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The Potential of Liquid Tofu Waste in Increasing Antioxidant Activity of Robusta Green Coffee

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Abstract

Robusta is the most widely cultivated coffee in Indonesia (90% of the total Indonesian coffee production). Antioxidant activity and polyphenol content of Robusta coffee is higher than Arabica coffee or the other plants. One of the efforts, for coffee market expansion is product diversification through decaffeination of coffee . Decaffeination is one of process to reduce caffeine content. Decaffeinated coffee (decaff coffee) began to become public demand. In addition to its more delicious taste, low caffeine coffee is beneficial for health because it is safer to eat. Coffee decaffeination can utilize organic solvents that contain proteases. Tofu waste was one of protease sources. So that, tofu waste had potential as solvent in decaffeination The objective of the study was to assess the antioxidant activity of Robusta green coffee after going through decaffeination by using tofu waste. The study was used Factorial Completely Randomized Design, i.e . concentration of tofu waste (30%,60%, 90%) and length of immersion (3, 6, 9 hours). Robusta green coffee antioxidant activity was examined by using DPPH method. The results showed that the highest antioxidant activity (17.6061) was in the treatment of 90% waste concentration and 9 hours of decaffeination time. In conclusion, the greater the concentration and the longer the decaffeination resulted in the higher antioxidant activity. This study provides information about coffee processing methods that can produce coffee with the best quality. So, It can increasing the value of domestic coffee products especially in the global market and utilizing tofu liquid waste to be more malleable.

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INTRODUCTION

Coffee is included in the genus Coffea which has about a hundred species including *Coffea arabica*, *C. robusta* and *C. liberica* (Varghese *et al*., 2014). However, out of a hundred species, there is only two species that have high economic value, namely *C. arabica* and *C. robusta*. The first coffee cultivated in Indonesia is *C. arabica* then followed with *C. liberica* and the last is *C.robusta*. Currently, the most grown coffee in Indonesia is Robusta, that is 90% of the total Indonesian coffee production (Rahardjo, 2012). This is because Robusta coffee is more resistant to *Hemelia vastatrix* (HV) than the other types (Wang & Ho, 2009).

Coffee contains many chemical compounds that act as antioxidants such as caffeine and chlorogenic acid (Smith *et al*., 2006). Caffeine is included in alkaloids of methylxanthin ie e 1,3,7 trimethyl xanthine. Caffeine levels of 10 g coffee powder (± 1-2 tablespoons) in 150 ml of water is 115 mg (Dollemore & Giuliucci, 2001). Chlorogenic acid is one type of polyphenolic compound that is included in powerful antioxidants in coffee (Naidu *et al*., 2008). Antioxidant activity and polyphenol content of Robusta coffee is higher than Arabica coffee or other plants (Johnston *et al*., 2003; Anggara & Marini, 2011).

One of the efforts, for coffee market expansion is coffee product diversification through decaffeination. Decaffeination is one of process to reduce caffeine content. Decaffeination aims to lower the levels of caffeine in coffee which resulted in decaff coffee (De-Azevedo *et a*l., 2008; Mulato, 2001). Low caffeine coffee (decaff coffee) began to become a public demand. In addition to more delicious taste, low caffeine coffee is also beneficial for health because it is safer to eat. One example of decaff coffee that has been known is *kopi luwak* (Hadipernata & Nugraha, 2012). *Kopi luwak* prices are quite expensive to be a constraint for people to consume them. The principle of making *kopi luwak* is the utilization of microorganisms that produce proteases in the digestion system of civet cat (Murthy & Naidu, 2011). Tofu waste was one of protease sources. So that, tofu waste had potential as solvent in decaffeination

