity by various mechanisms such as scavenging other reactive species such as $OH•_2$, $NO•_2$, N_2O_3, ONOOH, and HOCl; and donating hydrogen atoms to free radicals. Some phenolics can interfere with the absorption of metals from the diet by reacting with $O•_2$ or by binding transition metal ions (especially iron and copper) and resulting in forms poorly active in promoting free radical reactions (Taubert et al., 2003; Zin et al., 2006). Chlorogenic acids were reported to be the most abundant phenolic acids for up to 14% of the dry matter of green coffee beans (Farah and Donangelo, 2006). Flavonoids are a class of phenolic compounds which are extremely common in the plant kingdom as their glycosides (Kumar et al., 2014). The flavonoids act through scavenging of oxygen-derived free radicals or chelating process (Nijveldt et al., 2001).

Meanwhile, the best condition of extraction for the highest caffeine was at 25 °C for 15 min with 80 s^{-1} UAE duty cycle and medium coffee grind size, with 1.127 ± 0.02 mg caffeine/mL. In comparison to our result, Ahmed et al., (2019) found the TPC was 1.549 to 2.420 mg GAE/mL and the TFC was 1.216 (cold drip technique) to 2.340 mg RE/mL obtained from Arabica coffee (mix variety, origin from Brazil) with ultrasonication combined with agitation. Our result confirmed that it was in accordance with the previous result. Different coffee variety, origin, and brewing methods might account for the different results. The ultrasonication method was disrupting the cell walls. Under the disruption process, the bound polyphenols which related with phenol and flavonoid content will increase (Abid et al., 2013; Bhat and Goh, 2017). It was concluded that the increase of extraction temperature would increase the target compounds in coffee until 25 °C. The highest temperature used in this research (25 °C) could not degrade the molecular structure of phenolic compounds, where the enzymatic activities of phenolic degradation process only occur when the temperature reaches at least at 30 – 40 °C (Carrera et al., 2012).

The result suggests that 15 minutes of extraction time was not statistically different from 25 minutes of extraction time for caffeine. The longer extraction time provides more chance of water to bind with caffeine, therefore increasing the mass transfer step in the process (Fuller and Rao, 2017). The higher UAE duty cycle will increase the intensity of ultrasound. This parameter is directly influenced to the amplitude of the transducer and the pressure of the sound wave, thus the collapse of the bubble will be more aggressive (Chemat et al., 2017). It is showed that the total phenolic, total flavonoid, and caffeine contents were higher at a higher UAE duty cycle due to the amplitude pressure of the ultrasound probe. The smaller size of coffee ground presents more possibility of polyphenols and alkaloids extracted perfectly. The finest coffee bean size has proven to increase the phenolic, flavonoid, and caffeine content in coffee brews. The bigger size of surface area will generate more target compounds movement from the extraction's material to the solvent(Cordoba et al., 2019).

The selected optimum condition of ultrasound-assisted extraction to maintain the higher total of all target compounds was at 25 °C for 25 min with 80 s^{-1} UAE duty cycle and medium coffee grind size. Compared with the traditional cold brew method (Table 3), the TPC, TFC, and caffeine were 2.12 ± 0.03 mg GAE/mL ($p < 0.05$), 1.71 ± 0,01 mg RE/mL ($p > 0.05$), and 0.79 ± 0.01 mg caffeine/mL ($p < 0.05$), respectively. The ultrasound-assisted cold brew coffee could maintain more phenolic compounds in coffee beverage than the traditional cold brew coffee. The loss of phenolic compound could occur in a period of long extraction time due to a longer contact with oxygen, which can affect the molecular shape of phenolic compound. The flavonoid content as a rutin equivalent showed a similar result between two brewing methods. Our results were in accordance with another experiment by Górecki and Hallmann (2020) where longer extraction time does not significantly change the rutin content. In addition, based on their experiment, the total flavonoid as a sum of epigallocatechin, and quercetin and its derivatives, the longer extraction time increased the total flavonoid. The method differences also play an important role in the caffeine content of cold brew coffee. In the ultrasound-assisted cold brew coffee, we observed a significantly higher concentration than the traditional method. The longer brewing time, the caffeine content decreased due to the formation of caffeine-phenolic compound complexes. The specific phenolic compound is chlorogenic acids (Cesaro et al., 1976).

Table 3. Total phenolic, flavonoid, and caffeine contents of cold brew coffees

Sample	TPC (mg GAE/mL)	TFC (mg RE/ mL)	Caffeine (mg /mL)
Ultrasound-Assisted Cold Brew Coffee	2.40 ± 0.14^{a}	1.69 ± 0.05^{a}	1.10 ± 0.02^{a}
Traditional Cold Brew Coffee	2.12 ± 0.03^{b}	1.71 ± 0,01^{a}	0.79 ± 0.01^{b}

Average in a column with different letters are significantly different (LSD test, $p < 0.05$)

