

Improvement of Sensory Quality: Alteration of Physicochemical Characteristics and Antioxidant Activity of Immature Robusta Coffee Treated with Acetic Acid

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Abstract

Immature green coffee beans, which originate from green coffee cherries, are defective beans that are difficult to distinguish from mature green beans. Roasting these immature green beans produces quaker beans, which can reduce coffee quality. Acid treatment has been studied to improve the aroma quality of coffee. Acetic acid, an organic acid in coffee produced during fermentation and commonly used as vinegar, requires further study to determine its effects on immature coffee beans. This study investigated the effect of acetic acid treatment on the sensory, physicochemical and antioxidant properties of immature Robusta coffee beans. Immature green beans were soaked in acetic acid solutions of varying concentrations (0, 1, 2, and 3 %) and durations (30, 60, and 90 min) at 35 °C. Immature coffee beans had lighter brown color after roasted, and have higher chlorogenic acid (65.39 mg/g), caffeine (26.08 mg/g) and total soluble phenolic contents (65.70 mgGAE/g) than mature beans ($p < 0.05$). The roasted coffee of CI3-90 was the best acetic acid treatment sample with the highest cupping score (82.9) and the most intensely brown color, which resulted in decreased brightness (38.64 to 30.54) and yellowness (29.91 to 25.79). The acetic acid treatment significantly reduced total soluble phenolic content (61.58 to 45.26 mgGAE/g), chlorogenic acid content (20.10 to 14.64 mg/g) and trigonelline content (7.40 to 6.07 mg/g) of immature roasted coffee beans. Nevertheless, acetic acid treatment did not significantly influence caffeine content or antioxidant activity in either the green or roasted immature coffee beans ($p > 0.05$).

Keywords: Antioxidant, Acetic acid, Coffee bean, Immature, Physicochemical, Robusta

Introduction

Indonesia is one of the 5 largest coffee-producing countries, with average production in 2018 - 2022 of 766.42 thousand tons [1]. The type of coffee that is more widely produced in Indonesia is robusta coffee which reaches 72.66 % of total coffee production [2]. The Lampung Province is one of the primary regions producing Robusta coffee in Indonesia [3]. Lampung Robusta coffee generally has a solid and persistently bitter taste [4]. Several factors can influence primarily the quality of coffee flavor, including the coffee variety, growing environment, harvest process, fruit maturity level, and post-harvest process. The maturity of coffee

cherries is a crucial factor that should be considered during harvesting [5].

However, many local coffee farmers in Lampung often harvest coffee cherries without considering their maturity level, leading to green coffee cherries being processed alongside ripe coffee cherries. Processed green coffee cherries produce immature green beans. The immature green beans are difficult to visually distinguish from mature green coffee beans. After roasting, immature green beans produced quaker beans [6]. According to Velasquez *et al.* [7], coffee from immature green beans has the lowest sensory quality. Rabelo *et al.* [6] reported that the presence of 2 % quaker

beans could increase the bitterness and astringent flavor of coffee beverages, thereby decreasing flavor quality.

Further research is needed to investigate the physical and chemical differences between immature and mature coffee beans. Considering that immature green coffee beans are difficult to distinguish from mature green coffee beans, additional treatments are needed to reduce the unpleasant flavor caused by the presence of immature beans. Several studies have explored methods for improving the flavor quality of coffee, including re-fermentation of low-grade quality Robusta coffee beans using kefir Afriliana *et al.* [8], steam pressure treatment of low-grade Robusta coffee beans (containing 8.9 % immature beans) Kalschne *et al.* [9], soaking treatment of mature robusta coffee beans with acetic acid solutions at various concentrations for 2 h Liu *et al.* [10] and soaking treatment of mature robusta coffee beans with different sugar solutions at various concentrations, at a pressure of 2 bar and temperature of 100 °C for 30 min [11]. Treatment carried out specifically for immature green coffee beans has not yet been studied.

Acidity is an essential attribute in the quality of coffee flavor and is one of the main parameters for the industry and Q-Grader in assessing coffee quality [12]. The pH and titratable acidity of coffee beans are closely related to their acidity, and even slight changes in pH can affect taste profiles and consumer preferences [13]. The acidity profile of coffee is influenced by the presence of organic acid compounds in coffee beans, such as citric acid, lactic acid, acetic acid, malic acid, and propionic acid. Several types of organic acids, such as citric, succinic, and malic acids, are naturally present in coffee green beans [12,13]. Besides, several types of organic acids in coffee are produced after the roasting process, such as acetic acid, lactic acid, and formic acid because of sucrose degradation during roasting [14,15]. The formation of these types of organic acids after roasting contributes to the flavor of brewed coffee. Since the presence of immature coffee beans can increase the bitterness of brewed Robusta coffee, treating these immature green beans with acid is a cost-effective and practical solution to reduce bitterness.