Immersion of coffee in pineapple fruit extracts resulted in decreased caffeine and improved quality of coffee beans (Triyana, 2014; Oktadina *et al.*, 2013; Lestari, 2004). Liquid tofu waste contains 9% protein, 0.69% fat, and 0.05% carbohydrates (Fatoni *et al*., 2008). So, that, the nutritional components of tofu waste still have high levels of protein which allow indigenous protease-producing microorganisms to grow in them (Sulistyaningtyas *et al*., 2017). Coffee decaffeination can utilize organic solvents which contain proteases. Protease produced by naturally occurring microorganisms in tofu waste with a high proteolytic activity (65.24 x 10^{-2} µmol tir. $ml^{-1}.min^{-1}$) even exceeds the activity of bromelin extracted from pineapple (55.24 x 10^{-2} µmol tir. $ml^{-1}.min^{-1}$) (Rahman & Indarto, 2013; Damayanti *et al*., 2004). In vitro research of antioxidant compounds in coffee shows that they can protect DNA, lipids, proteins through the mechanism of trapping free radicals thereby reducing the risk of chronic disease (Bonita *et al*., 2007). Therefore, a study of the antioxidant activity of decaff coffee needs to be done. This study aimed to assess the antioxidant activity of Robusta green coffee after the decaffeination by using liquid tofu waste.

The benefits of this research for science was providing knowledge about decaffeinated method through the immersion of green beans in tofu liquid waste. This study also expected to provide an information about coffee processing methods that produce coffee with the best quality so as to increase the value of domestic coffee products especially in the global market and utilization tofu liquid waste to be more malleabe.

METHODS

The study used Robusta coffee from coffee plantation in Ambarawa Central Java, tofu waste from a known home industry in Semarang Central Java, paper label, filter paper Whatman No. 41, A. salina Leach, methanol,distilled water and DPPH

Preparation of Liquid Tofu Waste Solvent

Liquid tofu waste was obtained from home industry in Semarang Central Java. The tofu waste was initially filtered so that it is free from dirty. Composition rasio between coffee and liquid tofu waste solvent is 1: 2 (w/v).

Preparation of coffee samples

The coffee fruit was taken from Robusta coffee plantation in Ambarawa, Central Java. Coffee fruit was picked by picking, the characteristic of ripe coffee fruit are red color with the same size and without any physical defect. The pickled coffee was stored at low temperatures. Furthermore, coffee fruit was peeled to get coffee beans with skin horns. The beans were weighed before the treatment (Widyotomo *et al* 2009; Widyotomo *et al* 2012) .

Decaffeination of robusta coffee beans

Robusta coffee beans that had been washed. After that, it was soaked in liquid tofu waste with the concentration of tofu waste (30%, 60%, 90%) and different length of immersion (3, 6.9 hours).

Robusta Green Robusta Coffee Powder Sampling

The green beans that had been soaked in tofu waste were then dried with the help of sunlight for three days. Dried green coffee beans then were milled by using a coffee grinder (Ashihara *et al*., 2008; Dziki *et al.,* 2016; Perva-Uzunalić*,* 2006; Tello *et al*., 2011).

Extraction of total antioxidant

The immersing coffee beans were washed by using distilled water to reduce the mucus. After that, coffee beans were dried for three days with the help of sunlight. The next stage was the maceration of coffee beans by using 70% methanol for 24 hours stored at 4°C. Maceration in methanol aimed to extract the antioxidant compounds present in coffee beans. The extraction process will cause the sample color change from clear to yellow.

Extraction results are filtered using filter paper. Filtrate is a liquid used to determine the antioxidant activity in Robusta green coffee. This filtrate is considered as a solution with a concentration of 1000 ppm. Furthermore, dilution is carried out to a solution concentration of 100-10 ppm.

Analysis of Antioxidant Activity

The method used to test the antioxidant activity of Robusta coffee beans was a test method by using DPPH free radicals. DPPH reagent was a purple reagent used to determine the presence or absence of antioxidants in a solution. If a sample contained antioxidants it would change the color to yellow. The yellowish level depended on the total antioxidant compound content of a sample. The more yellow the higher the total antioxidant compound levels. Sample absorbance measurements by using spectrometry were preceded by maximal wavelength measurements. Maximum wavelength measurements were made by measuring the absorbance of methanol blank sample with DPPH reagent added.

