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Investigation of Coffee Instant Premium by Subcritical Water Hydrolysis: Enhance of Caffeine and Antioxidant Contents

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Abstract

The current study observed a basic investigation into instant coffee production through subcritical water (SBW) hydrolysis. A series of SWB research in lab-scale were carried out to optimize the product qualities by adjusting the process parameters such as temperature, solvent amounts, and time for hydrolysis. The caffeine, antioxidant, pH changes, and morphologies of modified and unmodified coffee powder (commercial products) were also evaluated by SEM. The result showed that the optimum conditions were observed for SBW process at 170°C/11.18 ml/20 min with obtained 0.48 mg/ml and 29.40 µg/ml for antioxidant and caffeine, respectively. SEM images represented physical degradation of coffee extracted powder during hydrolysis. Further, the amount of coffee in the blend with mineral amount could be important sources of minerals to the human diet, as evident by EDX analysis. Further, the change of chemistry surface was also confirmed by FTIR. In summary, flow-through SBW hydrolysis of coffee extract promises for enhance caffeine and antioxidant.

Keywords: antioxidant, caffeine, coffee instant, subcritical water hydrolysis, commercial products

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1. Introduction

Coffee is one of popular and widely consumed beverages in the world [1]. Recent research indicates that consistent use of coffee (approximate 2 to 4 cups each day) is related to a substantially reduced chance of mortality, colorectal cancer growth, liver damage and cirrhosis, as well asvserious illnesses (including dementia, Parkinson's disease, diabetes of type 2, and cardiovascular disease)v [2-3]. In the world, more fifty countries are contributed in supplying coffee as main agricultural produce [4-5].Thus, the development of brand coffeehouse has gaining popular recently. The customer interest of instant coffee approximately 400 billion cups a year and speculated periodically consumed by 40 percent of population in the world [6]. Instant coffee is more serviceable and cleaner to prepare than not instant coffee. These essential factors led to costumer's preferences to choose instant coffee over not instant coffee [7]. Generally, the robusta coffee is more selected than arabica coffee in production of instant coffee due to more economical and has strong bitter flavor [8-9]. Further, for those of people on a diet, robusta coffee may be a better option because it contain fewer sugar and fat than arabica coffee. The conventional coffee-making process is simple, roasted bean is grounded and then extracted by hot boiling water before drinking [10]. However, the techniques to extract coffee utilizing hot boiling water was only discharge volatile components in coffee, while the non-volatile components were not extracted [11-12]. Many studies reported to extracted the non-volatile components from coffee due to the essential portion abundantly found as non- volatile components, which offers benefit for enhance foam formation, sensory taste, and aroma intensity of coffee [13]. Common technologies to extracted coffee through chemical usages such as acids, alkalis, catalysts or organic solvents [14], but the main crucial drawbacks of chemical usage are long processing time, harmful, and required high cost [15-16]. Subcritical water (SBW) hydrolysis is a potential alternative for extracted coffee. SBW is defined "hot compressed water" as liquid water held at temperature over 100 and 374°C and pressure higher than its vapor pressure [17]. In these conditions, water could perform as less hazardous solvent and smaller corrosion risk than organic solvent and acidic minerals [18-19].

SBW has been applied effectively used to extract polyphenols from plant sources, as well as antioxidant [20]. Recently, it has been used to extract flavonoids from berries, polyphenols from herbs, and essential oils from seed [21]. As previous studies mentioned that SBW method is safer, faster, and more economical method than conventional extraction methods [10-22]. Hence, SBW is found to be appropriate for enhancing properties of coffee. In the past few years, significant work has been reported on the coffee extraction both utilizing conventional and non-conventional methods [23-24]. However, only few study SBW method to coffee extract. The effect of SBW temperature, solvent ratio, and time were investigated systematically. The SBW-treated solids were analyzed using scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR) to observe the modification of structural and chemical changes in the coffee solids material during SBW treatment. Moreover, the obtained of antioxidant activities and caffeine concentration during SBW process were also evaluated.

2. Materials and Methods

2.1 Materials

Red coffee beans were supplied by a local coffee shop in the traditional market of Yogyakarta. First, the red coffee beans were peeled and allowed to dry at ambient temperature to prevent the degradation of compounds in coffee. Next, the red coffee beans were categorized by size. The obtained of coffee beans were roasted, then crushed by a house blender and sieved in 250 µm. Before further experiments, the coffee powders were stored in plastic zip-lock bags in a refrigerator at -20°C. All other materials were high quality and obtained from available distributors.