Acid treatment of coffee beans has been previously studied by Liu *et al.* [10]. They reported that the aroma quality of Robusta coffee increased after acid

pre-treatment with acetic acid at varying concentrations of 0 - 5 % for 2 h [10]. In addition, Lee *et al.* [16] reported that the treatment of Robusta coffee beans with organic acids (malic, citric, tartaric, and succinic acids) and their combination with sonication reduced the production of negative volatile compounds (pyrazine, pyrrole, and phenol) while increasing the amount of positive volatile compounds (furan). Acetic acid is a type of acid that is widely known and utilized by the public as vinegar. This acid has sour, salty, and vinegar-flavored sensory properties [12]. High acetic acid content in coffee can reduce the quality of coffee flavor because coffee becomes more bitter and pungent aromas appear [17]. Acetic acid content at low to moderate concentrations can contribute to fruity and wine flavors in coffee [17,18]. However, further study is needed to evaluate the effects of acetic acid treatment on immature Robusta coffee beans.

Coffee flavor is related to its precursor compounds in green beans, including chlorogenic acid, lipids, caffeine, trigonelline, amino acids, reducing sugars, and organic acids [19]. These compounds are among the types of bioactive compounds that not only contribute to the flavor of coffee but also to the antioxidant properties of coffee brews. Currently, coffee is consumed not only because of its distinctive flavor but also because of its functional health benefits [20,21]. Maillard Reaction Products (MRPs), such as heterocyclic volatile compounds (pyrrole, thiazole, furans and thiopenes) and melanoidin can also contribute to the antioxidant activity of coffee [22-24].

Coffee bean processing can influence their physical from farms into cups and can affect their physical properties and chemical content, including their flavor characteristic [21]. Additional treatment during processing may affect the chemical composition of coffee beans. The effect of acid treatment on antioxidant activity has been studied in other food products. Sritongtae *et al.* [25] studied the effects of citric acid pretreatment on rice beans (*Vigna umbellata*) and found that pre-treatment of rice beans with 1 % citric acid increased the total phenolic and vitamin B contents. In addition, Ashkan *et al.* [26] reported that sliced button mushrooms soaked in citric acid with varying concentrations of citric acid for 10 min maintained their bioactive compound content and antioxidant activity

during storage compared to those not treated with citric acid.

Our previous study Fardenan *et al.* [27], has studied the effect of acetic acid on immature coffee beans, where the concentration of acetic acid used was different from that used in this study. However, the shortcomings of our previous study were that it used mixed coffee cherries (not fully immature green beans), the analysis was only carried out on roasted coffee samples, and the characteristics of their antioxidant properties were not studied. Although acid treatment of immature Robusta coffee beans is believed to improve coffee flavor quality, there is limited research on its effects in terms of physicochemical characteristics and antioxidant activity. Therefore, the research gap or novelty in this study encompasses the specific acetic acid treatment conducted on immature Robusta coffee beans derived from fully green cherries and the subsequent observation of the effects of the treatment on alterations in sensory quality, coffee color, bioactive compound content, and functional properties of antioxidant activity which have not been previously investigated. This study aimed to examine the impact of acetic acid treatment on the sensory quality, physicochemical properties, and antioxidant activity of fully immature Robusta coffee beans, given its significance in determining coffee flavor quality and potential health benefits.

Materials and methods

Chemicals and reagents

Chemicals of analytical grade, including acetic acid glacial 100 % (Merck #100063), ethanol absolute (Merck #100983), deionized water, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Folin–Ciocalteu reagent (Merck #109001), gallic acid (sigma G7384), nylon syringe filter Millipore Millex 0.45 μm , and some chemicals with liquid chromatography grade such as acetonitrile (Merck #1.00030), Methanol (Merck #1.06007), and aqua pro-injection.

