

Blood glucose response of robusta coffee with different roasting levels: light and dark

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ABSTRACT

The aims of this study were to analyze the total chlorogenic acid and total caffeine in robusta coffee roasted at light and dark levels; and the effect on clinical blood glucose response after its consumption. Original robusta coffee from Lampung was roasted into light and dark. Chlorogenic acids and caffeine in coffee brew were determined by using RP-HPLC, while α -glucosidase inhibitor was measured by UV-VIS spectrophotometer technique. The ethics of this research was approved by the Ethical Commission for Research Involving Human Subjects, IPB University. Blood glucose levels were assayed in ten healthy subjects after overnight fasting and consumption of coffee brew with and without references food (glucose and white bread). When light-roasted coffee was compared to dark-roasted coffee, the 3-CQA (O-caffeoylquinic acid) content reduced by 39%, 4-CQA by 41%, and 5-CQA by 44% in terms of chlorogenic acids. The amount of caffeine in the coffee brew (2.75–2.81 g/100 g) was unaffected by the roasting level. Inhibition of the α -glucosidase activity of light-roasted coffee the brew was 85% higher than dark-roasted coffee. While coffee brew alone did not significantly suppress postprandial glucose absorption, the combination of light-roasted coffee with glucose led to a greater reduction in blood glucose levels, particularly ~ 2.4 mg/dL/min during the 45–60 minute post-consumption period. These results suggest that lightly roasted Robusta coffee may have potential in modulating postprandial glycemic response in healthy individuals when consumed acutely.

Key words: α -Glucosidase; carbohydrate; coffee; chlorogenic acids; caffeine.

1 INTRODUCTION

Coffee has been one of the cultural heritage that has long been consumed in Indonesia since the beginning of the 18th century (Schenker; Rothgeb 2017). Coffee is growing in popularity and has an impact on social life, which can be seen by how easily to spot a cafe and the variance of coffee around (Purnomo et al., 2021). Coffee contains bioactive compounds like phenolic acids, alkaloids, and other chemical components of the Maillard reaction (Saud; Salamatullah, 2021). These functional properties have been proven to be beneficial for health, therefore coffee may be categorized as functional foods (Alongi; Anese, 2018). Bioactive compounds of coffee brew have several activities, such as antioxidant, anti- α -glucosidase, and anti-glycation (Herawati et al., 2019a, Mohamed et al., 2024; Alongi; Celayeta; Vriz; 2021). Numerous experiments showed that coffee brew could regulate glucose metabolism. Daily coffee consumption alters the pattern of glucose absorption in the small intestine (Johnston; Clifford; Morgan, 2003; Iriondo-Dehond et al., 2021), and the prolonged effects bring down the danger of type 2 diabetes (Lin et al., 2011; Bevilacqua et al., 2023).

Roasted coffee has been found to contain around 1000 volatile compounds (Vezzulli; Lambri; Bertuzzi. 2023). The chemical substances are suggested to have biological effect by influencing the glucose metabolism due to chlorogenic acids and caffeine contents (Johnston Clifford; Morgan, 2003;

Moisey et al., 2008; Al-mssallem; Brown 2013; Yanagimoto et al., 2022). Chlorogenic acids, a major phenolic acid in coffee, were reported to exert a pivotal role in glucose homeostasis. In-vitro analysis showed the ability of chlorogenic acids to act as α -glucosidase inhibitor (Zhang et al., 2016; Herawati et al., 2019a). The top three chlorogenic acids in the coffee brew are 5-O-caffeoylquinic acid (5-CQA), 4-CQA, and 3-CQA (Rojas-González et al., 2022). Caffeine, the primary alkaloid in coffee, stimulates the central nervous system, raises blood circulation, and increases respiration. These compounds are predicted as the main reason for coffee's popularity (Herawati et al., 2019b).

The efficacy of chlorogenic acids and caffeine in blood glucose response has been studied through in-vivo analysis. Intravenous injection of 5 mg/kg body weight/day chlorogenic acids into insulin-resistant, hyperglycemic, and obese Zucker rats showed a decrease in blood glucose concentration after three weeks (Rodriguez de Sotillo; Hadley, 2002). Oral consumption of caffeine causes a glucose tolerance improvement and blood glucose level reduction of diabetic rats.