2.2 Extraction procedure by SBW

The extractor has an internal volume of 10 l with maximum working conditions 178°C and 0.7 MPa. To remove the residual oxygen content, the extractor was equipped with nitrogen gas. While, the temperature difference of extraction process was monitored by thermocouples (APPA Digital Thermocouple Thermometer 55II), located at the outlet of extractor. Prior to the experiment, the extractor tank was flushed twice by nitrogen gas in order to ensure absent of oxygen content in extractor. Then, 350 g of the desired coffee powder were mixed with the water as solvent and placed inside the extractor before it was sealed. Again, the nitrogen gas was pumped to remove residual air. The entire system had been completely filled with water and entirely vacuum pressured. Next, the heating device was active to obtain the appropriate temperature process. The details of the condition process were mentioned in Table. 1. Further, obtained coffee extract was cooled in condenser, approximately required 15 minutes of continuous water flow in order to decrease the temperature. At regular intervals of 4 minutes, samples of coffee extracts collected for 20 minutes and then kept in glass tubes before analysis.

2.3 pH measurement

The pH alteration of coffee extract from SBW process was measured by a digital pH meter (HI98107, Hanna Instrument, and USA) at room temperature.

2.4 Antioxidant analysis with DPPH

The antioxidant analysis was performed by DPPH (2, 2-diphenyl-1-picrylhdrazyl) method, as described in previous study (Dos Santos and de Oliveira, 2001). The coffee extract from SBW process was mixed with DPPH solution. Then, the absorbance of reaction mixtures was recorded at 492 nm by a micro plate reader after incubation for 30 minutes. Antioxidant concentration in coffee extracts was obtained by calculating Trolox equivalent antioxidant capacity (TEAC) (mmol TE/g dry coffee extract).

2.5 Caffeine analysis

2g was mixed in 150 ml hot water boiling for 30 minutes. After homogenization, separate the solution into filtrate and retentate. Then, 1.5 g $CaCO_3$ was mixed with filtrate solution and extracted by chloroform as solvent. Prior measurement of absorbance using UV-Vis spectrophotometer (Shimadzu Co., Japan) at wavelength 271 nm, the extract solutions was washed twice to remove chloroform residue.

2.6 Scanning electron microscopy - Energy Dispersive X-ray (SEM-EDX)

The surface structure and mineral composition of coffee samples before and after SBW process was characterized by SEM-EDX (SEM, JEOL, JSM-6510 LA, and Japan). Before analysis, the samples were coated by a nanometer-thick gold film using sputter coater for increase their performance. SEM-EDX analysis were carried out in vacuum condition with a 5 kV acceleration voltage. Different magnifications were taken in order to ensure the consistency of the SEM imaging results.

2.7 Fourier transform infrared spectroscopy (FTIR)

The chemical structures of the membrane surface were characterized by a Fourier transform infrared spectroscopy (FTIR; Spectrum Two™ FTIR Spectrometer, PerkinElmer, Waltham, MA, USA). 122.

3. Results and discussion

3.1 Antioxidant content

The antioxidant content of coffee extracts in terms of DPPH are shown in Figure 1. The DPPH radical scavenging activity varied between 5.24 to 29.39 µg/ml. The results demonstrate that the antioxidant activity of the coffee extract decrease with the increasing solvent concentration. The high oxidant levels in samples were obtained by lower solvent concentration. Meanwhile, the best antioxidants were in the variable treatment of 170°C/11.18 ml/20 min with antioxidant levels 29.39. Meanwhile, the lowest antioxidant was in the variable treatment 170°C/20 ml/11.18 min, with an antioxidant level of 5.24. The average antioxidant in sixteen samples was 16.52. From the diagram above, the temperature does not affect the achievement of antioxidants, but time and adding ml to the product are very influential and cause the results to fluctuate. However, the high temperatures greatly influence the levels of antioxidants produced.