Coffee samples and treatments

The main materials were immature and mature Robusta green coffee beans obtained from 1 coffee farm of local farmers in Liwa, West Lampung, Lampung Province, Indonesia. The harvest and post-harvest

process for coffee cherries follows the methods commonly used by local farmers in Liwa Lampung. The harvest process was conducted manually between June and August 2022. The post-harvest of coffee cherries was processed using the natural dry process method to produce green coffee bean samples. In the natural dry process method, the harvested coffee cherries were immediately dried by sun-drying for 7 days (depending on the weather), then continued with hulling and packaging. The dried green bean samples were further sorted manually to separate broken beans, black beans, and impurities. The dried green coffee beans were kept in a plastic bag and transferred to the Gadjah Mada University campus for further treatment and analysis. The green bean samples were kept in a sealed jar and then stored in a cool room at a temperature of 3 °C.

Acetic acid treatment on immature green coffee bean was carried out according to the method of Liu *et al.* [10] with modifications. The immature green coffee beans were placed in a beaker glass. Acetic acid solution was added at various concentrations (0, 1, 2, and 3 %) at a ratio of 1:5 w/v. The beans were soaked using shaker water bath at a temperature of 35 °C for 30, 60, and 90 min. After the treatment process, the green beans were drained from the acid solution and each sample was dried at a temperature of 50 °C for 7 h in a cabinet dryer until the moisture content reach about 12 % db. The physicochemical characteristics of green and roasted coffee beans were tested to determine their physical and chemical characteristics after treatment process.

Roasting and milling

Coffee bean samples were roasted on a medium level (Agtron Scale 55) at 240 °C for 14 min using a gene café roaster machine (Gene Café CBR101, Ansan, Korea). The roasted coffee beans were ground using a Latina N-600 coffee grinder and sifted through a 20-mesh sieve. The ground coffee samples were stored in vacuum packaging for further analysis.

Color analysis

The color analysis was performed according to the method described by Yeager *et al.* [28]. Coffee bean color was analyzed using a Chromameter Konica Minolta CR-400 (Ramsey, NJ, USA). Coffee bean color was analyzed after grinding the beans into powder to

ensure more uniform sample conditions. The color characteristics of green and roasted beans before and after acetic acid treatment were determined by the CIEL* a* b* value. The L* value represents lightness and ranges between 0 (dark) and 100 (bright). A positive or negative a* value indicates a red or green color proportion, respectively. The b* value indicates blue (–) and yellow (+) color proportions [29]. The measurements were performed in triplicate.

Chemical properties of coffee beans

pH measurement

pH analysis was performed according to the method described by Liu *et al.* [10]. Coffee green bean powder (2 g) was dissolved in 15 mL of 95 °C hot water and stirred. The mixture was centrifuged at 4000 rpm for 15 min, followed by incubation at room temperature for 1 h. The pH of the supernatant was measured using a Mettler Toledo single-channel, sensor LE438 (Germany). The measurements were performed in triplicate.

Analysis of chlorogenic acid, caffeine, and trigonelline

The sample preparation for analysis of chlorogenic acid, caffeine, and trigonelline content was carried out based on the method developed by Lucia Margarita *et al.* [30]. The coffee powder (200 mg) was dissolved in 20 mL of hot pure water at approximately 95 °C. All coffee samples were ultrasonicated for 5 min in a digital ultrasonic cleaner bath at 40 KHz and 100 W (Guangzhou, China). The mixture was filtered through a Whatman filter paper (No. 1). A total of 1 mL of filtrate was placed into a 10 mL volumetric flask, and water for injection (1:1, v/v) solution was added to the mark. The sample was filtered using a Millipore nylon filter with a diameter of 25 mm and 0.45 µm pore size. All samples were sonicated for 10 min prior to HPLC analysis.

The HPLC conditions were as follows: a Shimadzu Prominence Series HPLC system (Kyoto, Japan) equipped with a column C18 (5 µm × 4.6 mm × 150 mm), autosampler (SIL-HTC, Shimadzu, Japan), binary pump (LC-20AD), and Photo Diode Array Detector UV-Vis SPD M-20A. Mobile phase A (0.01 % acetic acid in aqua pro-injection) and mobile phase B (2

% acetic acid in HPLC-grade acetonitrile) were used. The elution gradient was defined as follows: 2.5 % B to 94 % B for 15 min, 94 % B to 100 % B for 1 min, 100 % B for 3 min, and 100 % B to 2.5 % B for 5 min. The Total analysis time was 25 min at a flow rate of 1 mL/min with an injection volume of 20 µL. The detector was set at a wavelength of 320 nm for chlorogenic acid identification, and 270 nm for caffeine and trigonelline identification.