The effect of coffee consumption on glucose metabolism has been conducted with several variables, such as the amount of coffee (Kempf et al., 2010), caffeinated or decaffeinated coffee (Johnston; Clifford; Morgan, 2003; Moisey; Robinson; Graham, 2010), carbohydrate load (Moisey; Robinson; Graham, 2010) and subjects (healthy and diabetic) (Alkaabi et al., 2013). Whereas the effect of

the roasting level on the robusta coffee on response blood glucose concentration has not been considered. It has been confirmed that the process of roasting affects the quality of coffee brew. Chlorogenic acids undergo a chemical modification during roasting (Vignoli et al., 2014; Herawati et al., 2019b). Chlorogenic acids may be lost up to 90% during roasting (Farah et al., 2006; Vignoli et al., 2014). Caffeine is a thermostable compound, but it might decrease during roasting (Budryn; Nebesny; Oracz, 2015). The roasting process is a significant factor that affects the bioactive compounds of coffee that possibly influence blood glucose uptake. We considered evaluating the acute effect of coffee brew with two- roasting (light and dark) levels consumption on a healthy subject's blood glucose response.

2 MATERIAL AND METHODS

2.1 Materials

Robusta coffee beans (*Coffea canephora*) were purchased from Maharaja, Lampung. The following reagents were obtained from Sigma Aldrich (Saint Louis, USA): 5-O-caffeoylquinic acid (5-CQA), 4-CQA, 3-CQA, rat intestinal acetone powder and acarbose. Formic acid (HPLC grade), water (HPLC grade), methanol (HPLC grade), sodium phosphate buffer, sodium carbonate and maltose were purchased from Merck, Darmstadt, Germany. Other chemicals were glucose reagent color (Wako Pure Chemical Industries, Osaka, Japan), glucose (Dextrose monohydrate food-grade, Qinhuangdao, China), and white bread (PT. Nippon Indosari Corpindo, Indonesia).

2.2 Sample Preparation

Coffee beans were roasted at two levels, light and dark, using a William roaster (William, W600i, Bali, Indonesia). Green beans (1 kg) entered the machine at 200°C as the initial temperature. As the roaster temperature rose, the process was stopped after light and dark roast levels were obtained. The duration and final temperature of light and dark roasted coffees were 9 min and 210°C and 12 min and 250°C, respectively. The roasted coffee level was determined by visual appearance (color intensity) and post-roasting yield (Alongi; Anese 2018). The color intensity of ground coffee was measured by chromameter (Minolta CR-400; Konica Minolta Sensing, Inc., Tokyo, Japan) based on $L^*a^*b^*$ values after the coffee was cooled down and the yield was weighed immediately. The roasted coffee was ground using a Eureka grinder (Eureka Mignon MK 2, Florence, Italy) into a medium scale. The grounded coffee (16.7 g) was brewed using filter coffee paper (V60), adding 200 mL of hot water (94°C). The ground coffee to water ratio was 1:12. The content of the total soluble solid of each beverage was determined using a refractometer (HM Digital, SCM-1000, Korea). The coffee brew was analyzed in

chlorogenic acids, caffeine content, α -glucosidase inhibitory activity, and the response of coffee consumption to blood glucose concentrations.

2.3 Determination of Chlorogenic Acid

The methodology used was a slight modification (Herawati et al., 2019b). Three main chlorogenic acids, isomers 3-CQA, 4-CQA, and 5-CQA, were determined using an RP-HPLC system completed with a UV/VIS detector (LC-20AD, SHIMADZU Corp., Kyoto, Japan). Coffee brew was filtered with a 0.45 μ m PTFE membrane (Sartorius, USA). The coffee filtrate was injected into the HPLC system has a C18 column at 20 μ L (Zorbax, 4.6 x 150 mm, 5 μ m). The mobile phase of the system was 95% methanol (A) and 0.05% formic acid (B), which is run at a rate of 1 mL/ min. Elucidation of the samples used a gradient mode of: 5% A (0 min); 90% A (0 to 30 min); 90% A (30 to 35 min); 5% A (35 to 40 min); and 5% A (40 to 45 min). Maximum absorbance of chlorogenic acids was detected at 320 nm. The calibration curve used for quantification was mixed with standard solutions, at concentration ranges of 32.25-500 mg/L in triplicate (LoD 3-CQA = 0.79 mg/L, R^2 3-CQA = 0.9967; LoD 4-CQA = 4.39 mg/L, R^2 4-CQA = 0.9958; and LoD 5-CQA = 6.41 mg/L, R^2 5-CQA = 0.9958).

