

Extraction and Evaluation the Biological Activities of Oil from Spent Coffee Grounds

Phung T. K. Le^{*a}, Quan T. H. Vu^a, Quan T. V. Nguyen^a, Khoa A. Tran^b, Kien A. Le^c

^aHo Chi Minh city University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam.

^bHo Chi Minh city University of Natural Resources and Environment, Ho Chi Minh City, Vietnam.

^cInstitute for Tropicalisation and Environment, Ho Chi Minh City, Vietnam.
 phungle@hcmut.edu.vn

Spent coffee grounds (SCG) have grown its popularity as a low-cost and versatile resource in multiple application fields. The economic potential of SCG is highlighted by its abundance and rich contents of various beneficial compounds, such as polyphenols, polysaccharides, amino acids, antioxidants, etc. This study aimed to evaluate the content of lipid hydroperoxide (LP), total phenolic content (TPC), flavonoid content (TFC) and antioxidant in SCG's extract. The coffee grounds were extracted using n-hexane and methanol in three different SCG/solvent (w/v) ratios: 1 : 10, 1 : 15 and 1 : 20. Both conventional and advanced extraction methods were employed, including Maceration (MAC), Soxhlet (SOX), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE). The highest contents of LP and TPC were 0.0191 mM and 13.466 mg gallic acid (AG)/g dry weight by using UAE at the ratio of 1 : 20. With the same ratio, TFC peaked the highest point at 31.15 mg quercetin/g dry weight when extracted by MAE method. The oil recovery yield was in the range from 9.42 % to 17.96 %. The phenolic recovery yield was between 5.76 % and 12.3 %. The results indicated the high antioxidant capacity of the extract.

1. Introduction

SCG are the main focus of many recent researches in various fields, such as compost, organic fertiliser, animal feed, biodiesel, fuel pellet and activated carbon production (Caetano et al., 2012). The extract of SCG contains large amounts of valuable compounds such as proteins, lipids, oils, carbohydrates, organic acids and alkaloids. The dried coffee contains proteins ranges from 11 - 16 % and free amino acids. The coffee seeds contain 7 - 16 % of lipids and oils depending on the coffee species (Dam and Harmsen, 2010). Polysaccharides from coffee are proven to lower cholesterol, control blood glucose level and prevent tumor diseases (Ballesteros et al., 2015). Cafestol and Kahweol, identified in the unsaponifiable fraction of SCG, have recently drawn attentions due to their beneficial health effects. In fact, cafestol has been identified as an anti-inflammatory compound, and together with kahweol, which possess anti-carcinogenic properties (Cavin et al., 2002). In 2013, a group of researchers found that SCG activated antioxidant and anti-tumor compounds (Acevedo et al., 2013). In some other studies, oil from SCG contains high concentration of linoleic acid, which has excellent emollient properties and is an essential component for the organisation and perpetuation of skin barrier (Fluhr et al., 2001). The high content of palmitic acid are detected in the oil from SCG, which protect skin from ultraviolet ray affection (Rabasco Alvarez et al., 2000). The most important antioxidant in coffee is methyl pyridinium, formed by the degradation of trigonelline during roasting (Votavová et al., 2009). Chlorogenic acid, which accounts for 8.2 % of green coffee beans, is found to be a promising source of functional supplement (Dam and Harmsen, 2010). The amount of soluble CGA decreases to 4.5 % after roasting due to the release of caffeic acid and quinic acid. CGA contributes to reduce the incidences of atherosclerosis, diabetes and various types of cancer. Compared to tea, the amount of caffeine in coffee is 50 - 70 % higher (Harland, 2000). The rich presence of various valuable compounds in SCG potentially plays important roles in the food, cosmetic, pharmaceutical and renewable energy industries.

For these reasons, the main purposes of this work were to compare the amount of bioactive compounds in SCG's extracts using multiple extraction methods, to evaluate the antioxidant capacity as well as the potential functional properties of SCG's extracts.

2. Materials and Methods

2.1 Materials

Spent coffee grounds were collected from Trung Nguyen Coffee shops in Ho Chi Minh City, serving Arabica and Robusta coffee on a regular basis. The materials, with over 80 % humidity, were allowed to air dry for several days before dried at 80 ± 5 °C in 3 h until reaching constant weight with nearly 3 % of moisture. Next, the dried materials were sieved for a range of particle sizes from 0.315 mm to 0.6 mm then stored in the dark at room temperature until use.