Chlorogenic acid, caffeine, and trigonelline levels were quantified using mixed external standards. These standards were prepared at concentrations of 0.5, 1.25, 2.5, 5, 7.5, 10, 12.5, 25, 50, and 75 ppm in a methanol: water (1:1, v/v) solvent mixture. The quantification was determined using the standard curve regression equation (peak area as a function of concentration) of the best curve, with a coefficient of determination (R^2) \geq 0.99. The limits of detection (LOD) of chlorogenic acid, caffeine, and trigonelline compounds were 2.02, 0.69, and 1.7 ppm, respectively. The limit of quantification (LOQ) of the analysis of chlorogenic acid, caffeine, and trigonelline compounds in this study were 6.13, 2.12, and 5.18 ppm, respectively. The measurements were performed in triplicate.

Total phenolic content analysis (TPC)

Total phenolic analysis in this study was performed using the Folin–Ciocalteu method and the extraction process described by Bobkova *et al.* [31], with slight modifications. A total of 1.75 g of coffee bean powder was dissolved in 30 mL of hot deionized water at a temperature of 95 °C. The mixture was stirred for 5 min using a magnetic stirrer at 500 rpm. The samples were filtered through Whatman No.41 paper, and the supernatant obtained was analyzed.

The coffee extract was diluted 50 times with deionized water and its total phenolic content was determined using gallic acid as the standard (0 - 100 mg/L). One milliliter of the extract sample solution was added to 5 mL of 2 % Na₂CO₃ and allowed to stand for 10 min. 0.5 mL of 10 % Folin reagent solution was added, and the mixture was allowed to stand for 30 min. The absorbance was measured at a wavelength of 765 nm using a spectrophotometer Thermo Fisher Scientific Genesys10S Uv-Vis 840- 209700 (Waltham Massachusetts, USA). The total phenolic content of the

coffee samples was measured in triplicate and expressed as mg gallic acid equivalent/g (mg GAE/g) of dry coffee.

Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH)-radical scavenging activity) assay

The analysis of antioxidant activity and the extraction process have been described previously by Bobkova *et al.* [31], with slight modifications. The extract (100 µL) was put into a test tube, added to 3.9 mL of 0.1 mM DPPH reagent, and mixed in a dark room for 30 min. The absorbance was measured at a wavelength of 517 nm using a spectrophotometer Thermo Fisher Scientific Genesys10S Uv-Vis 840-209700 (Waltham Massachusetts, USA). The Antioxidant activity expressed as % radical DPPH scavenging activity, was calculated according to the following equation:

$$\% \text{ DPPH Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100 \% \quad (1)$$

where A0 is the control absorbance of DPPH solution (without sample), and A1 is the final absorbance of the DPPH solution after reaction with the sample.

Sensory analysis

Sensory analysis was based on the cupping test protocol of the Specialty Coffee Association of America (SCAA) [32]. Sensory testing was conducted by 5 expert panelists from Indonesian Coffee and Cocoa Research Institute (ICCRI), Jember, East Java, Indonesia. The number of expert panelists involved in this study was based on the method of Scholz *et al.* [33], who used 5 professional panellists as a reference for the cupping test. A total of 8.25 g of roasted coffee powder from each sample was brewed with 150 mL of hot water (95 °C) for 4 min. Each sample was repeated 5 times so that the brewed coffee was served in 5 bowls. When finished, the coffee brew was cooled at room temperature to 60 - 70 °C. A total of 20 mL of brewed coffee was served in a cup and assigned a 3-digit code. The sensory test was conducted by evaluating the taste and aroma of coffee by (a) sniffing the dry coffee powder, (b) tasting the coffee sample after 3 min of brewing, and (c) evaluating the brew 8 - 10 min after brewing. Assessment of cupping test results was performed by giving a score between 1-10 for each sensory parameter and a

description of the flavor characteristics of each sample tested. A score of 1 was the lowest score and 10 was the highest score. The attributes assessed were aroma, flavor, aftertaste, bitterness, acidity, body, balance, clean cup, uniformity, taint-faults and overall. This sensory analysis has obtained ethical clearance approval from the medical and health research ethics committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada-Dr. Sardjito General Hospital with number KE/FK/0734/EC/2023.

Data analysis

This study used 2 factors: completely randomized factorial design with acetic acid concentration and treatment duration as independent variables. All data were analyzed using SPSS Statistics software version 26 (IBM SPSS Statistics 26, IBM company, New York, USA). The analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to identify significant differences ($p < 0.05$) for each parameter if the data is normally distributed and homogeneous. All data were summarized as mean values and standard deviations (SD).