The purpose of this method was to determine concentration parameters to give 50% effect of antioxidant activity (IC50). This can be achieved by interpreting experimental data from the method. The pure isolates obtained were tested for their antioxidant activity by DPPH method. The sample was dissolved in methanol (concentration 10-1000 ppm), reacted with 0.2 mM DPPH, incubated for 30 min at room temperature, then had the absorbance measurement at 515 nm wavelength. Antioxidant activity was calculated as a percentage of inhibition to DPPH (percentage of "scavenging effect"), ie:% inhibition = [1 (absorbance of a sample / absorbance of blank)] x 100%. The value of IC50 was the sample concentration required to deliver 50% inhibition (Naidu *et al*, 2008; Saboo *et al*., 2013; Tillah *et al*., 2017).

RESULTS AND DISCUSSIONS

Immersion was done for 3, 6, 9 hours with the concentration of tofu waste by 30%, 60% and 90%. Control treatment in the study was coffee beans soaked in distilled water for 3, 6 and 9 hours. The liquid tofu waste had a characteristic odor of tofu, thick and low pH. Therefore, the tofu waste used was the new liquid tofu waste. Immersing coffee beans in tofu waste was an effort to decrease caffeine in Robusta coffee so it is safer for human body consumption.

Table 1. Quantitative Analysis of Antioxidant Activity of Robusta Green Coffee Bean (IC50) in Various Concentration and Length of Immersion using DPPH method

Concentrations (%)	Length of immersion (hour)		
	K1 (3 hour)	K2 (6 hour)	K3 (9 hour)
L1 (30%)	24.4931	23.6946	20.6338
L2 (60%)	23.2441	22.5515	19.0413
L3 (90%)	22.3427	19.2089	17.6061

The highest absorbance value of the blank indicates the maximum wavelength. Based on the absorbance result, the maximum wavelength is 517 nm. Samples that have been reacted with DPPH then incubated in a dark room with 37ºC for 25 minutes. After that, the absorbance sample was measured by spectrophotometry.

Antioxidant activity (IC50) of Robusta green coffee bean after decaffeination process in tofu waste with different concentrations and length of immersion is shown in Table 1. Based on Table 1 it is known that increasing decaffeination causes increased antioxidant activity. The highest antioxidant activity resulted from Robusta green coffee after decaffeination in tofu waste ranged from 17.6061 - 24.4931. Robusta coffee

with decaffeination in 90% waste concentration for 9 hours has the highest antioxidant activity of 17,6061. A natural ingredient is said to contain very active antioxidants, if it has an IC50 of less than 50 ppm (Budryn *et al.*, 2017; Fitmawati *et al.*, 2017; Gebeyehu & Bikilla, 2015; Iswari & Susanti, 2016).

All treatments produce Robusta green coffee with high antioxidant activity. This is because natural antioxidants in green coffee was very active. Chemical compounds in Robusta coffee which are responsible for causing antioxidant activity such are phenols, saponins and alkaloids (Dziki *et al.*, 2017; Naidu *et al.*, 2008). Phenol compounds contained in Robusta green coffee are tannins and flavonoids (Varghese *et al*, 2014). The amount of chemical compounds in coffee causes high antioxidant activity (Zlotek *et al.*, 2016). Increased antioxidant activity is due to the hydrolysis of chemical compounds in green coffee during decaffeination. The hydrolysis process causes the chemical compounds to break down into simpler molecules or change into other compounds (Naidu *et al.*, 2008). These changes affect the amount of natural antioxidant compounds in the coffee.

