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The effects of *Lactobacillus plantarum* addition to robusta coffee (*Coffea canephora*) during wet fermentation

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Abstract. *Swasti YR, Leong LP, Purwijantiningsih E, Pranata FS. 2024. The effects of Lactobacillus plantarum addition to robusta coffee (Coffea canephora) during wet fermentation. Biodiversitas 25: 3132-3140.* Robusta coffee (*Coffea canephora* L.) from the slopes of Merapi volcano in Indonesia is processed using a wet fermentation method. Its chemistry compound quality depends on the spontaneous microorganism during wet fermentation. This study aimed to investigate the effect of *Lactobacillus plantarum* in increasing the antioxidant activity and reducing the concentration of caffeine and mutagenic compounds during fermentation. After fermentation, the chemical compounds of green and roasted beans were determined. The furfuryl alcohol compound and protein were only analyzed in the roasted beans. Results showed that the addition of *L. plantarum* was able to alter the Chlorogenic Acid (CGA) concentration of robusta coffee. The concentration of CGA in the unroasted robusta coffee with the addition of *L. plantarum* after 6-hour of fermentation tends to be higher than in without the addition of *L. plantarum*, while caffeine concentration was slightly reduced. The CGA in unroasted and roasted coffee with 12-hour fermentation period resulted in the highest radical ABTS scavenging capacity. Moreover, caffeine in unroasted and roasted coffee with 6-hour fermentation period showed high radical ABTS scavenging capacity. The furfuryl alcohol content tends to be stable.

Keywords: Antioxidant, caffeine, chlorogenic acid, *Lactobacillus plantarum*, robusta coffee, wet fermentation

INTRODUCTION

Indonesia is the sixth largest coffee exporter in the world. In 2023, Indonesia produced 794,762 tons of coffee, and the main coffee beans are robusta (*Coffea canephora* L.) and arabica (*Coffea arabica* L.). Robusta and arabica make up 70.18% and 26.95% respectively of the coffee export (Darmawan 2023). Caffeinated coffee consumption (~120 mL/day) has a positive impact on health (Maso et al. 2021). The consumption of caffeinated and decaffeinated coffee can reduce weight gain compared to the consumption of coffee with sugar and cream (Henn et al. 2023). Further, the consumption of coffee does not raise blood pressure and does not negatively affect insulin sensitivity (Surma and Oparil 2021; Moon et al. 2021).

The increase in coffee consumption triggers studies investigating health-promoting compounds in coffee. Three techniques can be employed in coffee processing - wet process, natural dry process, and semi-dry process. The wet process is divided into two methods, wet fermentation and mechanical demucilage (Brando and Brando 2014). Wet fermentation is a spontaneous process in coffee pulp involving bacteria especially Lactic Acid Bacteria (LAB), yeast and filamentous fungi (López et al. 2013; Nasanit and Satayawut 2015; Campanella et al. 2017). Dominant LAB in spontaneous fermentation is *Leuconostoc mesenteroides* and *Lactobacillus plantarum* (now known as *Lactiplantibacillus plantarum*) (Echegaray et al. 2023; Elhalis et al. 2023). *L. plantarum* is found in human colon, fermented foods, colon and feces of civet consuming coffee cherry (Fitri et al. 2021b; Garcia-Gonzalez et al. 2022). It is able to ferment a wide range of carbohydrate especially mucilage (Neto et al. 2018; Wang et al. 2019). It eases the mucilage removal and further speeds up the postharvest handling prior to coffee bean drying. Wet fermentation with a controlled process through the addition of *L. plantarum* 8 log CFU/ml is able to shorten fermentation period from 24 h to 10 h; it reduces more pH than *L. mesenteroides* because *L. plantarum* produces higher acetic acid after 72 h and higher lactic acid (Pereira et al. 2020; Cassimiro et al. 2023). Dried coffee beans inoculated with *L. plantarum* have the same glucose and fructose content as dried coffee beans in spontaneous fermentation (Neto et al. 2018).

The removal of mucilage simultaneously produces bioactive and aroma compounds. Bioactive compounds are produced through the breakdown of phenolic complexes into simple compounds (Tolonen et al. 2004). *L. plantarum* increases the concentration of compounds with strong antioxidant activity (Li et al. 2019). Additionally, *L. plantarum* in wet fermentation increases the total phenolic compounds (Latief et al. 2023). It is able to convert protocatechuic acid (hydroxybenzoic acids) through decarboxylation in green coffee to catechol, which acts as an antioxidant (Rodríguez et al. 2008; Macit et al. 2021). It also increases antioxidant activity in robusta coffee compared to arabica coffee because robusta contains much more chlorogenic acid, 4,5-dicaffeoylquinic acid, and caffeine (Therdtatha et al. 2023). Prolonged fermentation may

produce smaller molecules which decrease concentration of antioxidant compounds (Palmieri et al. 2018). In addition, *L. plantarum* is also able to decrease furfuryl alcohol (Dimitrovski et al. 2015; Kamda et al. 2015).