Results and discussion

Color characteristics of immature robusta coffee beans after acid treatment

Coffee bean color is one of the most critical physical criteria for determining the coffee bean quality. The effects of acetic acid treatment on the color characteristics of green and roasted beans were investigated (**Figure 1**). The results showed that values of L*, a*, and b* for untreated immature and mature green coffee beans did not differ significantly, suggesting that using the green bean color parameter to differentiate between the 2 is difficult. After roasting, the L* and b* values of untreated immature roasted coffee beans were significantly greater than those of mature roasted coffee beans, indicating that the color of immature roasted coffee beans tends to be yellowish-brown or light brown. Immature roasted coffee beans with a lighter brown color indicate that the brown color did not develop properly during roasting. These coffee beans are known as quaker coffee beans.

After acetic acid treatment, immature coffee green beans treated with higher concentrations of acetic acid and longer treatment times had slightly increased L* and b* values, whereas the a* value remained unchanged

significantly. Immature green beans treated with 3 % acetic acid for 90 min had the highest L values, indicating that the green beans became brighter. The a* value, which did not change significantly, suggests that the intensity of the green color in immature green beans remained constant after treatment. Furthermore, immature coffee beans treated with 1 - 3 % acetic acid exhibited higher b* values than untreated beans and those treated with 0 % acetic acid. An increase in the b* value indicates that the color of immature green beans becomes yellowish. These results are in line with the study of Koca *et al.* [34], which also showed that green pea placed in acidic conditions (pH 5.5) turned

yellowish due to the degradation of chlorophyll into pheophytins. Acetic acid treatment on immature coffee beans was also studied in terms of color change after roasting. The data in **Figure 1** shows that treating immature beans with 3 % acetic acid for 90 min led to a significant decrease in L, and b values of roasted immature coffee beans. The samples that treated with 3 % acetic acid for 90 min had similar L, a, and b values to those of mature roasted coffee beans. These results indicate that treating immature coffee beans with 3 % acetic acid for 90 min after roasting can produce a darker brown color than untreated immature beans or other treated samples.