Indigenous microorganism of tofu waste might have a proteolytic activity for coffee decaffeination such as in *kopi luwak*. This study was showing the coffee processing methods that produce decaffeinated coffee with a high antioxidant content. In this study, the use of liquid tofu waste is also an attempt to civet cat exploitation in *kopi luwak* industry. Decaffeination of green coffee using liquid tofu waste was become new decaffeination method. This green coffee product has equivalent quality compared to *kopi luwak*.

Green coffee is a coffee which was not roasted. So, It is still containing high antioxidants to avoid free radical which is harmful for human health. Tofu liquid waste contains protease enzyme producing bacteria that potentially fight free radicals. Free radicals is a very reactive molecule because it has an odd (unpaired) numbers of electrons its outer orbit. So, it was could react with body cell molecules by binding to cell electrons. It can cause reaction chains that produce new free radicals. Antioxidants can react to free radical by reducing concentration of oxygen, preventing formation of single oxygen which is more reactive, preventing initiation of first chain by capturing primary radical as hydroxyl radical, binding the metal ion catalyst, decomposing primary radical products to be non-radical compounds, and disconnecting hydroperoxide chain (Fitriana *et al.*, 2016). This causes the antioxidant activity of green coffee increase after decaffeination by using liquid tofu waste. The antioxidant compound in coffee was increasing due to the protease enzyme that works during the immersion process in the liquid tofu waste. Therefore, decaffeination by using tofu waste can increase the amount of antioxidants and antioxidant activity in green coffee.

Benefits of this study for society was decaffeinated green coffee more healthier than roasted coffee because of its antioxidants. Soaking of green coffee in tofu waste can produce low caffeine green coffee, almost similar to *kopi luwak*. Traditional farmers will easily produce decaffeinated coffee with simple and economic method. Decaffeinated green coffee in tofu waste takes less time than *kopi luwak* belong to 12 hours. Therefore, decaffeinated green coffee in tofu waste is more beneficial for both health and economy.

CONCLUSION

Analysis of antioxidant activity by using DPPH on Robusta green coffee decaffeinated with a 90% (v / v) tofu waste as a solvent for 9 hours shows that the highest antioxidant activity is 17.6061. This result shows that the decaffeination of green coffee beans by using tofu waste affect the antioxidant activity in Robusta coffee. The longer the immersion of Robusta coffee beans and the higher the concentration of tofu waste resulted in the higher the antioxidant activity. Benefits of this study was provided information about coffee processing methods that produce decaffeinated coffee with a high antioxidant. This study is also introducing the use of liquid tofu waste as effort to decrease civet exploitation in *kopi luwak* industry.

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REFERENCES

Anggara, A., & Marini, S. (2011). *Kopi Si Hitam Menguntungkan Budi Daya dan Pemasaran.* Yogyakarta Indonesia: Cahaya Atma Pustaka..

Ashihara, H., Sano, H., & Crozier, A. (2008). Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. *Phytochemistry*, *69*(4), 841-856.

Bonita, J. S., Mandarano, M., Shuta, D., & Vinson,

J. (2007). Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacological research*, *55*(3), 187-198.

Budryn, G., Zakłos-Szyda, M., Zaczyńska, D., Żyżelewicz, D., Grzelczyk, J., Zduńczyk, Z., & Juśkiewicz, J. (2017). Green and roasted coffee extracts as antioxidants in βTC3 cells with induced oxidative stress and lipid accumulation inhibitors in 3T3L1 cells, and their bioactivity in rats fed high fat diet. *European Food Research and Technology*, 1-12.

Damayanti, A., Hermana, J., Masduqi, A., & FTSP-ITS, J. T. L. (2004). Analisis Resiko Lingkungan Dari Pengolahan Limbah Pabrik Tahu Dengan Kayu Apu (*Pistia stratiotes* L.) Environmental Analysis From Tofu Waste Water Treatment By Water Lettuce (*Pistia stratiotes* L.). *Jurnal Purifikasi*, *5*(4), 151-156.