2.4 Caffeine Analysis

The filtration of beverages was performed using a 0.45- μ m cellulose acetate filter membrane (Sartorius; Germany). The 20 μ L of filtrate samples were injected to the RP-HPLC system completed with UV/VIS detector (Agilent Technologies, Santa Clara, USA) using C18 column (Zorbax, 4.6 x 150 mm, i.d.5 μ m particle size). The caffeine of coffee brew was quantified by HPLC-based on the developed method by (Herawati et al., 2019b), with slight modifications. The isocratic elution condition was composed of HPLC-grade methanol and HPLC-grade water with a ratio of 30:70. The flow rate of the mobile phase was 0.8 mL/min, and the quantification of caffeine was measured at 280 nm. The quantification was based on six standard curves of caffeine (5-100 mg/L) (in triplicate, LoD = 2.1 mg/L, R^2 = 0.996).

2.5 Assay of α -Glucosidase Inhibition

The α -glucosidase inhibition of coffee brew was determined according to the previous method by Herawati et al. (2019b), with slight modifications. Rat intestinal α -glucosidase in acetone powdered form dissolved at 100 mg/mL concentration in 1 mL of 0.1 M sodium phosphate buffer (pH=6.9) in 1.5 mL microcentrifuge tubes. The suspension was centrifuged at 10.000 x g for 60 min at 4°C (Hettich Mikro 22 R, Beverly, USA). A buffer solution of 0.1 M phosphate buffer (pH 6.9) was used to dilute the crude enzyme extract at a dilution factor of 10.

Briefly, 25 μL coffee brew, 100 μL phosphate buffer (0.1 M), 100 μL maltose (0.25 M), and 100 μL diluted enzyme solution were incubated for 40 min at 37°C. Then, the 250 μL Na_2CO_3 (0.2 M) was later introduced to terminate the reaction. Acarbose and DMSO were used as positive control and blank samples, respectively. The 20 μL solution was added to 1500 μL glucose color reagent and incubated for 5 min at 37°C. The wavelength was read at 505 nm (absorbance) by UV-VIS spectrophotometer. The glucose standard curve used 25-200 mg/dL concentrations. The inhibitory activity was calculated based on (Herawati et al., 2019a).

2.6 Blood Glucose Response

This study was reviewed and approved by the Ethical Commission for Research Involving Human Subjects, IPB University (233/IT3.KEPMSM-IPB/SK/2019), based on the protocol completion and informed consent. Analysis was conducted in three steps: (1) subject recruitment, (2) submission, and approval ethical clearance, (3) glycemic index test. There were 14 candidate subjects, recruited from IPB University; however, only ten persons qualified based on inclusion and exclusion criteria, Table 1. Ten healthy subjects were two male and eight females. The inclusion criteria of the subject were healthy male and female, 20-45 years old, body mass index (BMI) 18.5-25 kg/m^2 , coffee tolerant, and willing to become research subjects by signing informed consent. While the exclusion criteria were inherited diabetes mellitus (type 1 and type 2), major chronic illness presence, pregnant, drinkers, and smokers.

Table 1: The data of subject criteria at inclusion parameters.

Subject criteria	Value
Age (y)	27.5 $\pm$ 4.81
Weight (kg)	56.90 $\pm$ 5.13
Height (m)	1.60 $\pm$ 0.05
BMI (kg/m^2)	22.33 $\pm$ 2.21

The blood glucose response was determined following FAO (FAO, 1998). The study was designed in a single-masked and randomized method. The effect of coffee was evaluated through three key aspects: (1) monitoring of glucose absorption, (2) assessment of carbohydrate digestion, and (3) ensuring no external glucose intake during the observation period. Subjects fasted for 10–12 hours, with only water permitted during this period. They arrived at the laboratory between approximately 08:00 and 09:00 a.m. After a 10-minute rest, fasting blood glucose levels were measured. Subsequently, each subject was given either a test food or a reference food. The test food consisted of brewed coffee, prepared by extracting 17 grams of roasted coffee

(light and dark roast) using 250 mL of bottled water at 94°C with the V60 drip technique.

The reference foods included: (1) glucose solution to assess the effect of coffee on glucose absorption, (2) white bread to evaluate the effect of coffee on carbohydrate digestion, and (3) no reference food to examine the effect of coffee alone without additional carbohydrate sources. Both glucose and white bread provided 50 g of available carbohydrates (AVCHO), as determined by proximate analysis. The glucose reference was prepared by dissolving 50 g of glucose in 200 mL of bottled water.

The use of reference foods containing 50 g of AVCHO is standard practice in glycemic response testing and is aligned with international guidelines (FAO, 1998) for evaluating the impact of foods and dietary components on postprandial blood glucose levels. Adherence to this protocol ensures subject safety and was conducted following ethical approval obtained from the institutional review board. All test and reference foods were consumed along with 250 mL of bottled water and ingested within 5 minutes. Postprandial blood glucose levels were measured at 15, 30, 45, 60, 90, and 120 minutes after food consumption.