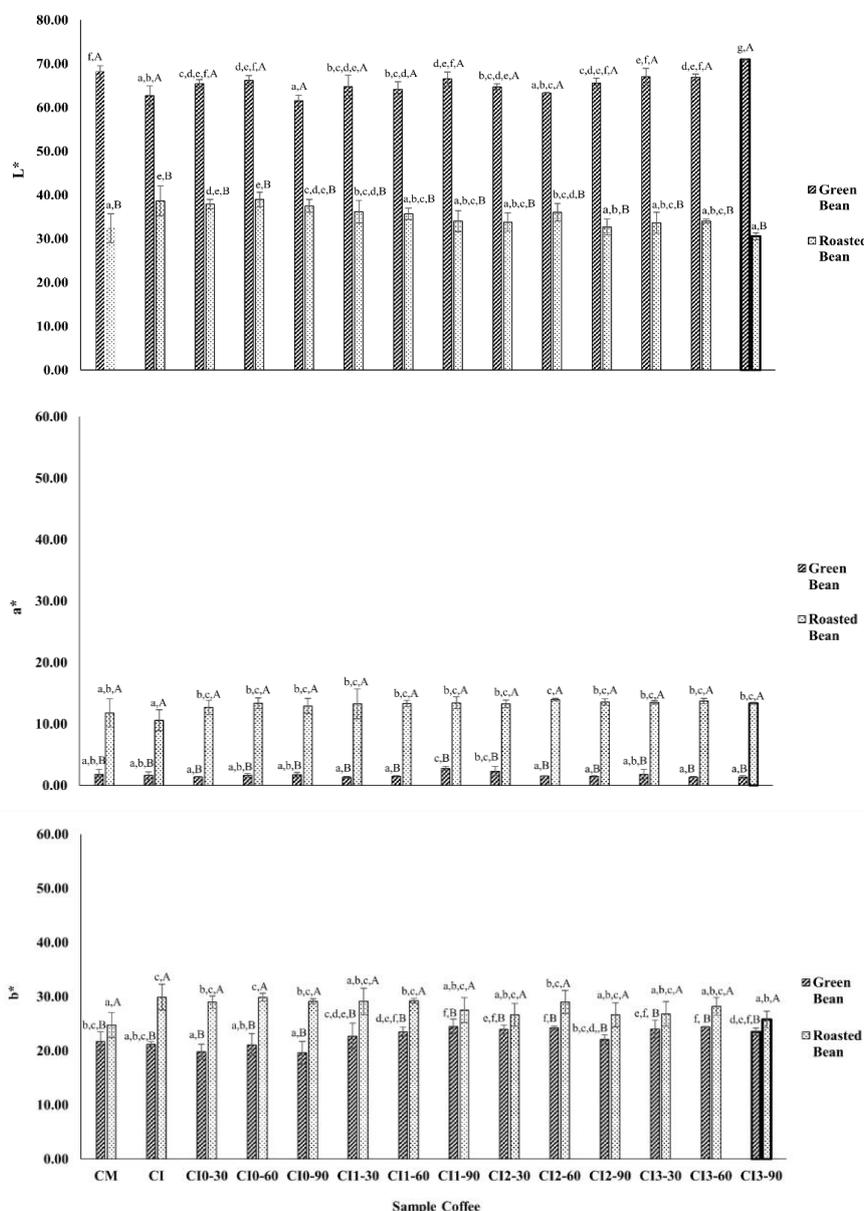


Figure 1 Color index (L, a, b) value of untreated coffee bean and acid-treated immature coffee bean. ^{a-f} Different lowercase letters on the same pattern bar indicate significant differences within each sample ($p < 0.05$). ^{A-B} Different

uppercase letters on bar for each sample, indicate significant differences within green and roasted beans ($p < 0.05$). Note: CM (Coffee Mature-untreated; positive control), CI (Coffee Immature- untreated; negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

Acid treatment can degrade polysaccharides like cellulose and hemicellulose into simple sugars, like glucose, fructose, and xylose [35]. These sugars react with amino acids in coffee green beans during roasting via the Maillard reaction. Amino acids are predominantly present in green coffee beans and serve as substrates for the Maillard reaction, resulting in a decrease in their content during coffee roasting [36]. As a result, the roasted immature coffee beans that were acid-treated tend to be darker brown than untreated ones. Additionally, the browning of coffee beans during roasting can be attributed to the formation of 5-hydroxymethylfurfural (5-HMF), an intermediate product of Maillard reaction [37]. 5-HMF is formed through the dehydration of glucose and hexose, which result from the degradation of cellulose and hemicellulose, respectively [35].

Mehaya and Mohammad [37] reported that 5-HMF compounds are formed by the decomposition of hexose sugars under acidic conditions and cause an increase in the brown color of thermally processed food products. Thus, if coffee green beans are subjected to more acidic conditions during roasting, it may enhance the formation of 5-HMF compounds, resulting in a darker brown color of the roasted immature coffee bean. **Figure 2** illustrates the change in roasted color of immature coffee beans after treatment with 3 % acetic acid for 90 min. Immature coffee beans treated with 3 % acetic acid for 90 min showed an increase in the intensity of the darker brown color during roasting, resembling the color of mature roasted coffee beans (**Figure 2**). This finding suggests that 3 % acetic acid treatment for 90 min is the best treatment for immature coffee beans based on the color parameters of roasted beans.

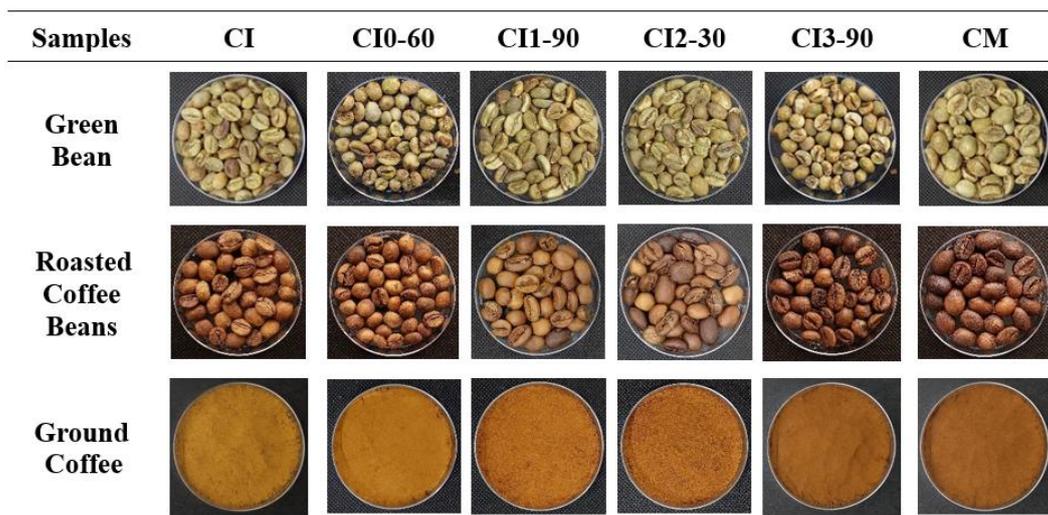


Figure 2 Color change of some acid-treated immature coffee samples compared to untreated coffee samples. Note: CM (Coffee Mature-untreated; positive control), CI (Coffee Immature-untreated; negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