Stability of Target Compounds

The stability of ultrasound-assisted cold brew coffee was done by measuring the TPC, TFC, and caffeine content in the optimized condition in a span of seven days. The observation for seven days was selected due to the lack of UHT treatment on this experiment. According to Sopelana et al. (2013), the microbial analysis of coffee brew showed a colony count number of 8.5 × 102 cfu/mL for mesophilic flora. This high number of microorganisms could reduce the freshness and the acceptance level of coffee brew (Kwok et al., 2020). From the Table 4, the TPC on day 0 and day 7 is relatively stable without any significant decrease ($p = 0.211$). Phenolic compounds

play an antioxidant role in a coffee beverage, that naturally known as thermolabile compounds (Coelho et al., 2014). The storage of coffee in 4 °C, successfully maintain the phenolic compounds. According to Sopelana et al. (2013), the phenolic compounds were fairly stable until 60 days of 4 °C storage. The changes were observed on ferulic acid, another phenolic acid compound, where be degraded into 4-venylguaiacol.

The storage time had no significant effect on the flavonoid content. The non-oxidative degradation reaction rate and the hydrolysis rate of quercetin glycosides during storage only occur at high temperature storage (80 °C), where storage in 4 °C and room temperature was relatively stable (Van Der Sluis et al., 2005). The stability of flavonoids was proven to be stable for 1 month in low pH conditions (Van Der Sluis et al., 2005).

Caffeine, an alkaloid compound normally related to the bitterness of coffee (Clarke, 1985), showed a significant decrease ($p < 0.05$) throughout the 7-day storage (Table 4). These results were in contrast with those obtained in previous research, where the caffeine content was relatively stable throughout 120-day storage at 4 °C under the influence of a UHT treatment (Sopelana et al., 2013). Caffeine was known as a stable compound and not time/temperature dependent, thus 7 days storage should possess a similar level of caffeine (Labbé et al., 2008). The decreasing caffeine content was possibly due to the error from the researchers, and there was no evidence that caffeine decreased during storage based on the previously published studies.

Table 4. Total phenolic, flavonoid, and caffeine contents of ultrasound-assisted cold brew coffee for 7 days storage

Sample	TPC (mg GAE/mL)	TFC (mg RE/ mL)	Caffeine (mg /mL)
Day 0	2.40 ± 0.14[a]	1.69 ± 0.05[a]	1.10 ± 0.02[a]
Day 7	2.38 ± 0.04[a]	1.69 ± 0.01[a]	1.05 ± 0.02[b]

Average in a column with different letters are significantly different (Paired T-test; $p < 0.05$)

Antioxidant Capacity

The IC_{50} value is the total amount of antioxidant content in a sample that can inhibit the radical compounds of selected assays by 50%. Thus, the lower IC_{50} value the higher antioxidant activity (Sánchez-Moreno et al., 1998). DPPH assay measures the scavenging capacity of antioxidants towards a stable free radical DPPH. The odd electron of a nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Kedare and Singh, 2011). Ascorbic acid was used as positive control that known having a strong antioxidant activity (Matuszewska et al., 2018). The IC_{50} to DPPH free radical from ultrasound-assisted sample was 7,487 mg/L, lower than the traditional cold brew coffee (9,219 mg/L) (Table 5). This was probably due to the higher concentration of phenolics found in the ultrasound-assisted sample (Table 3). Compared with ascorbic acid, the IC_{50} value was significantly low than the samples. These results conduct the assumption that the ultrasound-assisted cold brew coffee sample and traditional cold brew coffee had a weak response on the ability of antioxidant in term of giving hydrogens atom to the stable free-radical compounds.

$ABTS^{\bullet+}$ assay or commonly known as Trolox Equivalent Antioxidant Capacity (TEAC), which measure both water-soluble and lipid-soluble antioxidants to scavenge the stable radical cation $ABTS^{\bullet+}$ (Pinto et al., 2005). The IC50 value of ultrasound-assisted sample by $ABTS^{\bullet+}$ showed the lowest antioxidant capacity (64,113 mg/L), indicating the traditional cold brew coffee TEAC (51,084 mg/L) had higher antioxidant capacity than the ultrasound-assisted sample. All cold brew coffees showed a lower inhibitory effect than Trolox (1 mg/L) indicating the samples fail to scavenge $ABTS^{\bullet+}$. The lower concentration of phenolics found in traditional cold brew coffee (Table 3) may contain high lipid-soluble antioxidants (tocopherols and esters of diterpene alcohols) than the water-soluble antioxidants, thus increasing the IC_{50} value.

Table 5. Antioxidant capacity of cold brew coffees compared with the standard compounds

Sample	IC_{50} Value (mg/L)	
	DPPH	ABTS
Ascorbic Acid	47	-
Trolox	-	1
Ultrasound-Assisted Cold Brew Coffee	7,487	64,113
Traditional Cold Brew Coffee	9,219	51,084

CONCLUSION

The optimum condition of ultrasound-assisted extraction of cold brew coffee to maintain the highest total phenolic, flavonoid, and caffeine content was at 25 ºC extraction temperature, 15 min extraction time, 80 s^{-1} UAE duty cycle, and medium grind size of coffee beans. The ultrasound-assisted cold brew coffee showed a promising coffee beverage product replacing the traditional cold brew coffee.

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