Blood glucose concentration was measured using a glucometer (GlucoDr.Auto A - ISO 15197:2013). The subject's blood was obtained through capillaries located at the fingertips. Capillary blood vessels have smaller variations of blood glucose concentration than taken from veins. Subjects were not allowed to consume any food and were advised to remain in the laboratory. The incremental area under the curve (iAUC) is the total area of areas A, B, C, D, E, and F in Figure 1. The figure presents a conceptual model of postprandial blood glucose changes over time, divided into sections (A–F) to demonstrate the integration method for calculating iAUC. The area under the basal glucose line (< 0 mg/dL) is not included in the calculation iAUC. The subject's blood glucose iAUC was calculated using the trapezoidal formula. The GI of the sample analysis was calculated as the (average of iAUC sample/average of iAUC references) X 100. The GI of each test food is expressed as the average and standard error (SE) of all subjects.

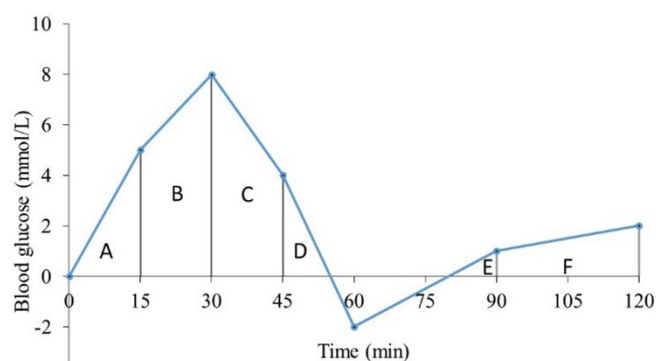


Figure 1: Blood glucose response curve adapted from (FAO, 1998).

2.7 Statistical Analysis

All values in this study were presented as means, standard error, and standard deviation (as stated in each figure). The difference between means of roasting temperature, weight loss of the sample, and subject criteria was analyzed using t-test (Confidence interval: 95%). The bioactive compounds of two-roasting levels samples were analyzed using a one-way analysis of variance (ANOVA). The glucose response was plotted to the graphic and the trapezoidal method was used to calculate the incremental area under the curve (iAUC). The difference in each period of blood glucose concentration in one treatment was analyzed using one-way ANOVA, followed by Fisher analysis (Confidence interval: 95%).

3 RESULTS

3.1 Roasted Coffee Bean Characteristics

The roasting process significantly affected the physical and chemical characteristics of Robusta coffee beans, Table 2. The weight loss of coffee beans was greater in dark-roasted coffee (15.95%) compared to light-roasted coffee (10.00%), confirming a higher degree of moisture and volatile compound loss. The color analysis showed that light-roasted coffee had a higher lightness value ($L^* = 43.95$) than dark-roasted coffee ($L^* = 27.48$), reflecting the Maillard reaction and caramelization effects during roasting. No significant difference was observed in total soluble content between the two roasting levels ($p > 0.05$).

Table 2: Roasting parameters and lightness degree of coffee.

Roasting level	Weight loss (%)**	Lightness Color (L^*)	Total soluble content (g/100 mL)
Light	10.00±0.28 ^b	43.95±0.74 ^a	2.37±0.12 ^a
Dark	15.95±0.35 ^a	27.48±1.51 ^b	2.57±0.15 ^a

**From green to roasted coffee beans.

Values are presented as mean of three data ± standard deviation. Numbers with different superscript letter within the same column differ significantly ($P < 0.05$).

3.2 Bioactive Compounds and α -Glucosidase Inhibitory Activity in Coffee Brew with Two Roasting Levels

Chlorogenic acids, caffeine content, and α -glucosidase inhibitory activity of coffee brew from the V60 technique are presented in Table 3. The concentration of chlorogenic acid compounds (3-CQA, 4-CQA, and 5-CQA) was significantly higher in light-roasted coffee than in dark-roasted coffee ($p < 0.05$). In contrast, caffeine content did not differ significantly between roasting levels, suggesting its thermal stability. The α -glucosidase inhibitory activity was 85% higher in light-roasted coffee compared to dark-roasted coffee, indicating that roasting decreases the potential of coffee to inhibit carbohydrate-hydrolyzing enzymes.