Aroma compounds produced from *L. plantarum* are more dominant than *L. mesenteroides*. Esterification reaction between alcohols and free fatty acids produces wine aroma (ethyl propionate) and pineapple aroma (ethyl hexanoate) (Pereira et al. 2016). It also produces honey and floral aroma (phenylacetaldehyde) from phenylalanine catabolism and apple skin aroma (1-hexonal) from 3-keto-hexanoyl-CoA conversion (Neto et al. 2018). *L. plantarum* produces chocolate and nutty sensory notes and *L. mesenteroides* produces dark chocolate and caramel sensory notes (Cassimiro et al. 2023).

The demethylase activity of *L. plantarum* is able to reduce caffeine during fermentation of coffee. Furthermore, in wet fermentation, it reduces caffeine because it produces protease (Sasaki et al. 1995; Fitri et al. 2021a; Latief et al. 2023). Proteolytic microorganisms such as *Pseudomonas putida* can reduce 80% of caffeine through N-demethylation pathway (Mazzafera 2002; Summers et al. 2015). The concentration of caffeine is higher in robusta coffee (2.54% dmb) than in arabica coffee (1.22% dmb) (Ky et al. 2001). *L. plantarum* is also able to remove mucilage layer from the cherry much more efficiently because *L. plantarum* consumes the sugar in the mucilage layer (Neto et al. 2018). The objective of this study was to investigate the effect of *L. plantarum* in enhancing antioxidant activity and reducing the concentration of caffeine and mutagenic compounds.

MATERIALS AND METHODS

Study area

Robusta coffee used in the research was collected from an organic plantation located on the slopes of Merapi Volcano in Yogyakarta, Indonesia. Microorganism *L. plantarum* was obtained from Food and Nutrition Culture Collection (FNCC) Universitas Gadjah Mada, Yogyakarta (Batch number 0095).

Procedures

Robusta coffee fermentation and roasting methods

Three hundred grams of peeled cherry robusta (*Coffea canephora* L.) coffee was inoculated with 10^7 *L. plantarum* at room temperature in an open container for 6, 12, and 18 hours. The beans were then dried in a greenhouse until the water content fell below 10% to obtain green beans. The green beans were then dark roasted using Gene Cafe Roaster 101 Red.

Extraction of ground coffee

Coffee beans were downsized using a coffee grinder. Fifty milligrams of the unroasted or roasted bean powder were added with 1 mL of 50% methanol in water for all analyzes except phenolic analysis using 100% methanol (Albouchi and Murkovic 2018). Then, it was extracted with sonicator Elmasonic for 30 min at 32 kHz. The supernatant extract was separated using centrifugation at 14000 rpm for 15 min and filtered with Whatman filter paper No. 41.

Determination of protein content

The protein content was analyzed using Kjeldhal method with Büchi instrument. Coffee powder sample (0.2 g) was digested with 20 mL H_2SO_4 until it became light green solution. Then, it was distilled with 40% NaOH and titrated with 0.1 N HCl until the solution became light pink (Wang et al. 2016).

Determination of phenolic content

Folin-Ciocalteu's solution was made by mixing 1.5 mL Folin-Ciocalteu's reagent and 1.5 mL aqua bidest ddH_2O. Na_2CO_3 solution was made by dissolving 5 g Na_2CO_3 in 100 mL aqua bidest ddH_2O. Folin-Ciocalteu's solution (0.0078 mL) and Na_2CO_3 solution (1125 µL), which were made previously, were added with 25 µL of extract sample. Then, it was incubated for 1 hour, and the absorbance was measured at 765 nm using a spectrophotometer Shimadzu UV-1800 (Jung et al. 2021). Standard curves were made using gallic acid at 75, 150, 225, 300 and 375 ppm.

Determination of furfuryl alcohol content

Twenty microliters of the coffee extract was used for HPLC (Shimadzu LC-10AT VP) analysis equipped with Purospher® RP-18 5µm LiChroART® 125-4 (Merck, Darmstadt, Germany) column and a UV detector at 228 nm. The column was set at a temperature of 25° C. The mobile phase used was 100% methanol (eluent A) and water (eluent B). The gradient was initially set at an A/B ratio of 25:75 from 0 to 0.01 min; the eluent then decreased to 0:100 from 0.01 to 5 min, and after that, the eluent was returned to 25:75 from 5 to 15 min (Swasti and Murkovic 2012). Furfuryl alcohol was purchased from Sigma Aldrich, USA.