Sensory characteristic

The effect of acetic acid treatment on immature coffee beans was also studied to determine changes in sensory characteristics and cupping score. The sensory analysis was carried out on control samples (untreated immature and mature beans) and all acetic acid-treated samples. The cupping test was performed based on the

cupping test protocol developed by the Specialty Coffee Association of America (SCAA). The cupping test results are listed in **Table 1**.

The cupping test for each coffee sample was carried out with 12 sensory attributes that were assessed, including aroma, flavor, aftertaste, acidity, bitter/sweet, mouthfeel/body, balance, uniformity, clean cup, taints,

overall, and final score. Based on the cupping test results shown in **Table 1**, the immature robusta coffee beans (CI) have a lower final score than the mature robusta coffee beans (CM). According to the SCAA protocol, if a coffee has a cupping final score greater than 80 (> 80), it is categorized as a specialty coffee [32]. The final score of the CI samples had a value of 78.65, and the CM sample had a value of 81.8, so that Lampung robusta coffee from mature beans was classified as specialty coffee. The flavors descriptions of the CI samples by the panellists were cereal, chocolaty, spicy-pepper-like, clove-like, and astringent aftertaste. The flavors description of CM samples was chocolaty, roasted, spicy-clove-like, caramelly, bitter, and astringent aftertaste. The results of the flavors description by the panellists indicated that both Lampung robusta coffees from immature and mature beans had spicy and astringent flavors characteristics.

Based on the final cupping test score for all treated coffee beans, it is known that after acetic acid treatment, the final score of immature coffee beans increased and approached the final score of mature coffee beans. This showed that there is an improvement in the flavor quality of immature robusta coffee beans. A previous study by Liu *et al.* [10], reported that mature Robusta coffee beans treated with acetic acid had a better aroma profile because the content of pyrazine and sulphur-containing compounds decreased. Immature coffee beans treated with 3 % acetic acid for 90 min (CI3-90) had the highest final cupping test score of 82.9. From a

statistical perspective, the cupping score of CI3-90 was not significantly different from that of the other acid-treated samples. Nevertheless, in terms of numerical value, the CI3-90 sample demonstrated the highest score among the samples evaluated. This observation serves as the foundation for determining that 3 % acetic acid treatment for 90 min constitutes the optimal treatment for immature coffee beans in this investigation. Immature coffee beans treated with 0 % acetic acid for 30 min (CI0-30) had the lowest final cupping test score of 75.2. The flavors description of sample CI3-90 by panellists was brown sugar, chocolaty, spicy-coriander seed-like, vanilla, rosella tea, acidic, and very sweet. Meanwhile, the flavors description of sample CI0-30 was nutty, roasted, spicy clove-like, chocolaty, and cereally.

These results support those of our previous study Fardenan *et al.* [27], which also obtained the best treatment with 3 % acetic acid for 90 min. Nevertheless, this outcome differs from a previous investigation conducted by Liu *et al.* [10], which conclude that the optimal sample was that treated with 2 % acetic acid for an extended duration of 2 h. The findings of the present study suggest that the enhancement of coffee flavor quality can also be achieved in immature coffee beans through this acid treatment. Furthermore, the cupping test results revealed that a concentration of merely 1 % acetic acid was sufficient to improve the flavor quality of immature Robusta coffee beans, yielding a cupping test value of > 80 .

Table 1 Sensory profile of immature green coffee bean (GI) after acetic acid treatment.