3.3 Blood Glucose Response

Ten subjects in this research, two males and eight females, with the average age, weight, and BMI were 27.5, 56.9, and 22.3, respectively. All subjects chosen were able to consume coffee. Figure 2 (a) presents blood glucose concentration after consuming coffee brew with glucose solution. The glucose concentrations in the blood at 0, 30, 60, 90, and 120 min were found to differ insignificantly ($P > 0.05$). On the other hand, blood glucose concentration at 45 min after robusta coffee ingestion was significantly different ($P = 0.006$). From the figure, the roasting level of coffee affected the blood glucose concentration rate at 45 min postprandial. The peak blood glucose response of glucose+light-roasted coffee brew was shifted to the right (45 min) compared to control and glucose + dark roasted coffee brew. The peak of blood glucose concentration in subjects after consuming sample glucose only, and glucose+light-roasted coffee brew sample was at 30 min, whereas the glucose+dark-roasted coffee brew sample was at 45 min.

The roasting level effect on blood glucose concentration was identified by calculating the rate of blood glucose concentration incline and decline periodically, as shown in Table 4. Elevated blood glucose concentration indicates the glucose absorption into blood, while the negative slope indicates the glucose absorption from blood to tissue by insulin.

Table 3: Chlorogenic acids and caffeine of coffee brew prepared from light- and dark-roasted beans.

Sample	Chlorogenic Acids (g/100g) *			Caffeine (g/100g)*	α -Glucosidase inhibition (%)
	3-CQA	4-CQA	5-CQA		
Light roasted coffee	1.04± 0.05 ^a	1.33±0.07 ^a	3.19±0.17 ^a	2.81± 0.27 ^a	44.95±8.28 ^b
Dark roasted coffee	0.63±1.29 ^b	0.78± 0.16 ^b	1.79±0.35 ^b	2.75±0.06 ^a	24.30±10.79 ^c
Acarbose 0.1 g/L	-	-	-	-	73.43±3.58 ^a
Acarbose 0.01 g/L	-	-	-	-	42.42±6.99 ^b

* Chlorogenic acid and caffeine were stated as g/100g soluble solid

Coffee concentration for α -glucosidase inhibition was 12.5 g eq./100 mL

Values are presented as means ($n_{\text{chlorogenic acids and } \alpha\text{-glucosidase}} = 3$; $n_{\text{caffeine}} = 2$) ± standard deviation. Numbers with different superscript letter within the same column differ significantly ($P < 0.05$).

Table 4 clearly shows that the roasting level of coffee affects the rate of blood glucose absorption. Blood glucose changes at 30–45 and 45–60 min were significantly different ($P < 0.05$). We assume that light-roasted coffee contains bioactive compounds that potentially extend blood glucose absorption and increase the rate of the glucose absorption to the tissue.

3.4 Inhibition of carbohydrate-hydrolyzing enzyme

Figure 2 (b) describes the bioactive compounds of coffee to inhibit carbohydrate hydrolysis to glucose. Bread, as complex carbohydrates, are hydrolyzed to glucose by α -glucosidase, which will increase blood glucose concentration. Inhibitors of α -glucosidase are associated with gastrointestinal symptoms that cause carbohydrate malabsorption (NIDDK 2021). The potential of coffee as an α -glucosidase inhibitor was analyzed by measuring the blood glucose responses after consuming bread (50 g carbohydrate available), followed by coffee brew (light- or dark-roasted), compared to control (bread only).

The effect of coffee roasting level on blood glucose concentration did not differ significantly ($P > 0.05$; at 0, 15, 30, 45, 60, 90, and 120 min). The peak of blood glucose concentration among three samples appeared at 30 min postprandial, whereas the maximum concentration was 131 mg/dL (bread), 149 mg/dL (bread+light-roasted coffee brew), and 145 mg/dL (bread+dark-roasted coffee brew). Table 4 expresses the different patterns of blood glucose concentration rate between samples and control. Blood glucose concentration did not rise significantly, indicating that it was not dependent on the roasting level. However, the rate of blood glucose concentration decline was significantly different ($t = 60\text{--}90$). The blood glucose concentration of control (bread only) from 45–120 min tended to stabilize, but the blood glucose concentration of bread with coffee still decreased gradually.

The rate of blood glucose absorption between coffee and simple or complex carbohydrate load was different as shown in Figure 2 (a) and (b). Coffee with complex carbohydrates had a lower blood glucose absorption rate than coffee with simple carbohydrates (glucose). Glucose had a higher glycemic index (GI) than bread, whereas the GI of glucose (50 g carbohydrate) was 100 (Brand-Miller et al., 2009) and bread (50 g carbohydrate) was 75. The higher glycemic index indicates the digested glucose that can be absorbed faster than bread (Brand-Miller et al., 2009).