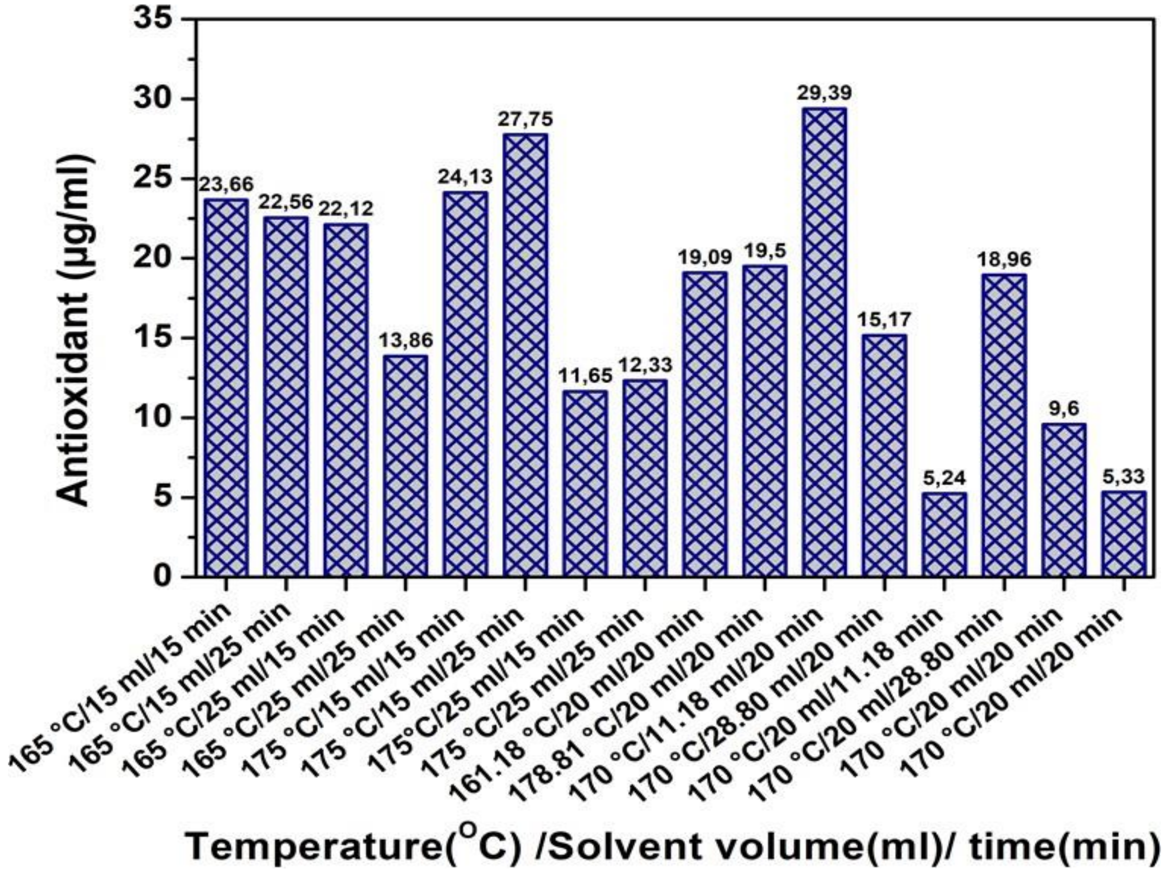


Figure 1. Antioxidant concentration in coffee extract result during SBW process.