The 2,2- diphenyl-1- picrylhydrazyl (DPPH) and quercetin were bought from Merck Co. (Germany). Folin-Ciocalteu phenol reagent and gallic acid (3,4,5-trihydroxybenzoic acid) were obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA). The equipment included Microwave (Sanyo EM-S2086W), supersonic bath (Power Sonic 410), a set of Soxhlet extractors, filtering evaporator, rotating evaporator (model R-215, BUCHI Labortechnik AG, Switzerland) and Ultraviolet – visible spectrophotometer (PG Instruments T70).

2.2 Methods

2.2.1 Extraction procedure

The extraction experiments were carried out by different methods such as Ultrasound-assisted extraction (UAE), maceration, Microwave-assisted extraction (MAE) and Soxhlet method. For each experiment, 10 g of SCG and an appropriate amount of n-hexane were loaded into 250 mL flask or a set of Soxhlet extractors. The amount of n-hexane depended on SCG/solvent ratio of 1 : 10, 1 : 15 and 1 : 20 w/v, corresponding to 100 mL, 150 mL and 200 mL of n-hexane. For all methods, with the exception of Soxhlet methods, the experiments were set at room temperature. The solvent was boiled and refluxed in the Soxhlet extractor at 65 ± 5 °C. Depending on the method used, the extract was taken and the same amount of new solvent was added after a specific amount of time. This process was repeated until the extract became colorless. At the end of each extraction, the extract was filtered through a 0.45 µm membrane filter. The solvent was then withdrawn from the extractor and evaporated in a Rotavapor equipped with a vacuum controller and a pump. Finally, SCG oil was weighed, re-dissolved in methanol and stored at -18 °C until the analysis for lipid hydroperoxide content. In the next stage, defatted SCG (the residue after extract removal) were dried at room temperature to remove solvent then it was extracted by methanol with the same ratios (1 : 10 w/v, 1 : 15 w/v and 1:20 w/v). Likewise, the extract was filtered through a 0.45 µm membrane filter then evaporated in a Rotavapor. The phenolic phase was weighed, re-dissolved in methanol and stored at -18 °C until the analysis for total content of phenolic, flavonoid and antioxidant capacity. There were three runs per experiment and the average values \pm standard deviations were presented.

2.2.2 Determination of total lipid hydroperoxide content

Unsaturated fatty acid compounds in coffee oil reduced due to self-oxidation of unsaturated fatty acid radicals. Fe^{3+} concentrations were determined by measuring absorbance at 500 nm and $\epsilon = 58,440 \text{ M}^{-1} \text{ cm}^{-1}$, due to the formation of complexes between Fe^{3+} and SCN^- .

2.2.3 Evaluation of antioxidant activity

DPPH (2,2- diphenyl-1- picrylhydrazyl) is the most common antioxidant assay. It is an abbreviation of 2,2-diphenyl-1-picrylhydrazyl, a stable free radical. An antioxidant, as hydrogen donor, can neutralise the free radical, reducing DPPH (purple) to DPPHH (yellow). Even though the decolorisation shows sign of reducing affect, a more accurate indicator for radical scavenging's strength is IC50. In this study, IC50 was specifically defined as the extract's minimal inhibitory concentration, at which 50 % of DPPH were inhibited. In general, the stronger the scavenging strength, the lower the IC50 value.

2.2.4 Determination of total phenolic content

Folin-Ciocalteu is a widely used to measure total phenolic content. The indicator is the color change of Folin-Ciocalteu reagent (FCR), from yellow to dark green, in the reaction with the hydroxyl of phenolic compound. In this study, phenolic content was determined, based on sample absorbance values and a calibration curve with gallic acid as the standard.

2.2.5 Determination of total flavonoid content

The total flavonoid content of a methanolic extract from defatted SCG was determined by aluminium chloride colorimetric method based on the formation of a complex.