The iAUC value between coffee brewed with and without bread was not significantly different ($P > 0.05$). The light- and dark-coated coffee brew did not inhibit the carbohydrate hydrolysis. In this study, a short-term consumption of coffee brew did not appear to inhibit the inhibition of α -glucosidase enzyme. However, epidemiology studies showed that habitual coffee consumption in a long-term intervention could reduce the risk of type 2 diabetes (Tuomilehto et al., 2004). Blood glucose concentration of coffee brew+bread or bread-only

consumptions after 120 min did not return to the baseline level, as bread was a starchy food with complex carbohydrate and was digested slowly (Brand-Miller et al., 2009).

Figure 2 (c) shows the changes in blood glucose concentration after consuming coffee brew without carbohydrate load. During measurement ($t = 120$ min), blood glucose concentration was highly fluctuated compared to the baseline (88 mg/dL). Table 4 shows no significant difference in blood glucose movement after a two-hour measurement at 0 mg/dL/min. The iAUC values of coffee brewed with light- and dark-roasted methods were not significantly different ($P < 0.05$).

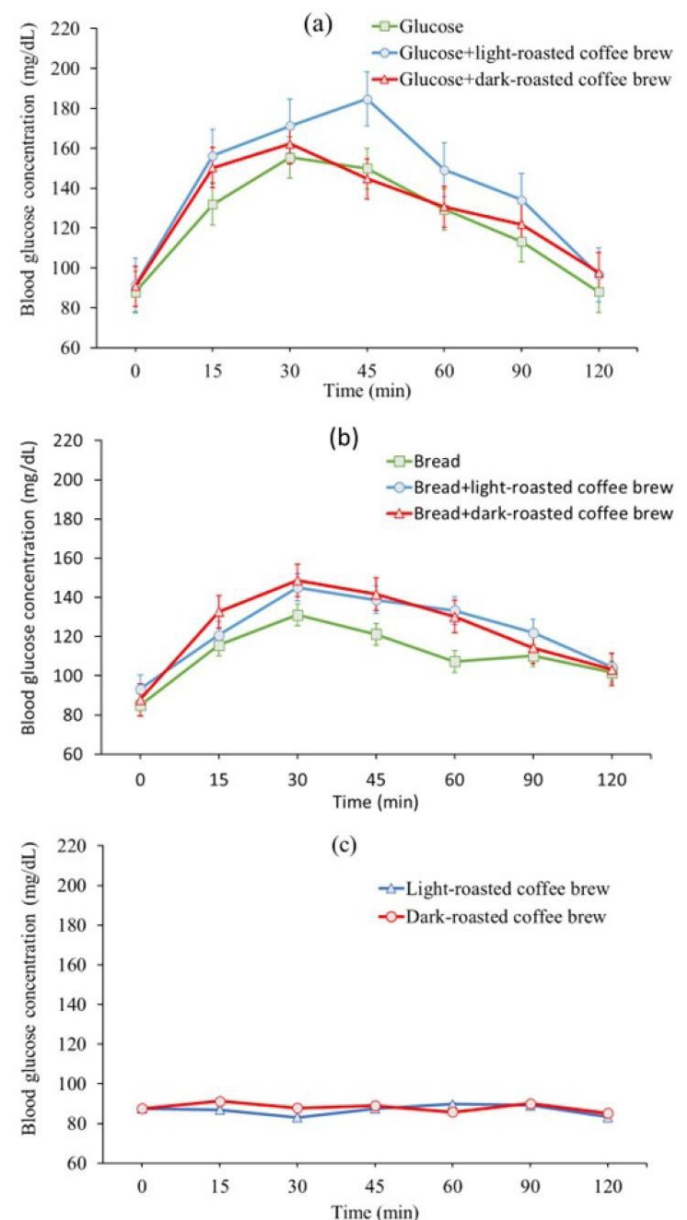


Figure 2: Blood glucose concentrations (mean $\pm$ SEM) before and after coffee ingestion with and without carbohydrate load to (a) glucose absorption, (b) rate of carbohydrate (bread) digestion and absorption, and (c) blood glucose levels after consumption of coffee brew (without carbohydrate), $n = 10$

Table 4: Rate of change in blood glucose levels and iAUC values after coffee ingestion.