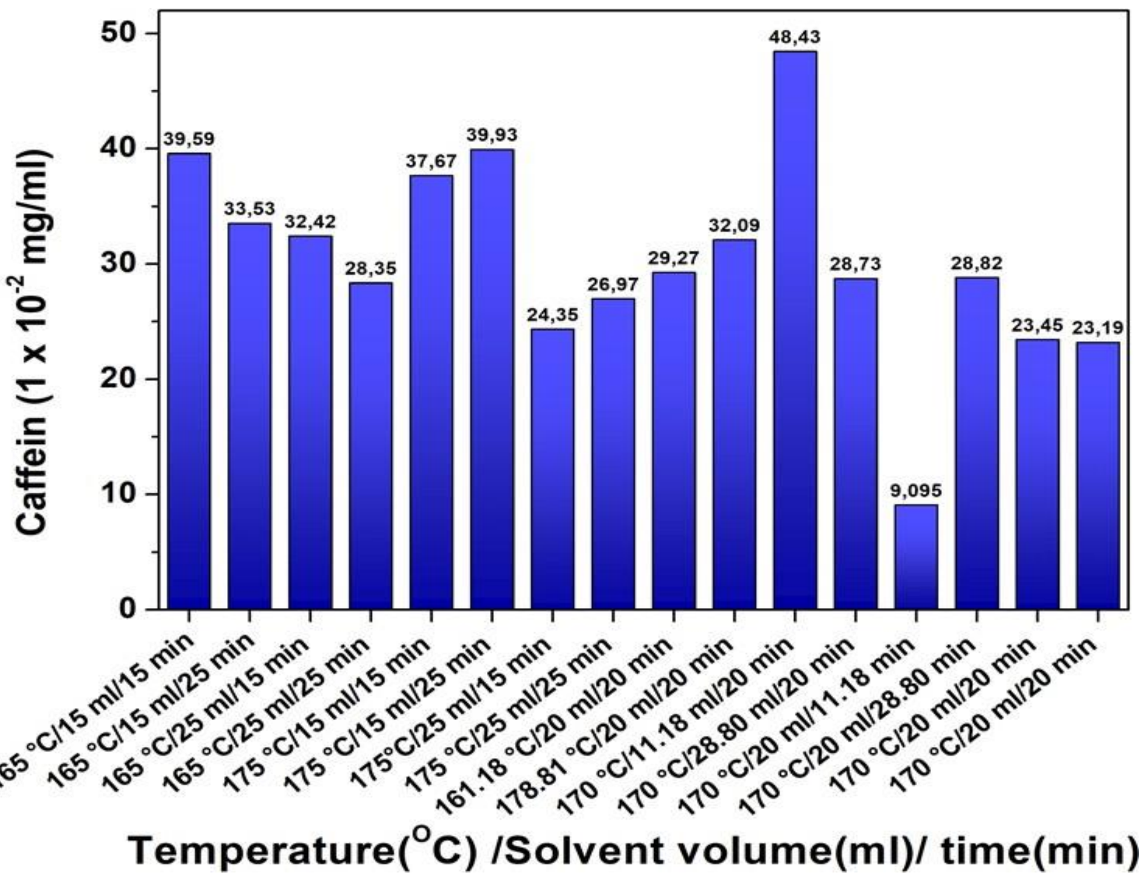


Figure 2. Caffeine concentration in coffee extract result during SBW process.