3. Results and discussion

3.1 Fatty acids composition

In Maceration and MAE experiments, the fatty acids compositions of SCG extracted oil were determined by GC-MS method equipped with a FID (Flame Ionization Detector). The analysis showed that the main constituents of the oils were saturated and unsaturated fatty acids, of which the glyceride portion was predominantly made up of palmitic, oleic and linoleic acids. Most of the fatty acids in diet are long-chain. Table 1 showed that in the total fatty acids extracted from SCG, the unsaturated accounted for over 60 %, nearly doubled that of the saturated. The presence of saturated acids in Maceration and MAE methods were 37 % and 38 %. In particular, the percentage of linoleic acid (C18: 2) was the highest, approximately 35.39 % in Maceration, and 33.68 % in MAE. High amount of linoleic acid (C18:2) presented in SCG oils is necessary for human body. Second to linoleic acid was palmitic acid (C16: 0), which was 28.73 % for Maceration samples and 31.55 % for others. Next was oleic acid (C18: 1), which was 25.78 % and 25.89 % for Maceration and MAE samples. The high concentration of oleic acid (C18:1) – one of the drug neurotransmitters, can enhance the nutrient absorption of human body. The minority of oil was other acids which accounted less than 10 %. Generally, the oil compositions obtained from the two methods, Maceration and MAE, were similar; nonetheless, slight fluctuations occurred in percentage values. These results were consistent with previous reports.

Table 1: The fatty acids of SCG extract using Maceration and MAE by GC-MS method

Content of Fatty acids (%)	Maceration	MAE
Decanoic acid (C10:0)	0.06	0.05
Dodecanoic acid (C12:0)	0.34	-
Tetradecanoic acid (C14:0)	0.59	0.45
Pentadecanoic acid (C15:0)	0.04	-
Hexadecanoic acid (palmitic acid) (C16:0)	28.73	31.55
9 -Hexadecenoic acid (C17:0)	0.10	-
Heptadecanoic acid (C17:0)	0.09	1.01
Octadecanoic acid (C18:0)	5.06	4.15
Cis - 9 - Octadecenoic acid (oleic acid) (C18:1)	25.78	25.89
Trans – 9 - Octadecenoic acid (C18:1)	0.09	-
Cis – 9,12 - Octadecadienoic acid (linoleic acid) (C18:2)	35.39	33.68
Cis – 9,12,15 - Octadecatrienoic acid (C18:3)	2.07	1.67
Eicosanoic acid (C20:0)	1.17	1.03
11 – Eicosenoic acid (C20:1)	0.18	-
Eicosatrienoic acid (C20:3)	-	0.30
Docosanoic acid (C22:0)	0.22	0.22
Tetracosanoic acid (C24:0)	0.09	-

3.2 The recovery yield of oil from a hexanoic extract, of phenolic from a methanolic extract

The recovery yields of oil and phenolic from extracts were calculated by the following formulas:

$$\text{Recovery yield of SCG oil} = \frac{\text{weight of oil from a hexanoic extract}}{\text{dry weight}} \times 100\% \quad (1)$$

$$\text{Recovery yield of phenolic phase} = \frac{\text{weight of phenolic from a methanolic extract}}{\text{dry weight}} \times 100\% \quad (2)$$

From Figure 1, it can be clearly seen that both MAE and Soxhlet showed the highest recovery yields than other methods. Maceration gave the lowest content of oil at most of the ratios. The recovery yield of oil significantly increased in ascending order of solid/solvent ratio. In general, the extracted oil using MAE was slightly higher than using Soxhlet. The maximum yield was achieved by MAE (17.96 %) at ratio of 1 : 15, which is higher than the result in previous report (15.1 %) with the same solvent (Obruca et al., 2014).

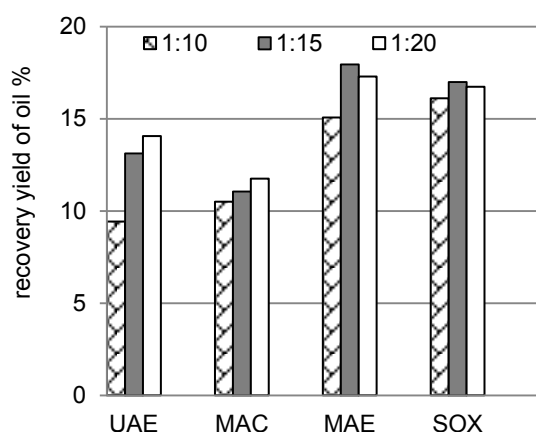


Figure 1: The recovery yield of oil vs. different solid/solvent ratios

To choose an optimal method for extraction, a comparison of the extraction time was summarised in Figure 2. It showed that Maceration was the most inefficient choice due to its time consumptions. MAE was highlighted with the least time consumption (30 min). The time consumption of Maceration was 16 times longer compared with that of MAE. Therefore, MAE was effective for extracting oil from SCG.

