INTEGRATED EXTRACTION BY PERCOLATION, DISTILLATION, AND SOXHLET EXTRACTION TO SEPARATE BIOACTIVE AND BIOENERGY COMPOUNDS FROM SPENT COFFEE GROUND

Askal Maimulyanti\textsuperscript{1,\,*} and Anton Restu Prihadi\textsuperscript{2}

\textsuperscript{1}Department of Analytical Chemistry, Politeknik AKA Bogor, Indonesia
\textsuperscript{2}Department of Quality Assurance of Food Industry, Politeknik AKA Bogor, Indonesia
\*Corresponding Author: askal_m@yahoo.com

ABSTRACT
The spent coffee ground has potential compounds applied in food and bioenergy. In this study, an integrated extraction to separate chemical compounds from the spent coffee ground was designed. Furthermore, the extraction apparatus was combined for percolation, distillation, and Soxhlet extraction. The distillation technique produces flavored coffee, Soxhlet extraction separates coffee oil; while percolation produces the coffee extract. Analysis of coffee aroma using distillation techniques obtained alpha furfuryl alcohol (52.16%), methyl pyrazine (15.63%), and 2.6-dimethyl paradiazine (9.62%). Analysis of coffee extract using the percolation technique obtained antioxidant compounds with IC\textsubscript{50} (56.61 µg/mL), polyphenols (1.25 mg/g), caffeine (7.88%), chlorogenic acid (1.25%), and tannins (21.22%). Analysis of coffee oil using the Soxhlet extraction technique obtained methyl ester compounds that have the potential for biodiesel such as linoleic acid methyl ester (39.90%), palmitic acid methyl ester (34.30%), oleic acid methyl ester (8.74%), and stearic acid methyl ester (8.66%). The integrated extraction has successfully separated many chemical components from spent coffee ground dan it has added value to be applied in food, beverage, pharmaceutical, and bioenergy.

Keywords: Spent Coffee Ground, Extraction, Bioactive Compounds.

INTRODUCTION
Coffee is the most popular beverage in the world. The coffee beverage produces a lot of solid waste and by-products that pollute the environment. Coffee powder waste which is usually called coffee grounds or spent coffee ground (SCG) is waste that comes from coffee brewing. The spent coffee ground has the potential to be used as a value-added product. Almost 50% of coffee production generates around 6 million tons of spent coffee ground per year.\textsuperscript{1} The produced nutrients waste has the potential to be used as a value-added product in multiple different processes. SCG has bioactive compounds, e.g. polyphenol, flavonoid, chlorogenic acid, and useful resource materials for biodiesel. The use of the spent coffee ground as a natural antioxidant and phenolic compounds. It can be used as food, nutritional supplement, and pharmacy application.\textsuperscript{2,4} SGC has the potential to be used as a source for biodiesel production, food industry,\textsuperscript{7} and bioethanol.\textsuperscript{8} Technology has been developed to extract the compounds from coffee and spent coffee grounds. The solvent extraction,\textsuperscript{9,10} sequential extraction,\textsuperscript{11} supercritical CO\textsubscript{2},\textsuperscript{12} soxhlet extraction,\textsuperscript{13} supercritical fluid extraction,\textsuperscript{14,15} and extraction by solid-liquid method.\textsuperscript{16} The chemical compounds of spent coffee grounds are dependent on the extraction process. This study aims to design an integrated extraction including percolation, distillation, and Soxhlet extraction. The chemical compounds of the extract products including coffee extract, coffee oil, and coffee aroma were analyzed.

Experimental
Design of Integrated Extraction
Extraction of bioactive compounds using integrated extraction was designed using distillation, percolation, and Soxhlet methods. The design of the apparatus can be seen in Fig.-1.
Hydro Distillation Process
The sample was placed in the extraction tube (B) and the solvent was placed in the tube (A). The solvent was heated and the vapor flowed into the sample in the extraction tube (B). The steam extracted aroma in the extraction tube (B) and condensed through the condenser (F). The aroma dissolved in the solvent accommodated in the receiver C tube. The solvent in receiver C was transferred to the receiver (D) without stopping the distillation process. The aromatic coffee aroma in the receiver tube (D) was analyzed by GCMS to determine the extracted active compound as an aromatic coffee extract.

Percolation Process for Coffee Extract in Water
The sample was placed in the tube (B) and the solvent was placed in the tube (A). The solvent was heated and evaporated and flowed into the extraction column B. After the percolation process, the solvent was evaporated. Evaporation was carried out into condenser (F) and the solvent was collected in the receiver column (C) and (D). The chemical compounds in the extracted coffee were analyzed.

The Soxhlet Extraction Process for Coffee Oil
The sample was placed in extraction tube B and the solvent was placed in extraction tube A. The solvent was heated and the vapor passed through the condenser (E) to the sample in the extraction column (A). The Soxhlet process continued until the extract was obtained in column (A). After the Soxhlet process was complete, the extract in extraction column A was concentrated by evaporating the solvent through the condenser (F). The solvent was received in columns (C) and (D) and the concentrated extract was in the extraction column (A). The extract of coffee oil was further analyzed using GC-MS.