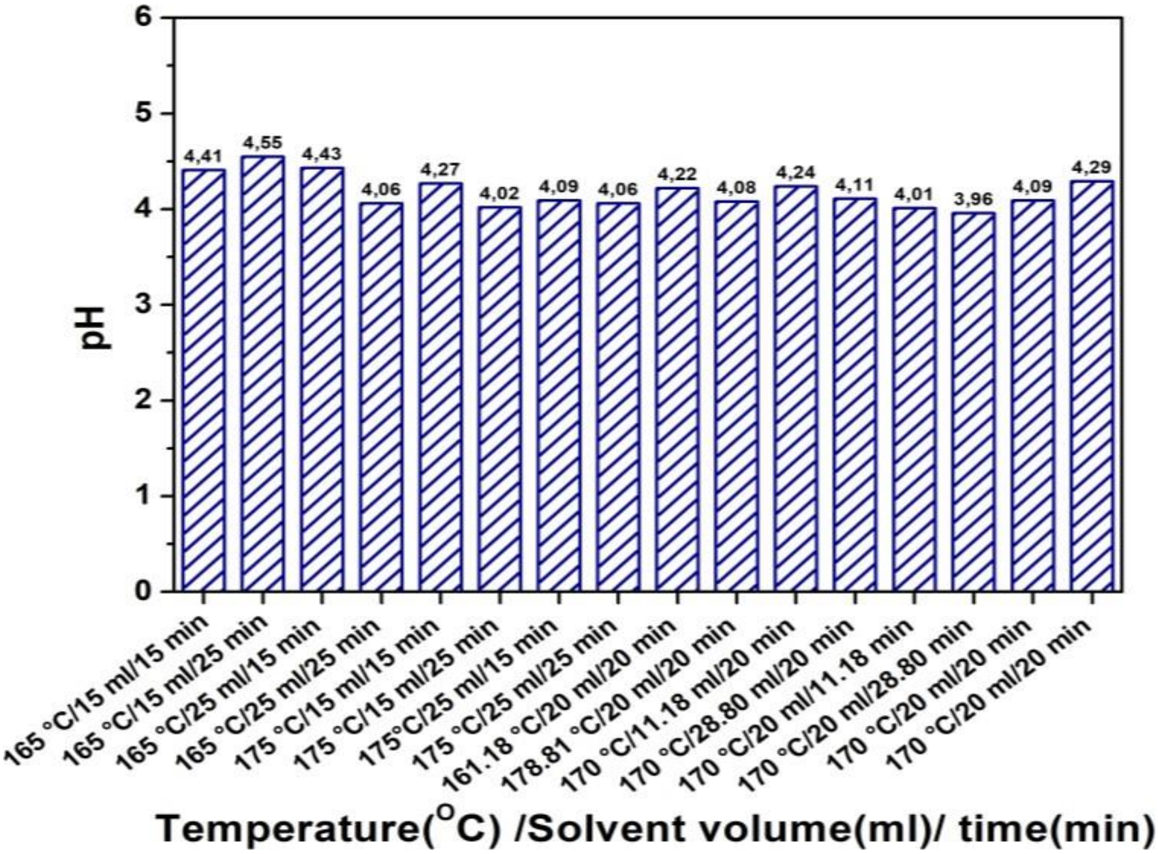
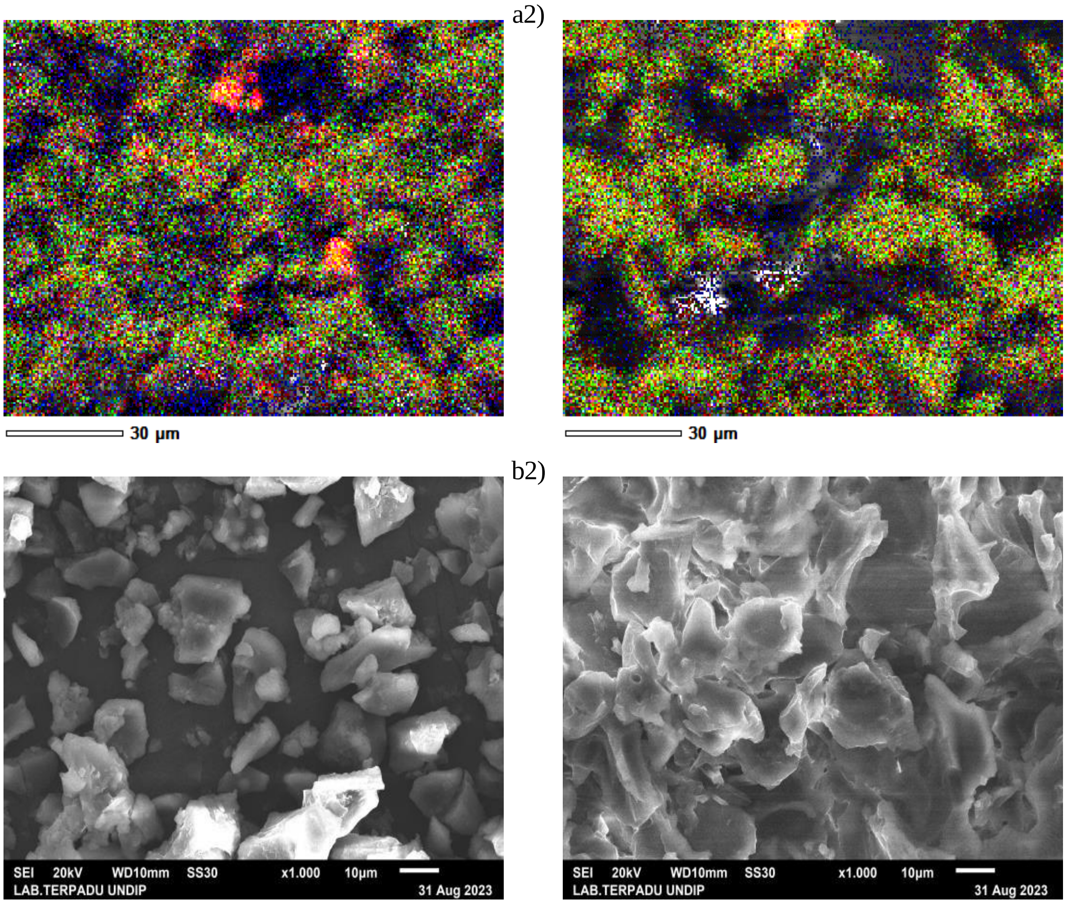


Figure 3. The result of pH in coffee extract result during SBW process

Unmodified coffee **Modified coffee**

a1) a2) b1) b2)