The efficiency of extraction of phenolic compounds from SCG was presented in Figure 3. The trends varied among methods when ratio of material to solvent increased. For UAE and Maceration, the yield increased when the amount of solvent increased. An opposite trend occurred in MAE. In the Soxhlet method, the yield rebounded to 9.04 % at 1 : 20 after reducing to 7.03 % at 1 : 15. Notably, MAE was shown to be the most effective with the highest amount of phenolic, up to 12.3 %, which was twofold as compared with the lowest amount (5.76 %) in Maceration.

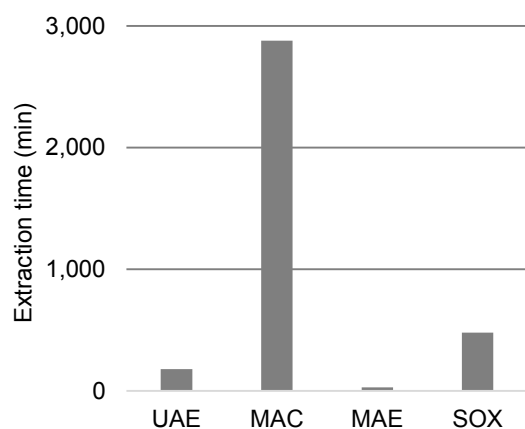


Figure 2: The extraction time of different methods

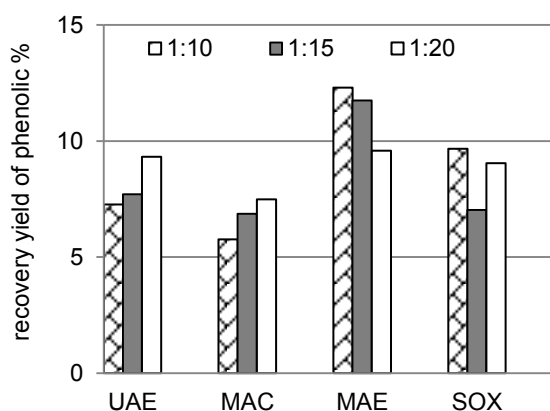


Figure 3: The recovery yield of phenolic vs. different solid/solvent ratios

3.3 The results of total lipid hydroperoxide content analysis and antioxidant capacity

The total lipid hydroperoxide contents of SCG oil extracted by different methods were shown in Figure 4. Despite the fact that SCG/solvent ratios considerably rose from 1 : 10, 1 : 15 to 1 : 20, the LP content was stable in Maceration, MAE and Soxhlet. The content slightly increased from 0.0153 mM to 0.0191 mM with the assistance of ultrasonic. The highest amount of LP was found in UAE, at the ratio of 1 : 20, which was a bit higher than that of MAE (0.0172 mM). It can be seen that increasing solvent did not affect the LP content in Maceration and Soxhlet methods. Figure 5 shows the effects of extraction methods and ratios on antioxidant activity. The activity can be evaluated through IC₅₀ value. The values ranged from 31.44 mg/L to 43.15 mg/L. It confirmed the antioxidant potential of SCG, which was reported in earlier papers (Obruca et al., 2014).