Analysis of Antioxidant Activity
The analysis of antioxidants used the 1-1-diphenyl-2-picrylhydrazyl (DPPH) method and the analysis use a UV-Vis spectrophotometer at a wavelength of 517 nm. A control solution was used as a comparison. DPPH solution of 0.5 mM was prepared in methanol, pipette 1 mL each, and put into a vial bottle. Samples were prepared with concentrations of 0, 10, 20, 30, 40, and 50 ppm in methanol and put into vials containing 0.5 mM DPPH solution. The solution was diluted with methanol to a volume of 5 mL. 17,18

Analysis of Caffeine and Chlorogenic Acid
A coffee extract solution of 10 mg/mL was analyzed using a reverse-phase HPLC column. The mobile phase consisted of 50 mM acetic acid in acetonitrile and 50 mM acetic acid in distilled water. Caffeine and chlorogenic acid were detected at absorption wavelengths of 270 and 325 nm, respectively.

RESULTS AND DISCUSSION
Extract from Distillation Process of Spent Coffee Ground
A distillation technique was carried out to obtain the aroma compounds in the spent coffee ground. Analysis of the volatile compound components in the extract can be seen in Fig.-2 and Table-1.
Hydro distillation is the method to extract volatile oil. Based on the experiment, the main volatile compounds were Alpha furfuryl alcohol (52.16 %), methyl pyrazine (16.63 %), and 2,6-dimethylparadiazine (9.62%). Other researchers reported the aroma compounds in spent coffee ground consists of the phenol and non-volatile acids, a group of neutral carbonyl, a group of carbonyl acid compounds, free amino acid groups, isoleucine, alanine, threonine, glycine leucine, and volatile acid groups.

The aroma of coffee contains volatile compounds derived from alcohol, furans, acids, aliphatic carbonyl compounds, and cyclic diketone. Simultaneous interactions with nitrogen-containing fragments can occur as pyrazine, pyridine, and imidazole compounds. Pyrazine is known as a substance that contributes to the aroma of baked goods. There are 81 pyrazine compounds that have been identified in the aroma of coffee with various concentrations. Pyrazine is a flavoring volatile compound and is often found in cocoa and coffee. Pyrazines in coffee contribute to a pleasant aroma of coffee. Several alkylated pyrazine compounds were found as aroma volatile compounds.

**Extraction from Percolation Process of Spent Coffee Ground**

Analysis of components from the spent coffee ground with the percolation method can be seen in Table-2.

| Component | Content |
|-----------|---------|
| Antioxidant activity IC$_{50}$ | 56.61 µg/mL |
| Polyphenol | 1.25 mg/g |
| Caffeine | 7.88 % |
| Chlorogenic acid | 1.25 % |
| Tannin | 21.22 % |
Percolation is a continuous process in which the saturated solvent is replaced by the fresh solvent. Extraction using percolation techniques was carried out using water at a heating temperature of 70°C and obtained a yield of 13.30%. The components in this extract contain antioxidant with IC$_{50}$ (56.61 µg/mL), polyphenol (1.25 mg/g), caffeine (7.88 %), chlorogenic acid (1.25 %), and tannin (21.22%). Tannin as tannic acid is a phenol compound that is dissolved in water.

Figure-3 shows that there was a response from the extract to antioxidant activity. There was a linear relationship between concentration and absorbance with $R^2 = 0.9476$ and equation $y = -0.0002x + 0.0302$. Antioxidant activity was expressed as IC$_{50}$ = 56.61 µg/mL.

Figure-4 shows the peak for caffeine with a retention time of 9.999 minutes and chlorogenic acid at 3.286 minutes. The analysis of this extract coffee obtained a caffeine content of 7.88% and chlorogenic acid of 1.25%. Caffeine is a natural compound contained in coffee beans. Caffeine is a pure alkaloid is the most popular compound in the coffee product. Caffeine is a naturally occurring alkaloid found primarily in coffee. Caffeine concentration ranges from 0.734 to 41.3 µg/mg of SCG extract obtained and 6-11.5 mg/g SCG from the coffee bar. Caffeine has various positive effects, such as increasing awareness and concentration temporarily, preventing asthma, and increasing digestive acid production. However, there are also various negative effects caused by caffeine including causing addiction, symptoms of illness such as insomnia, reduced calcium absorption, and increasing blood pressure. Caffeine can be separated from the coffee grounds by extraction. The method better known as the process of separating caffeine from coffee grounds is solid-liquid extraction using a water solvent. This is done because water can dissolve caffeine in large enough quantities. The benefits CGA can prevent diabetes and atherosclerosis.
Extract Spent Coffee Ground with Soxhlet Extraction Technique

The extraction technique by Soxhlet extraction is carried out using a non-polar hexane solvent. The result obtained was 14.86%. The components were extracted as coffee oil. The results of the analysis of antioxidants in coffee oil can be seen in Fig.-5.