Determination of caffeine content

Twenty microliters of the supernatant and caffeine standard (Sigma-Aldrich Chemical Co., Steinheim, Germany) were used for HPLC (Shimadzu LC-10AT VP) analysis equipped with Purospher® RP-18 5µm LiChroART® 125-4 (Merck, Darmstadt, Germany) column and a UV detector at 272 nm (Jeon et al. 2019). The column was set at a temperature of 25° C. The mobile phase used was 100% methanol (eluent A) and water (eluent B). The gradient was initially set at an A/B ratio of 25:75 from 0 to 0.01 min; the eluent then decreased to 0:100 from 0.01 to 5 min, and after that, the eluent was returned to 25:75 from 5 to 15 min and with a flow rate of 1 ml/minute.

Determination of chlorogenic acid content

The supernatant extract was separated using centrifugation (Eppendorf 5430 R) at 14000 rpm for 15 min at 4° C temperature. The supernatant was passed through RC Agilent syringe filter (0.2 µm). Five microliters of the supernatant and chlorogenic standard (Sigma-Aldrich Chemical Co., Steinheim, Germany) were injected into ultrasphere C18 150 x 4.6 mm Phenomenex column (Torrance, CA, USA). The column was set at temperature of 35° C. The mobile phase used in the analysis was 100% acetonitrile (eluent A) and 2% formic aid in water (eluent B). The gradient was initially set at an A/B ratio of 5:95 from 0 to 0.01 min; the eluent then decreased to 0:100 from 0.01

to 5 min, and then the eluent was returned to 5:95 from 5 to 15 min. The flow rate is 1 mL/minute. The ELSD Agilent was set at the evaporation temperature 40° C, nebulization temperature 40° C, gas flow 1.5, and the gain 1.

Determination of radical ABTS scavenging

The stock ABTS (Sigma-Aldrich, St. Louis, MO) solution consisted of 0.7 mM [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS), which was oxidized with 2.45 mM potassium persulfate in absolute ethanol for 12 h (Shui and Peng 2004). The 0.7 mM stock ABTS radical solution (0.6 mL) was diluted with 0.7 mL ethanol before adding 4 µL coffee extract. The mixture was mixed using a vortex and then subsequently measured at 734 nm using a Shimadzu UV-1800 spectrophotometer.

Determination of radical DPPH scavenging

Antioxidant capacity was measured using a spectrophotometer Shimadzu UV-1800. Two milligrams of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical powder (Himedia, Thane West Maharashtra, India) were dissolved with 100 mL methanol. Subsequently, 1.8 mL of the DPPH solution was added to 30 µL extract coffee and then incubated for 1 hour and measured at 517 nm using spectrophotometer Shimadzu UV-1800 (Jung et al. 2021).

Determination of antioxidant capacity using UPLC 1260 infinity Agilent

The ABTS solution of 0.7 mM was oxidized by 2.45 mM potassium persulfate in ethanol absolute for 12 h (Shui and Peng 2004). One milliliter sample was mixed with 3 mL of oxidized ABTS solution. Then, the solution was filtered using RC Agilent syringe filter (0.2 µm). Five microliter of the supernatant was injected into ultrasphere C18 150 x 4.6 mm Phenomenex column (Torrance, CA, USA). The column, mobile phase gradient, mobile phase flow rate, ELSD condition are the same as in chlorogenic acid content determination using UPLC 1260 infinity Agilent.

Data analysis

The research data was analyzed with Analysis of Variance (ANOVA) using SPSS version 15 program to determine the significant difference between all treatments. If there is a significant difference among data produced in this research, the data will be analyzed further using Duncan's Multiple Range Test (DMRT) at a 95% confidential level.

RESULTS AND DISCUSSION

Protein content

The protein content of untreated roasted coffee was around 17% and of treated roasted coffee with *L. plantarum* about 18% (Figure 1.A). These results are consistent with the previous research showing that the protein content of roasted robusta coffee is 16.08% (Moreira et al. 2017). The protein content increases after the addition of *L. plantarum* and it is likely due to the ability of *L. plantarum* to produce amino acids, such as methionine, lysine, threonine, and tryptophan (Lim et al. 2019). The addition of *L. plantarum* with 12-hour fermentation tended to increase the total protein because 12-15 hours was the optimum growth time of *L. plantarum* (Barragán et al. 2020). Fermentation of coffee beans increases the number of amino acids (Oktavianawati et al. 2020).