De-Azevedo, A. B. A., Mazzafera, P., Mohamed, R. S., Melo, S. A. B., & Kieckbusch, T. G. (2008). Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents. *Brazilian Journal of Chemical Engineering*, *25*(3), 543-552.

Dollemore, D., & Giuliucci, M. (2001). Rahasia Awet Muda bagi Pria. *Jakarta: PT Gramedia Pustaka Utama*.

Dziki, D., Gawlik-Dziki, U., Ró□y□o, R., Siasta□a, M., & Kowalczyk, D. (2016). Quality of wholemeal wheat bread enriched with green coffee beans. *Croatian journal of food science and technology*, *8*(2), 112-119.

Fatoni, A., Zusfahair., & Lestari, P. (2008). Isolasi dan Karakterisasi Protease Ekstraseluler dari Bakteri dalam Limbah Cair Tahu. *Jurnal Natur Indonesia*, *10*(2), pp.83-88.

Fitmawati, F., Sofiyanti, N., Roza, R. M., Isnaini, I., Irawan, Y. R., Winata, D. R., & Dewi, A. P. K. (2017). Antioxidant Activity of Dominant Plants Species in Obat Pahit from Lingga Malay Ethnic in Riau Archipelago. *Biosaintifika: Journal of Biology & Biology Education*, *9*(2), 325-331.

Fitriana, F., Amirullah, A., Ilmy, N., Pratiwi, D.N., Resmilasari, A.A. & HM, N.A. (2016). Potensi Limbah Air Tahu Asal Kota Maros Sebagai Sumber Bakteri Penghasil Enzim Protease Dalam Melawan Radikal Bebas. *As-Syifaa Jurnal Farmasi*, *8*(2), pp.33-40.

Gebeyehu, B. T., & Bikilla, S. L. (2015). Determination of caffeine content and antioxidant activity of coffee. *American Journal of Applied Chemistry*, *3*(2), 69-76.

Hadipernata, M & Nugraha, S.(2012). Identifikasi Fisik, Mikrobiologi Biji Kopi Luwak sebagai Acuan Teknologi Proses Kopi Luwak Artifical. In *Prosiding Seminar Nasional Intensif Riset Sinas* (pp. 117-121).

Iswari, R. S., & Susanti, R. (2016). Antioxidant Activity from Various Tomato Processing. *Biosaintifika: Journal of Biology & Biology Education*, *8*(1), 129-134.

Johnston, K.L., Clifford, M.N dan Morgan, L.M. (2003). Coffee Acutely Modifies Gastrointestinal Hormon Secretion and Glucose Tolerance in Human: Glycemic Effect of Chlorogenic Acid and Caffeine. *J. Clinic Nutrition* 79 (4): 728-733.

Lestari, H. (2004). *Dekafeinasi Kopi Robusta dengan sistem pengukusan-pelarutan* (Doctoral dissertation, [Yogyakarta]: Universitas Gadjah Mada).

Mulato, S. (2001). *Pelarutan Kafein Biji Robusta Dengan Kolom Tetap Menggunakan Pelarut Air***.** *Jakarta Indonesia: Pelita Perkebunan*.

Murthy, P & Naidu, M. (2011). Improvement of Robusta Coffee Fermentation with Microbial Enzymes. *J of Applied Sciences* 3 (4): 130-139. ISSN 2079-2077

Naidu, M. M., Sulochanamma, G., Sampathu, S. R., & Srinivas, P. (2008). Studies on extraction and antioxidant potential of green coffee. *Food Chemistry*, *107*(1), 377-384.

Oktadina, F.D., Argo, D.B & Hermanto, M.B. (2013). Pemanfaatan Nanas (*Ananas comosus L. Merr*) untuk Penurunan Kadar Kafein dan Perbaikan Citarasa Kopi (Coffea sp) dalam Pembuatan Kopi Bubuk. *J Keteknikan Pertanian Tropis dan Biosistem* 1 (3): 265-273.