![Fig.-5: Correlation between Concentration of Extract and % Inhibition](image)

The relationship between concentration and % inhibition shows that the greater the concentration of the extract, the greater the inhibitory power against free radicals. This shows that the higher the extract concentration, the higher the antioxidant properties. Linear relationship is obtained with the value of $R^2 = 0.9776$ with the equation $y = 0.1499x + 5.4743$. To see the oxidation ability with the IC$_{50}$ value, namely the ability of 50% antioxidant activity, it was obtained by extrapolating the % inhibition at the 50% value in the above equation. The IC$_{50}$ value was obtained at 297.04 µg/mL. Thus, the extract of spent coffee ground in water with maceration techniques showed the presence of active substances with antioxidants at an IC$_{50}$ value of 297.04 µg/mL. This shows the ability of the active substance to oxidize free radicals as much as 50% obtained at a concentration of 297.04 µg/mL. The results of the analysis of non-polar components in oil coffee extract can be seen in Fig.-6 and Table-3.

![Fig.-6: Chromatogram of Coffee Oil Extract](image)

### Table-3: Identification of Active Compounds from Coffee Oil

| Compounds                        | tR    | % area |
|----------------------------------|-------|--------|
| Palmitic acid methyl ester       | 31.427| 34.30  |
| Linoleic acid methyl ester       | 33.809| 39.90  |
| Oleic acid methyl ester          | 33.879| 8.74   |
| Elaidic acid methyl ester        | 33.919| 0.80   |
| Stearic acid methyl ester        | 34.21 | 8.66   |
| Cis-13-eicosenoic acid methyl ester| 36.16 | 0.42   |
| Eicosanoic acid methyl ester     | 36.526| 3.44   |
| Nanodecanoic acid methyl ester   | 39.531| 0.65   |
Based on the results of the analysis of active substances from the extracts of spent coffee ground, the Soxhlet technique using hexane could separate non-polar substances so that coffee oil products were produced. The main component produced was a methyl ester compound with the following composition: Linoleic acid methyl ester (39.90%), Palmitic acid methyl ester (34.30%), and stearic acid methyl ester (8.66%). The biodiesel from the spent coffee ground with the Soxhlet technique was 21.50%.

Part of the coffee plant that has the potential to be used as raw material for biodiesel after psycho-chemical testing is coffee ground. Biodiesel is biodegradable and contains sulfur. In coffee oil, there are 81.3% triglycerides as the main component. The fatty acids in the wax coating are different from coffee oil. In coffee oil, there are triglycerides with linoleic fatty acid and palmitic acid. The diterpenes contain palmitic acid and linoleic acid. Fats and their derivatives in coffee beans include triglycerides, free fatty acids, diterpenes, free diterpenes, triterpenes, sterols, sterol esters, tocopherols, phosphatides, and 5-hydroxytryptamine and their derivatives. Biodiesel can be obtained through triglyceride transesterification reactions and free fatty acid esterification reactions depending on the quality of vegetable oil used as raw material. Transesterification (commonly referred to as alcoholysis) is the conversion step from triglycerides to alkyl esters, through a reaction. The use of oil from coffee grounds began to attract attention after the high content of essential fatty acids was recognized. The antioxidant content in coffee oil can also inhibit the rate of oxidation so that it can be used in the food industry. Fresh coffee beans contain oil in the range of 8 to 18% depending on the type of coffee beans. A fresh robusta coffee bean can contain 9.0 to 13% fat and its value can increase to 11 to 16.0% after going through the roasting process. Triglyceride is the largest component in coffee beans with a content of 75% by dry weight. Coffee grounds have an oil content of between 15 and 25% by weight which can be used for biodiesel raw materials, and palmitic acid is a major component in coffee grounds oil, i.e. 44.5%. The process of extracting oil (solute) in coffee grounds can be done through leaching. The extract must have high solubility in the solvent so that the extracted content can be maximized. Several extraction methods have been used to obtain oil from coffee beans. Soxhlet extraction is the method commonly used to extract oil from coffee beans. The Soxhlet method is generally used to determine the oil content of coffee beans. Apart from the Soxhlet method, several other methods have also been developed as technical applications for coffee bean oil extraction, including supercritical fluid extraction, and two-phase solvent extraction assisted by ultrasonic waves.

CONCLUSION

The design of an integrated extraction device consists of distillation, percolation, and Soxhlet techniques. The device consists of 2 extraction tubes, 2 condensers, 2 receiver tubes, equipped with electric heating and temperature control, a pump, and stirring. The extracts from the distillation technique resulted in flavored coffee, Soxhlet separated the oil coffee and percolation separated the coffee extract from the spent coffee ground. These extracts contain active compounds that use in beverages, pharmaceuticals, and bioenergy.

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Maimulyanti and Prihadi