These results are close to previous research that green robusta coffee contains 18.03% protein and roasted robusta coffee contains 14.50% protein (Norazlin et al. 2023). The protein content of green bean liberica coffee was 14.42% and the concentration tended to be stable for 8-month storage (Ismail et al. 2013). The protein content of green arabica coffee was 13.56% (Kitzberger et al. 2016). The concentration of protein in arabica coffee remains stable for 3 years in the amount at approximately 14% (Barbosa et al. 2019). The protein content in the present study tends to be higher than other research results. This is probably due to the high-altitude soil of the robusta coffee plantation. The coffee samples of this research were taken from the plantation at an altitude of about 900 masl. The previous research found that the concentration of nitrogen tends to be higher in the high-altitude soil (He et al. 2016).

Furfuryl alcohol content

The results showed that furfuryl alcohol content was high at the beginning of fermentation with the addition of *L. plantarum*, but then decreased as the fermentation time increased. However, there were no significant differences between spontaneous fermentation (24-48 mg/100 mg) and fermentation with the addition of *L. plantarum* (32-73 mg/100 mg) (Figure 1.B and Figure 2). It may be caused by glucose and fructose concentration do not change during wet fermementation coffee with the addition of *L. plantarum* (Neto et al. 2018). The concentration of furfuryl alcohol in this research is within the safe level of daily consumption - below 53 mg/kg (Abbott 2011). One cup of espresso coffee only needs about 7.5 mg of coffee powder (Caprioli et al. 2014). The increase in furfuryl alcohol content may be due to the ability of *L. plantarum* to produce amino acids (Oktavianawati et al. 2020). Amino acids reacts with reducing sugar during heating due to the Maillard reaction producing furfuryl alcohol, which was reactive to DNA and could lead to have mutagenic effect (Glatt 2000; Yaylayan 2006; Ozolina et al. 2011). The 12-hour fermentation resulted in the lowest furfuryl alcohol. This is likely due to the growth of *L. plantarum*, which causes a tenfold decrease of furfuryl alcohol (Dimitrovski et al. 2015; Kamda et al. 2015).

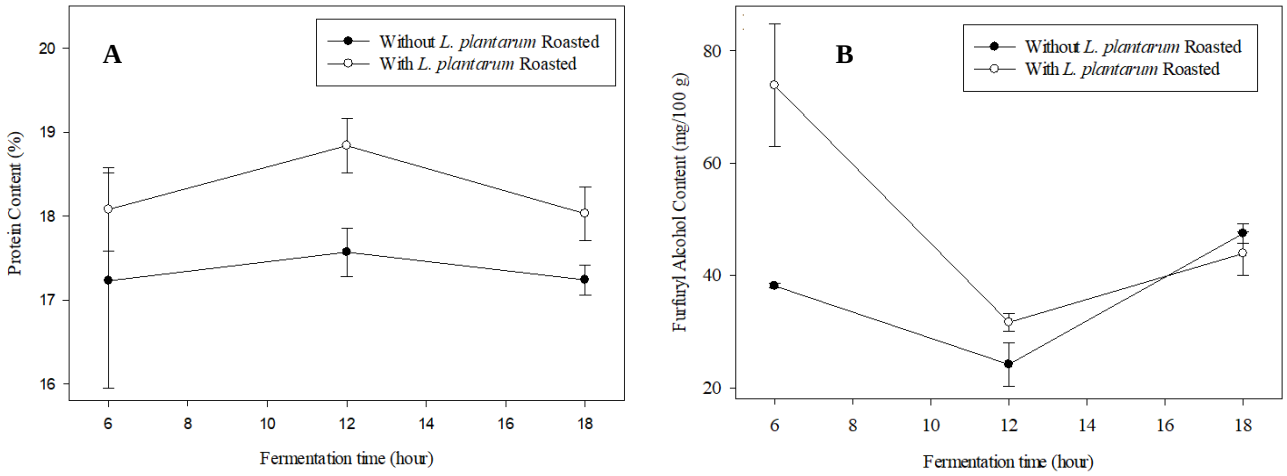

Figure 1. A. Protein; B. Furfuryl alcohol content of untreated and treated roasted robusta coffee bean with the addition of *L. plantarum*