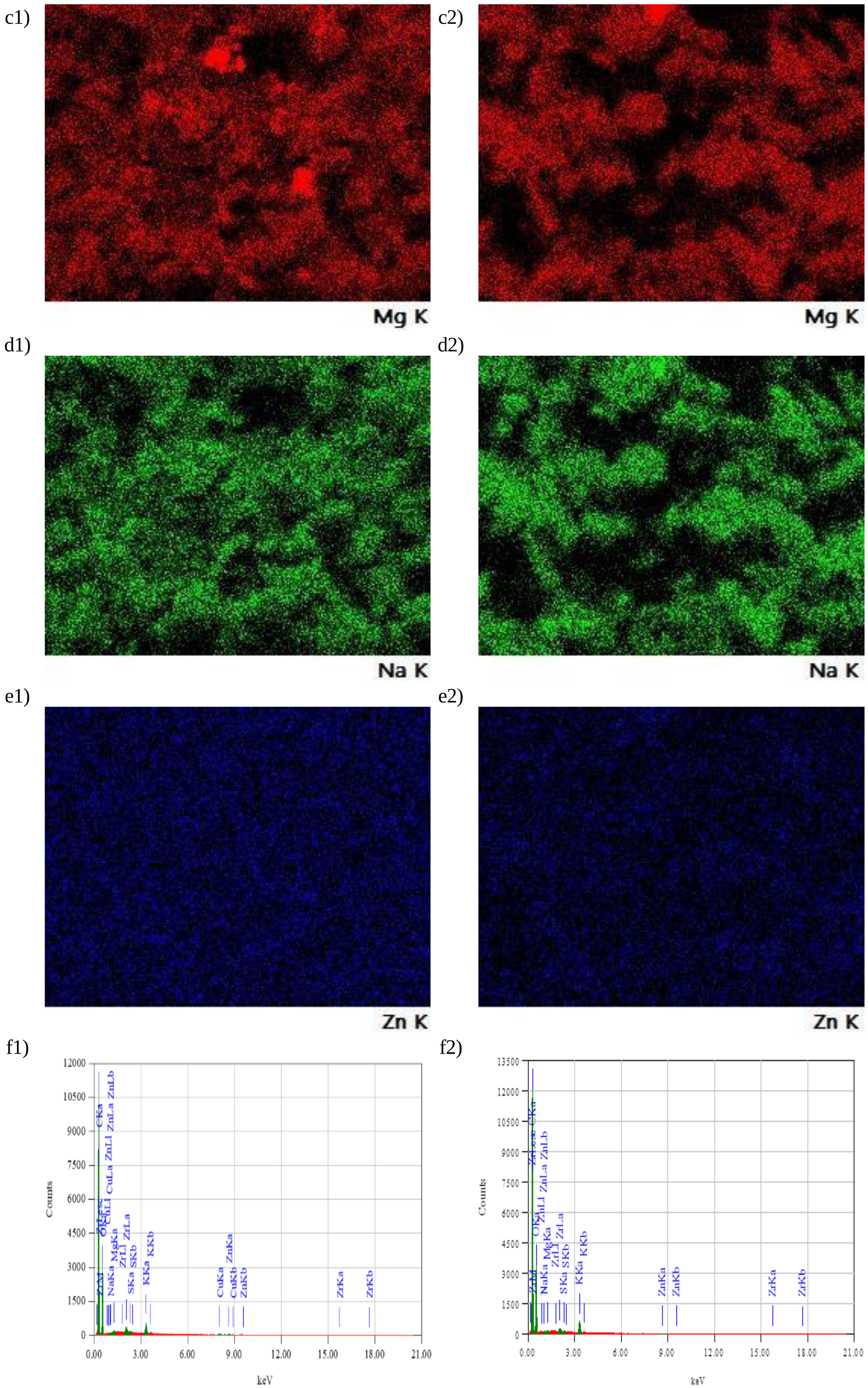


Figure 4. Images obtained by SEM-EDX on coffee extract powder unmodified (left) and modified coffee extract powder unmodified (right). Modified coffee extract after the SBW process at 170°C (temperature), 11.18 ml (solvent water), 20 min (time SBW).