Sample	Rate of blood glucose concentration (mg/dL/min) at time						iAUC
	0-15	15-30	30-45	45-60	60-90	90-120	
Observation of glucose absorption							
Glucose solutions	2.9±0.62 ^a	1.6±1 ^a	-0.4±1.06 ^b	-1.4±0.7 ^{ab}	-0.5±0.4 ^a	-0.8±0.3 ^a	4639±1104 ^a
Glucose solutions + light roast of coffee brew	4.3±2.5 ^a	1±2.5 ^a	0.9±1.0 ^a	-2.4±1.9 ^b	-0.5±0.9 ^a	-1.2±0.6 ^a	6303±2799 ^a
Glucose solutions + dark roast of coffee brew	4±1.7 ^a	0.8±1.3 ^a	-1.2±0.8 ^b	-0.9±1.5 ^a	-0.3±0.6 ^a	-0.8±0.8 ^a	4753±1433 ^a
b. Observation of carbohydrate digestion							
Bread	2±1.2 ^a	1±1.3 ^a	-0.7±0.8 ^a	-0.9±1.1 ^a	0.1±0.5 ^a	-0.3±0.4 ^a	3209±1466 ^a
Bread + light roast of coffee brew	3±1.3 ^a	1.1±1.3 ^a	-0.5±1.3 ^a	-0.8±1.4 ^a	-0.5±0.6 ^b	-0.4±0.7 ^a	4381±1308 ^a
Bread + dark roast of coffee brew	1.8±1.5 ^a	1.6±0.9 ^a	-0.4±0.5 ^a	-0.4±1.1 ^a	-0.4±0.5 ^{ab}	-0.6±0.4 ^a	3807±1427 ^a
c. No external source of glucose							
Light-roasted coffee brew	0	0	0	0	0	0	278±340 ^a
Dark-roasted coffee brew	0	0	0	0	0	0	502±483 ^a

Values are presented as mean ± standard deviation. Numbers with different superscript letter within the same column differ significantly ($P < 0.05$).

4 DISCUSSION

This study demonstrated that the level of coffee roasting significantly influenced the concentration of key bioactive compounds. Light-roasted Robusta coffee retained higher amounts of chlorogenic acids — including 3-CQA, 4-CQA, and 5-CQA — which were reduced by 39%, 41%, and 44% respectively in dark roast samples. The results of this study are in accordance with those found by Alongi et al. (2018) that roasting reduces CGA content, with dark roasting causing a greater reduction than light roasting. Additionally, the α -glucosidase inhibitory activity of light-roasted coffee was 85% higher than that of dark-roasted coffee, suggesting a stronger potential to modulate carbohydrate digestion. The study by Alongi et al. (2021) demonstrated that in vitro-digested coffee exhibited a more effective inhibitory effect on the α -glucosidase enzyme compared to undigested coffee, regardless of the roasting level. This finding is further supported by molecular docking results, which show that chlorogenic acid compounds have a high binding affinity for α -glucosidase. This study complements previous research by incorporating the coffee roasting factor and evaluating its inhibitory activity both in vitro against α -glucosidase and in vivo in ten human subjects. Prior to in vitro and clinical evaluations, the physicochemical changes in coffee due to roasting are first discussed.

4.1 Physical and Chemical Changes During Roasting

The roasting process in this study affected weight loss and color lightness, showing statistically significant differences between light- and dark-roasted coffee. The mass reduction of the beans during roasting occurs due to the loss of moisture

content and carbohydrate degradation (Mehaya; Mohammad 2020). At the initial stage, water in the bean matrix evaporates, causing the volume of the coffee beans to increase. As roasting progresses, organic compounds and CO₂ begin to release as a result of pyrolysis and degradation reactions (Herawati et al., 2019b). High degradation occurs in polysaccharides, amino acids, sugars, and chlorogenic acids, while lipids and organic acids relatively increase. The heat generated by the roasting process induces a caramelization reaction, which affects the aroma and brownish color. Another major reaction impacting coffee's flavor and color during roasting is the Maillard reaction (Tarigan et al., 2022).

Another physical characteristic evaluated was the total soluble content. In our study, no significant differences were observed among light-, medium-, and dark-roasted Robusta coffees, indicating that the overall extractable solids remain relatively constant despite variations in roasting intensity. However, the types of soluble carbohydrates may vary due to thermal degradation and Maillard reactions (Lopes et al., 2020). While higher porosity and finer particle size contribute to more efficient extraction, intense dark roasting conditions can degrade complex carbohydrates into smaller, structurally different molecules, such as furfural and melanoidins. Consequently, although the quantity of extractable carbohydrates may remain unchanged, the bioactive profiles and functional properties of these compounds may differ substantially between light and dark roasts.