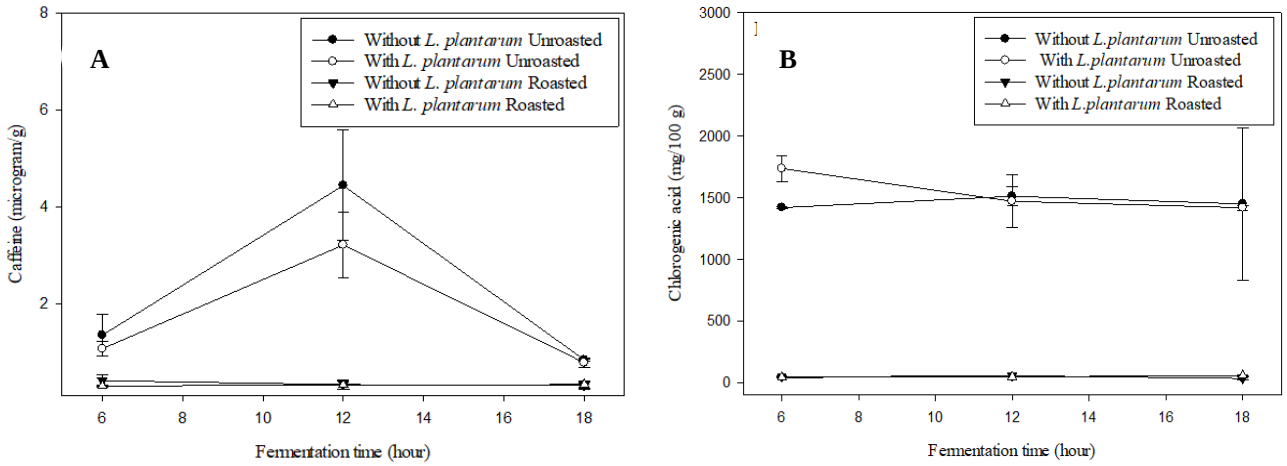

Figure 3. A. Caffeine content; B. Chlorogenic acid content of untreated and treated roasted with robusta coffee bean *L. plantarum*

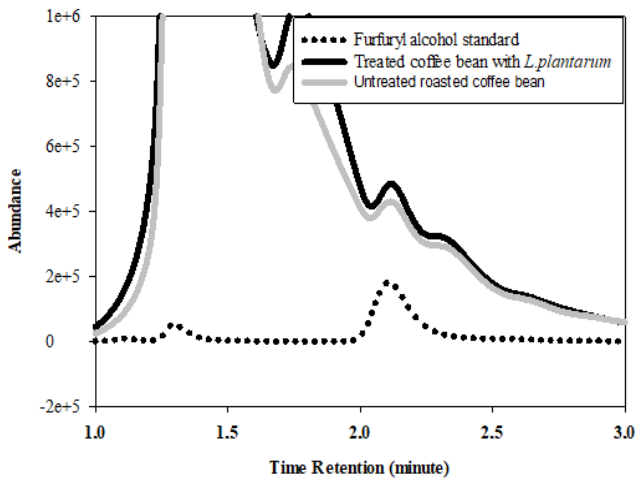

Figure 2. Furfuryl alcohol content of untreated and treated roasted robusta coffee with *L. plantarum*

Caffeine content

The concentration of caffeine in unroasted and roasted beans showed a tendency to decrease as the fermentation period increased (Figure 3.A). However, the concentration of caffeine (1-4 µg/g to 1-3 µg/g in green coffee and 0.3 µg/g to 0.4 µg/g in roasted coffee) was not affected by the addition of *L. plantarum*. The concentration of caffeine in unroasted robusta coffee was higher than that in roasted coffee. This study found a lower caffeine level of the robusta roasted coffee compared to the previous study of which caffeine level was 1.26 mg/g (Norazlin et al. 2023). This may be due to different types of coffee. In this study, organic coffee was used for experiments. Organic coffee contains lower caffeine compared to conventional coffee (Król et al. 2020; Deveci et al. 2023). However, the addition of *L. plantarum* did not significantly change the concentration of caffeine. The 18-hours fermentation showed the lowest caffeine content in the unroasted beans. The lowest caffeine content is likely due to the ability of *L. plantarum* to remove mucilage (Dimitrovski et al. 2015;

Neto et al. 2018). Besides, fermentation is also able to reduce the caffeine in tea (Horie et al. 2017).

Chlorogenic acid

The concentration of Chlorogenic Acid (CGA) in untreated green (1419-1512 mg/100 g) and roasted (34-55 mg/100 g) robusta coffee beans showed a tendency to decrease as the fermentation period increased (Figure 3.B). The treated green (1418-1736 mg/100 g) and roasted robusta coffee beans with *L. plantarum* showed a tendency to increase as the fermentation period increased (41-58 mg/100 g). Fermentation of coffee beans increases the amount of chlorogenic acid (Oktavianawati et al. 2020). These results are higher than previous research in green robusta coffee which is 239 mg/100 g and lower in roasted one which is 70 mg/100 g (Norazlin et al. 2023).

As the fermentation period increased, the reduction of CGA showed significant results in unroasted robusta coffee, but the addition of *L. plantarum* reduced the CGA insignificantly. Chlorogenic acid could be reduced by the addition of *L. plantarum* as it could metabolize it to produce caffeic acid (Fritsch et al. 2016; Le et al. 2020), which has higher antioxidant activity (Sato et al. 2011). The concentration of CGA in unroasted robusta coffee was higher than the roasted one. The high-heat roasting reduces the CGA (Šilarová et al. 2019). The concentration of CGA in unroasted or roasted robusta coffee beans was even much higher than the concentration of caffeine.