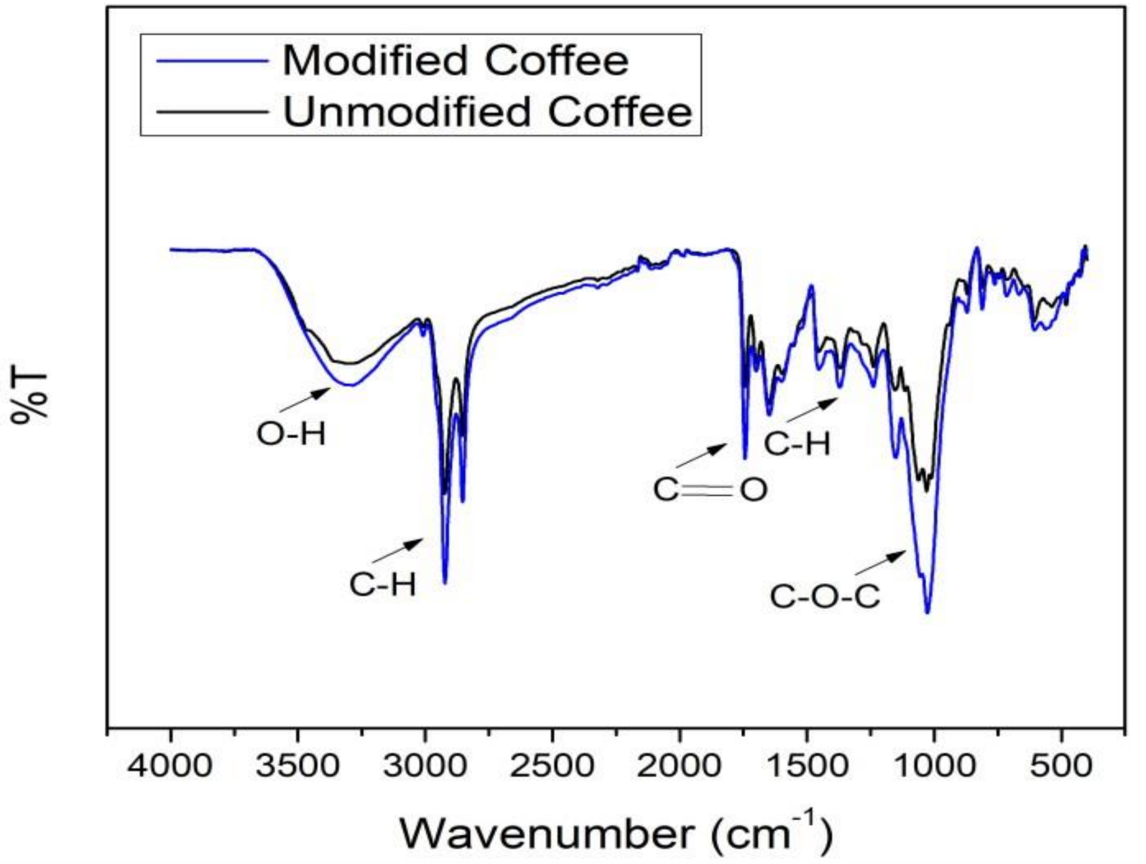


Figure 5. FTIR spectra of unmodified and modified coffee extracts. The condition of extraction a 170°C (temperature), 11.18 ml (solvent water), 20 min (time SBW).

Table 1. The details of condition process during SBW

Run	Temperature (□C)	Solvent (water) (ml)	Time (min)
1	165	15	15
2	165	15	45
3	165	25	15
4	165	25	45
5	175	15	15
6	175	15	45
7	175	25	15
8	175	25	45
9	170	20	30
10	161	20	30
11	179	11.18	30
12	170	28.8	30
13	170	20	30
14	170	20	4
15	170	20	56
16	170	20	30

Table 2. The elements of coffee extracts before and after SBW modification process at 170°C (temperature), 11.18 ml (solvent water), and 20 min (time SBW).

Elements	Mass%	
	Unmodified coffee extracts	Modified coffee extracts
C	64.97	64.24
O	32.80	34.15
Na	0	0.03
Mg	0.09	0.06
S	0.08	0.08
K	0.78	0.96
Cu	0.40	-
Zn	0.28	0.02
Zr	0.6	0.46

High temperatures result in the degradation of certain phenolic compounds. This results in products containing phenol structures that are very stable in subcritical water. It can be concluded that the coffee produced is good because it has high antioxidants.

3.2 Caffeine content

It can be seen from Figure 2, the highest caffeine level is obtained by 170°C/11.18 ml/20 min at 48.43. Meanwhile, the lowest caffeine level was provided by 170°C/20 ml/11.18 min at 9.09. The average caffeine level in this study was 27.46. It can be seen from Figure 2, the highest caffeine level is obtained by 170°C/11.18 ml/20 min at 48.43. Meanwhile, the lowest caffeine level was provided by 170°C/20 ml/11.18 min at 9.09. The average caffeine level in this study was 27.46. This is directly proportional to antioxidants, which means that the caffeine levels in the coffee due to this research are pretty high. The antimicrobial mechanism of caffeine can be explained by its ability to bind specifically to single stranded DNA and inhibit excision repair mechanisms [25]. Caffeine levels are a significant barrier to increasing consumption. Coffee can also pose a public health problem, with consumer dependency [26]. The usual human dose is two cups per day.