4.2 Chlorogenic Acids and α -Glucosidase Inhibition

The results indicated that chlorogenic acid concentrations were significantly reduced in dark-roasted coffee compared to light-roasted coffee. Specifically, the degradation rates of 3-CQA, 4-CQA, and 5-CQA were 39%, 41%, and 44%,

respectively. This decline aligns with previous findings that show chlorogenic acids are thermolabile and degrade progressively with increasing roasting temperature (Vignoli et al., 2014; De Souza Costa et al., 2024). The content of 5-CQA was found to be higher than 3-CQA and 4-CQA. The content of 3-CQA can increase along with the decrease of 5-CQA during the ripening and roasting process (De Souza Costa et al., 2024). In contrast, caffeine, a thermostable alkaloid, remained relatively stable across roasting levels (Tsai; Jioe 2021).

In terms of α -glucosidase inhibition, light-roasted coffee brew demonstrated an 85% higher inhibitory effect than dark-roasted coffee. The α -glucosidase enzyme plays a crucial role in carbohydrate hydrolysis, and its inhibition can slow glucose absorption into the bloodstream. These results suggest that light-roasted coffee may have a stronger potential to modulate postprandial glucose levels due to its higher chlorogenic acid content (Alongi; Anese, 2018).

4.3 Blood Glucose Response and Glucose Absorption

The study analyzed the acute effects of coffee brew consumption on blood glucose levels in ten healthy individuals. Results showed that while neither light- nor dark-roasted coffee significantly suppressed glucose absorption, the rate of blood glucose changes varied depending on roasting level (Gamboa-Gómez et al., 2024). Blood glucose concentration declined more rapidly (-2.4 mg/dL/min) between 45-60 minutes after consuming light-roasted coffee, compared to dark-roasted coffee and the control group. This effect may be attributed to chlorogenic acids' ability to enhance insulin sensitivity and inhibit glucose transporters in the intestine (Al-mssallem; Brown 2013). However, the absence of significant differences in overall glucose absorption rates suggests that acute coffee consumption alone may not be sufficient to exert a strong glycemic control effect.

Additionally, caffeine has been reported to influence glucose metabolism by stimulating catecholamine release and increasing cyclic adenosine monophosphate (cAMP) levels, which in turn affects insulin function (Rodak; Kokot; Kratz, 2021). While some studies have suggested that caffeine may impair glucose tolerance in the short term (Moisey et al., 2008), others have indicated that chronic coffee consumption could improve insulin sensitivity and reduce diabetes risk (Tuomilehto et al. 2004). The results from this study suggest that the interaction between caffeine and chlorogenic acids may influence glucose metabolism, but further long-term studies are needed to clarify these mechanisms.

4.4 Effect on Carbohydrate Hydrolysis and Glycemic Response

To further evaluate coffee's role in glucose metabolism, the study measured blood glucose response following the

consumption of coffee with complex carbohydrates (white bread). The results showed that neither light- nor dark-roasted coffee significantly inhibited carbohydrate digestion. The peak blood glucose concentration was observed at 30 minutes for the control and light-roasted coffee, while for dark-roasted coffee, it was slightly delayed to 45 minutes. This delay could be associated with differences in polyphenol composition, as melanoidins formed during the roasting process may also contribute to glucose metabolism (Bellesia; Tagliazucchi 2014).

While the immediate effects of coffee consumption on glucose absorption were not significant, epidemiological studies have suggested that long-term coffee intake can reduce the risk of type 2 diabetes by improving insulin sensitivity (Bevilacqua et al., 2023). The present findings support the hypothesis that bioactive compounds in coffee, particularly chlorogenic acids, could have beneficial metabolic effects, but the extent of these benefits may depend on habitual consumption patterns and individual metabolic responses.

5 CONCLUSIONS

Light-roasted coffee brew contains more chlorogenic acid and shows stronger α -glucosidase inhibition (73.43%) than 0.01 g/L acarbose (44.95%), while caffeine levels remain unaffected by roasting. Short-term studies indicate no inhibition of glucose absorption, inhibit carbohydrate hydrolysis and modulate glucose from the liver. However, light-roasted coffee enhances glucose uptake into tissues within 45 minutes postprandial. Future studies should explore long-term effects on insulin sensitivity and glycemic control, including synergistic roles of bioactive compounds to optimize roasting for health benefits.

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7 AUTHORS' CONTRIBUTIONS

Conceptual Idea: Giriwono, P.E.; Herawati, D.; **Methodology design:** Tarigan, E.B.; Giriwono, P.E.; **Data analysis and interpretation:** Tarigan, E.B.; **Supervision:** Giriwono, P.E.; Andarwulan, N.; Herawati, D.; **and Writing and editing:** Tarigan, E.B.; Giriwono, P.E.; Herawati, D..

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