The CGA tend to be higher in roasted robusta coffee with the addition of *L. plantarum*. It is likely due to the pectinolytic activity of *L. plantarum*, which is able to degrade pectin in the mucilage of coffee beans and the decline of mucilage level facilitates the metabolite of *L. plantarum* going inside the coffee beans to increase the amount of CGA with a strong antioxidant capacity (Garcia et al. 1991; Ryu et al. 2019; Yalçınkaya and Kılıç 2019). The increase in CGA concentration possibly occurred in decaffeinated coffee (Jeszka-Skowron et al. 2016). Fermentation of coffee beans increases the amount of chlorogenic acid (Oktavianawati et al. 2020).

Phenolic content

The phenolic content of untreated and treated green beans of robusta coffee with *L. plantarum* were 6.52-7.00 mg/g and 6.45-6.87 mg/g, respectively. The dominant compound in green coffee was chlorogenic acid (7.68%). Green coffee also contained bioactive compound such as procyanidin A1, which is more dominant than other procyanidin (Oktavianawati et al. 2020). The total phenolic of robusta coffee is lower than that of arabica coffee (Kaur et al. 2018). The phenolic content of green coffee robusta extracted with water solvent was 4.2 mg/g, which was much higher than that of ethanol solvent in the amount of 0.95 mg/g (Upadhyay et al. 2012). These phenolic compounds decreased during roasting. The phenolic content of green arabica coffee was reduced after roasting from 52.79 mg/g to 30.39 mg/g (Mehaya and Mohammad 2020).

The phenolic content of untreated and treated roasted robusta beans with *L. plantarum* was 3.53-3.66 mg/g and 5.98-6.18 mg/g, respectively (Figure 4.A). These results are close to previous research, which found a phenolic content of 8.28 mg/g in roasted coffee beans (Król et al. 2020). Treated roasted beans with *L. plantarum* produce higher phenolic content than untreated. This may be due to the ability of *L. plantarum* to produce phenolic compounds such as caffeic acid, which has antioxidant activity and a higher melting temperature than chlorogenic acid (Cavin et al. 1997; Rodríguez et al. 2009; Degrain et al. 2020). In addition, *Lactobacillus johnsonii* NCC 533 is able to converse chlorogenic acid to caffeic acid (Bel-Rhlid et al. 2013).

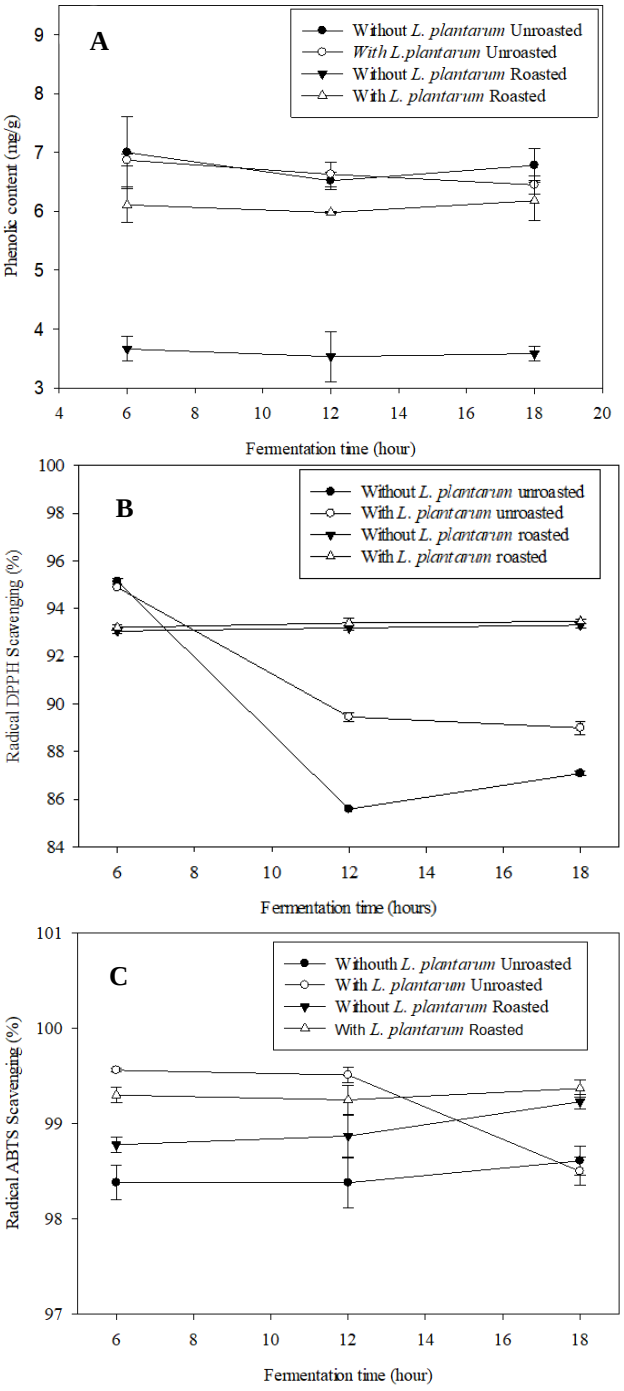

Figure 4. A. Phenolic content; B. Radical DPPH scavenging; C. Radical ABTS scavenging of untreated and treated roasted robusta coffee beans with *L. plantarum* addition