Perva-Uzunali□, A., Škerget, M., Knez, □., Weinreich, B., Otto, F., & Grüner, S. (2006). Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. *Food Chemistry*, *96*(4), 597-605.

Rahardjo, P. (2012). Panduan Budidaya dan Pengolahan Kopi Arabika dan Robusta. Jakarta Indonesia:Penebar Swadaya.

Rahman, A., & Indarto, C. (2013). Aktivitas Proteolitik Mikroorganisme Limbah Padat Pengolahan Tahu.Madura Indonesia:Universitas Trunojoyo Press.

Saboo, S. S., Tapadiya, G., Farooqui, I. A., & Khadabadi, S. S. (2013). Free radical scavenging, in vivo antioxidant and hepatoprotective activity of folk medicine Trichodesma sedgwickianum. *Bangladesh Journal of Pharmacology*, *8*(1), 58-64.

Smith, B. D., Gupta, U., & Gupta, B. S. (2006). 1 Arousal and Caffeine: Physiological, Behavioral, and Pathological Effects. *Caffeine and Activation Theory: Effects on Health and Behavior*, 1.

Sulistyaningtyas, A.R., Prihastanti, E. and Hastuti, E.D. (2017). Performa Green Bean Kopi Robusta (Coffea robusta Lindl. Ex De Will) setelah Perendaman Limbah Tahu dengan Jenis dan Konsentrasi yang Berbeda. *Buletin Anatomi dan Fisiologi*, *2*(2), pp.148-152.

Tello, J., Viguera, M., & Calvo, L. (2011). Extraction of caffeine from Robusta coffee (Coffea canephora var. Robusta) husks using supercritical carbon dioxide. *The Journal of Supercritical Fluids*, *59*, 53-60.

Tillah, M., Batubara, I., & Sari, R. K. (2017). Antimicrobial and Antioxidant Activities of Resins and Essential Oil From Pine (Pinus merkusii,

Pinuso ocarpa, Pinus insularis) and Agathis (Agathis loranthifolia). *Biosaintifika: Journal of Biology & Biology Education*, *9*(1), 134-139.

Triyana, N.R.S. 2014. Pengaruh Ekstrak Buah Nanas (*Ananas comosus* (L). Merr) dan Lama Perendaman Terhadap Kadar Kafein dan Performa pada Biji Kopi Robusta (Coffea robusta Lindl. Ex de Will). *Skripsi*. Jurusan Biologi, Fakultas Sains dan Matematika, Universitas Diponegoro Semarang.

Varghese, M., Ho, L., Wang, J., Zhao, W., Levine, S., Ono, K., & Pasinetti, G. M. (2014). Green coffee as a novel agent for Alzheimer's disease prevention by attenuating diabetes. *Translational Neuroscience*, *5*(2), 111-116.

Wang, Y & Ho, C. (2009). Polyphenolic Chemistry of Tea and Coffee: A Century of Progress. *J. Agric Food Chem* 57: 8109-8114. DOI:10.1021/jf804025c.

Widyotomo, S., Mulato, S., Purwadaria, H. K., & Syarief, A. M. (2009). Decaffeination process characteristic of Robusta coffee in single column reactor using ethyl acetate solvent. *Pelita Perkebunan (a Coffee and Cocoa Research Journal)*, *25*(2).

Widyotomo, S., Purwadaria, H. K., & Ismayadi, C. (2012). Peningkatan mutu dan nilai tambah kopi melalui pengembangan proses fermentasi dan dekafeinasi. In *Prosiding Seminar Hasil Penelitian Insentif Riset. Kementerian Riset dan Teknologi*.

Złotek, U., Karaś, M., Gawlik-Dziki, U., Szymanowska, U., Baraniak, B., & Jakubczyk, A. (2016). Antioxidant activity of the aqueous and methanolic extracts of coffee beans (Coffea arabica L.). *Acta Scientiarum Polonorum Technologia Alimentaria*, *15*(3), 281-288.