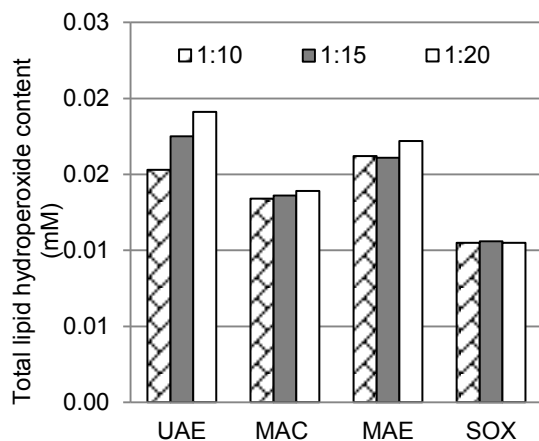


Figure 4: The results of total lipid hydroperoxide content analysis

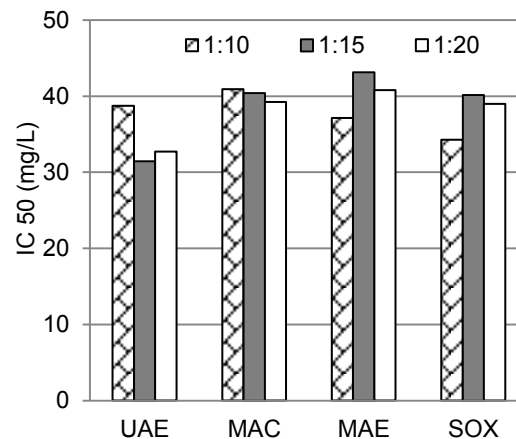


Figure 5: Antioxidant capacity of extracts vs. different ratios

3.4 The results of total phenolic content (TPC) and total flavonoid content (TFC) analysis

The analysis found that total phenolic content versus various ratios did not fluctuate in samples in MAE and Soxhlet (Figure 6). However, there were increasing trends in others. In detail, with UAE at 1 : 20, the highest value of TPC reached 13.466 mg AG/ g dry mass; this value was much lower than the result of Vignoli et al. (2011) (16.67 ± 0.34 mg AG/ g). The lowest amount was obtained from Maceration (about 10 mg AG/g dry weight). According to Jenkins and colleagues (Jenkins et al., 2014), the reasons lies in the differences in species, climates, soils or geographical regions, etc.

It was apparent from Figure 7 that TFC of Maceration was much lower than that of others. In fact, the difference among methods was considerable. The measured flavonoid content ranged from about 20.60 mg to 29.53 mg quercetin/g dry weight whereas the TFC of the extracts from SCG bar and capsule ranged from 2.11 to 8.03 mg quercetin/g (Panusa et al., 2013). With the same method, the highest value was 31.15 mg quercetin/g dry weight at the 1 : 20 ratio, which was approximately 1.5 times the lowest value at the 1 : 10 ratio. Figure 7 describes a general trend, when the solid/ solvent ratio went up, the total flavonoid content also increased. This trend highly resulted from the effects of heat. Heating caused the molecular flavonoids to be easily separated from the raw materials, so in UAE, MAE and Soxhlet methods, the TFC values were high. The Maceration method prevented the effect. Thus, flavonoid's extraction was quite challenging.

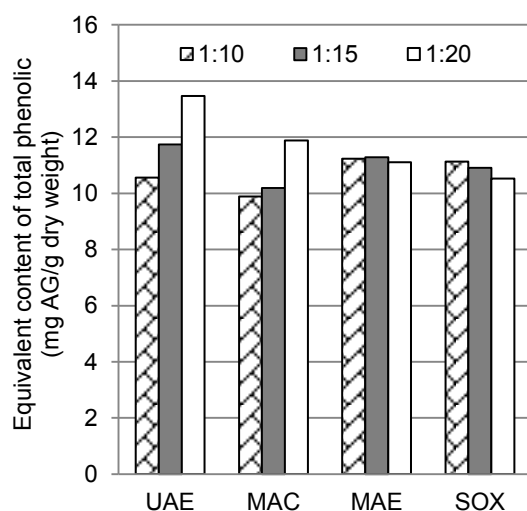


Figure 6: The results of total phenolic content analysis

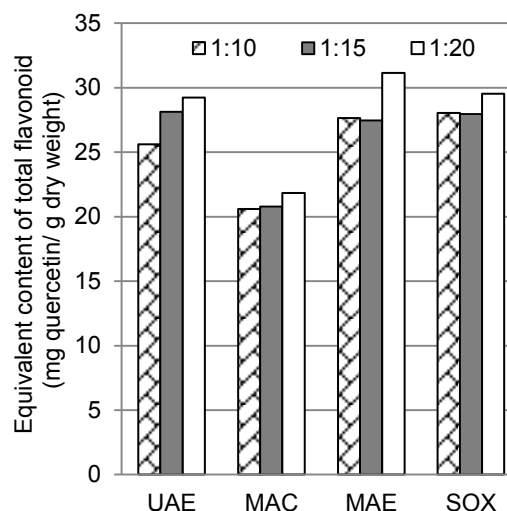


Figure 7: The results of total flavonoid content analysis

4. Conclusions

This paper only focused on measuring the content of bioactive compounds in SCG and the efficiency of each extraction methods. The associated cost performance will be studied in future studies. This study found that 90 % of SCG was composed of palmitic, oleic and linoleic acids. MAE was the optimal method to obtain the highest recovery of both oil and phenolic, based on its time and solvent efficiency. In addition, the highest amount of total lipid hydroperoxide and total phenolic content were achieved by UAE. SCG was also confirmed as a highly potential antioxidant source, with 30 - 40 mg/L.

Acknowledgements

This research was funded by JICA Project for AUN/SEED-Net under grant number HCMUT CRC 1501.

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