Coffee's phenolics are reduced by up to 80% during 12 months of storage (Król et al. 2020). The total phenolic did not change during the roasting if the V 60 method was employed for the extraction (Várady et al. 2020). The phenolic content declined slightly at roasting temperature of 225˚C for 19 min (13.34 mg/g). However, the total phenolic content increased at the roasting temperature of 210˚C for 14 min (Diviš et al. 2019).

Antioxidant activity in radical DPPH and ABTS scavenging

Although the total phenolic did not significantly differ with *L. plantarum* addition in unroasted coffee, it resulted in significant ability of radical DPPH (88.99 to 94.89% in unroasted coffee and 93.21 to 93.46% in roasted coffee) and ABTS (98.50 to 99.37% in unroasted and roasted coffee) scavenging (Figure 4.B, 4.C). This may be due to the ability of *L. plantarum* to produce amino acids and ultimately induce Maillard reaction product. Maillard reaction produced melanoidin that is able to act as antioxidant (Delgado-Andrade et al. 2010; Lim et al. 2019). The increase in antioxidant activity is also likely due to the metabolism of *L. plantarum* during fermentation, which increases the phenolic content and antioxidant compounds (Rodríguez et al. 2009; Degrain et al. 2020). The addition of *L. plantarum* with 12-hour fermentation tends to increase radical DPPH and ABTS scavenging in unroasted and roasted. This is likely due to the optimum growth of *L. plantarum*, which increases caffeic acid content at 12-hour fermentation (Sato et al. 2011; Dimitrovski et al. 2015).

The ability of chlorogenic acid and caffeine robusta coffee in radical ABTS scavenging (%)

The ABTS radical scavenging capacity of CGA in green robusta coffee untreated with *L. plantarum* addition during the fermentation and treated with *L. plantarum* (3-9% in untreated and 13-21% in treated) was higher than in the roasted one (0.77-12% in untreated and 0.76-11 in treated) (Figure 5.A, 6.A, and 6.B). The highest capacity was obtained during 12-hour fermentation process. The addition of *L. plantarum* in a sample containing CGA increased the ability to inhibit inflammation and oxidative stress (Palócz et al. 2016).

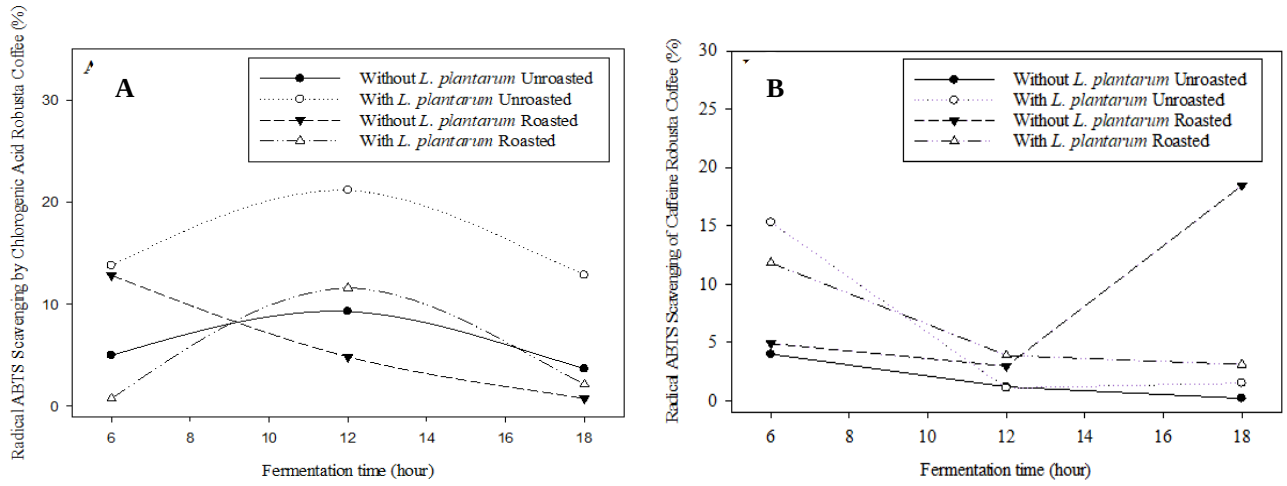

Figure 5. The ability of chlorogenic acid (A) and caffeine robusta coffee (B) in radical ABTS scavenging analyzed with UPLC-ELSD