3.3 pH result

From Figure 3, the highest pH was obtained by of 165°C/15 ml/25 min, namely 4.55. Meanwhile, the lowest pH was performed by 170°C/20 ml/28.80 min, namely 3.96. The average pH produced by the sixteen variables was 4.18. From this result, it showed that the pH of the hydrolyzate decreases with increasing reaction temperature, while pH decreases when extraction temperature and pressure increase. At high temperatures, the product in the initial stages of the process decomposes into organic acids and contributes to a reduction in pH of the extract.

3.4 Morphologies of coffee extracts

As listed in Table 2, the chemical composition of unmodified and modified coffee extracts was observed by EDX analyses. The EDX examination results demonstrated that the unmodified coffee extracts contain the elements carbon (C), oxygen (O), natrium (Na), magnesium (Mg), sulfur (S), kalium (K), cupper (Cu), zinc (Zn), zirconium (Zr), with concentration of 62.87%, 32.80%, 0%, 0.09%, 0.08%, 0.78%, 0.40%, 0.28%, and 0.6%, respectively. Compared to the unmodified coffee extracts, the modified coffee extracts contained a new element of Na with concentrations of 0.03%. Meanwhile, the element of Cu disappeared. This could be due to the SBW process may break the Cu elements and produce Na elements from coffee seed. Moreover, the modified coffee extracts had a higher oxygen content than unmodified coffee extracts. The increase in oxygen content may help the modified coffee extracts blend easily to the water. 171 Figure 4 showed SEM images (a1-f1) and (a2-f2) for unmodified and modified coffee extract, respectively. The subcritical water conducted at 170°C/11.81 ml/20 min. The results show that the surface of the unmodified coffee extract is brighter than those of the samples that underwent SBW treatment, consistent with the increased antioxidant content in the treated samples.

The previous study mentioned that SBW treatment decreases the sample oil content, thereby decreasing the appearance of artificial brightness. Further, at higher magnifications in Figure 4 (b1) have irregularly shaped and exhibit more roughness than Figure 4 (b2). Meanwhile, the increase oxygen content in modified coffee extract could affect the taste, aroma and overall quality of coffee beans, as showed in Figure 4 (f1-f2). Further, the chemical composition was found in modified coffee extracts such as Na, Mg, Zn, and Zr. These minerals are usually considered as minerals in coffee beans [26]. Nonetheless, caffeine, the classic coffee identifier, is highly variable within coffee, being especially reliant on the coffee species utilized, with robusta coffee having about double the caffeine content of Arabica coffee. Robusta coffee is the most commonly used raw coffee for instant coffee production due to its higher solid soluble yield, higher caffeine content, and, most importantly, lower price. Consequently, SEM images demonstrated physical degradation of the coffee material from SBW process.

3.5 Chemical groups of coffee extracts

FTIR was used to analyze the chemical structure of the membranes surfaces in order to confirm the successful of modification process. In Figure 5, the spectra in FTIR from the wavenumber range of 400 cm^{-1} to 4000 cm^{-1} of the modified and unmodified coffee extract samples. The presence of a peak at 3300 cm^{-1} can be attributed to the O-H groups in coffee extracts. The FTIR spectra of coffee extract both modified and unmodified demonstrated peaks in four important wavenumbers, i.e., 2923, 1741, 1353, and 1020 cm^{-1}, which were assigned to the C-H, C=O, C-H, and C-

O-C, respectively. These findings are correspond with those of previous publication [27]. Compared to the peak of unmodified coffee extracts, the peak of modified coffee extracts demonstrated sharper in terms of O-H groups in wavenumber range from 3125 up to 2425 cm^{-1}. This indicates that O-H groups after modification has increased. Moreover, this also confirmed by previous result in section 3.4. From SEM-EDX result, the elements of O have been increased from 32.80% to 34.15%.

4. Conclusions

To study the value of coffee factory leftovers to produce chemicals and biofuels, coffee powder and extracted cake were treated under flow through SBW conditions. The optimum conditions were observed for SBW process at 170°C/11.18 ml/20 min with obtained 0.48 mg/ml and 29.40 µg/ml for antioxidant and caffein, respectively. SEM was used to examine coffee extract powder surface powder before and after treatment SBW. SEM images represented physical degradation of coffee extract powder during hydrolysis. Further, the amount of coffee in the blend with mineral amount could be an important source of minerals to the human diet, as proved by SEM- EDX. Meanwhile, FTIR confirmed the successful modification process with chemical structure. In summary, flow-through SBW hydrolysis of coffee extract promises for enhance caffein and antioxidant.

Conflict of interest

Disclose any potential conflict of interest appropriately. The authors declare no conflict of interest.

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