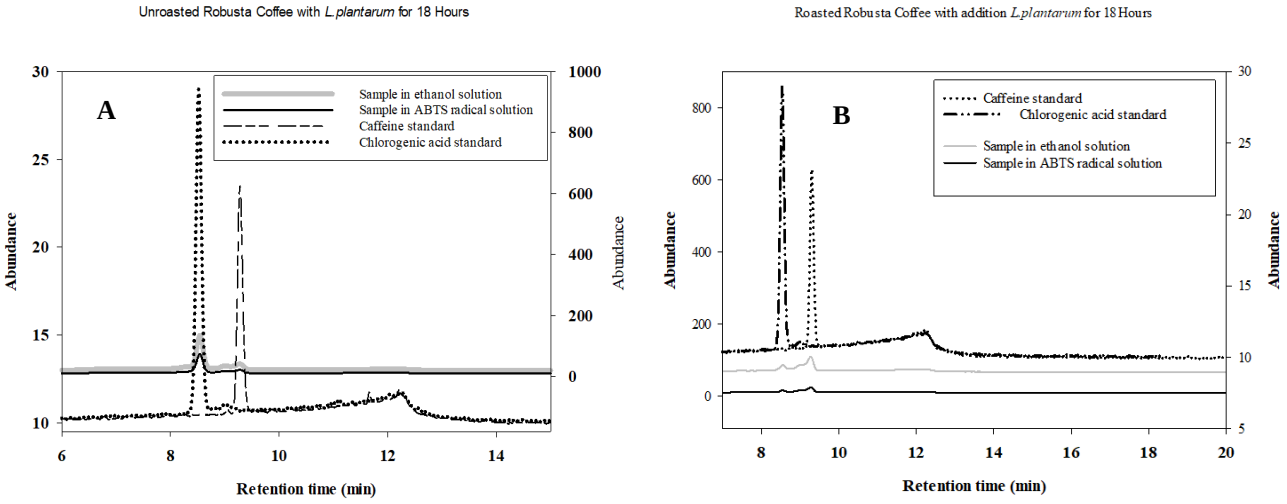

Figure 6. Chromatogram antioxidant activity of caffeine and chlorogenic acid of unroasted (A) and roasted (B) robusta coffee with the addition of *L. plantarum*

The unroasted robusta coffee with the addition of *L. plantarum* showed a tendency to increase the radical ABTS scavenging capacity. *L. plantarum* was one of LAB that was able to enhance antioxidant capacity by modifying phenolic compounds to be more active in radical scavenging (Zhao and Shah 2016). *L. plantarum* is able to enhance the antioxidant capacity of phenolic compounds (Landete et al. 2014). The CGA is more active in the sample undergoing the high heat treatment compared to the sample without high heat treatment although the concentration is lower (Kamiyama et al. 2015). The most active radical ABTS scavenging capacity of CGA was obtained through 12-hour fermentation period with the addition of LAB. This result is in line with the previous research, which found an increase in the number of LAB in the incubation period of 10-15 hours (Avallone et al. 2001). 12-hour fermentation was the best fermentation period to obtain the highest antioxidant capacity, as *L. plantarum* was able to reduce fermentation period from 24 to 12 hours (Pereira et al. 2016). A high concentration of CGA in coffee was able to reduce negative emotional effects (Haskell-Ramsay et al. 2018).

The addition of *L. plantarum* during fermentation was able to enhance the radical ABTS scavenging capacity of caffeine (Figure 5.B). The scavenging capacity of caffeine from the beans with the addition of *L. plantarum* during the fermentation (1.11-15%) was higher than without the addition of *L. plantarum* (0.23-4%) in unroasted coffee beans (Figure 6.A). The scavenging capacity of caffeine in the roasted bean with the addition of *L. plantarum* during fermentation (3.12-11.83%) was nearly the same as the one without the addition of *L. plantarum* (2-18%) (Figure 6.B). The highest capacity of caffeine to scavenge radical ABTS was obtained through 18-hour fermentation in unroasted coffee and 6-hour fermentation in roasted coffee. Caffeine has antioxidant capacity in rat brain (Abreu et al. 2011; Metro et al. 2017).

In conclusion, the addition of *L. plantarum* showed an increase of protein, chlorogenic acid, phenolic content, and antioxidant activity. Furthermore, it also slightly reduced caffeine content, but the furfuryl alcohol content remained stable.

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