Attributes	CI	Immature (CI) Treated Samples											CM	
		0 - 30	0 - 60	0 - 90	1 - 30	1 - 60	1 - 90	2 - 30	2 - 60	2 - 90	3 - 30	3 - 60		3 - 90
Aroma	7.45 ± 0.48 ^b	6.9 ± 0.63^a	7.45 ± 0.51 ^b	7.55 ± 0.37 ^b	7.8 ± 0.41 ^b	7.45 ± 0.27 ^b	7.75 ± 0.21 ^b	7.6 ± 0.22 ^b	7.75 ± 0.18 ^b	7.6 ± 0.38 ^b	7.7 ± 0.21 ^b	7.7 ± 0.11 ^b	7.85 ± 0.34 ^b	7.65 ± 0.22 ^b
Flavor	7.25 ± 0.53 ^{a,b}	6.95 ± 0.6 ^a	7.4 ± 0.34 ^{a,b,c}	7.6 ± 0.38 ^{b,c}	7.6 ± 0.45 ^{b,c}	7.5 ± 0.53 ^{a,b,c}	7.7 ± 0.27 ^{b,c}	7.75 ± 0.31 ^{b,c}	7.55 ± 0.41 ^{a,b,c}	7.55 ± 0.33 ^{a,b,c}	7.65 ± 0.34 ^{b,c}	7.55 ± 0.48 ^{a,b,c}	7.9 ± 0.38^c	7.9 ± 0.49 ^c
Aftertaste	7.15 ± 0.7 ^{a,b}	6.9 ± 0.63 ^a	7.4 ± 0.49 ^{a,b,c}	7.4 ± 0.45 ^{a,b,c}	7.6 ± 0.58 ^{b,c}	7.55 ± 0.51 ^{a,b,c}	7.7 ± 0.33 ^{b,c}	7.7 ± 0.27 ^{b,c}	7.5 ± 0.4 ^{a,b,c}	7.45 ± 0.45 ^{a,b,c}	7.45 ± 0.33 ^{a,b,c}	7.5 ± 0.31 ^{a,b,c}	7.95 ± 0.37^c	7.7 ± 0.33 ^{b,c}
Acidity	7.35 ± 0.45 ^{a,b}	6.95 ± 0.54 ^a	7.35 ± 0.38 ^{a,b}	7.35 ± 0.52 ^{a,b}	7.5 ± 0.4 ^{b,c}	7.5 ± 0.47 ^{b,c}	7.5 ± 0.4 ^{b,c}	7.65 ± 0.29 ^{b,c}	7.3 ± 0.25 ^{a,b}	7.65 ± 0.7 ^{b,c}	7.55 ± 0.33 ^{b,c}	7.45 ± 0.41 ^{b,c}	8 ± 0.4^c	7.7 ± 0.21 ^{b,c}
Bitter/Sweet	7.35 ± 0.42 ^b	6.85 ± 0.45^a	7.4 ± 0.45 ^b	7.3 ± 0.6 ^b	7.45 ± 0.45 ^b	7.4 ± 0.49 ^b	7.6 ± 0.29 ^b	7.6 ± 0.29 ^b	7.4 ± 0.29 ^b	7.5 ± 0.64 ^b	7.55 ± 0.33 ^b	7.35 ± 0.34 ^b	7.9 ± 0.52 ^b	7.7 ± 0.21 ^b
Mouthfeel	7.45 ± 0.48 ^{a,b}	6.95 ± 0.62 ^a	7.4 ± 0.52 ^{a,b}	7.3 ± 0.45 ^{a,b}	7.55 ± 0.57 ^{a,b}	7.35 ± 0.42 ^{a,b}	7.45 ± 0.33 ^{a,b}	7.7 ± 0.48 ^b	7.5 ± 0.4 ^{a,b}	7.45 ± 0.6 ^{a,b}	7.5 ± 0.4 ^{a,b}	7.4 ± 0.22 ^{a,b}	7.65 ± 0.22 ^b	7.75 ± 0.43 ^b
Balance	7.3 ± 0.54 ^{a,b}	6.85 ± 0.65 ^a	7.4 ± 0.45 ^{a,b}	7.35 ± 0.52 ^{a,b}	7.5 ± 0.35 ^b	7.5 ± 0.47 ^b	7.65 ± 0.29 ^b	7.6 ± 0.22 ^b	7.55 ± 0.37 ^b	7.45 ± 0.6 ^{a,b}	7.6 ± 0.42 ^b	7.55 ± 0.48 ^b	7.75 ± 0.33 ^b	7.65 ± 0.22 ^b
Uniformity	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a
Clean Cup	10 ± 0.00 ^a	10 ± 0.00	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a	10 ± 0.00 ^a
Overall	7.35 ± 0.55 ^{a,b}	6.85 ± 0.54 ^a	7.4 ± 0.45 ^{a,b}	7.35 ± 0.52 ^{a,b}	7.6 ± 0.58 ^b	7.5 ± 0.47 ^b	7.6 ± 0.22 ^b	7.6 ± 0.22 ^b	7.5 ± 0.00 ^b	7.45 ± 0.6 ^{a,b}	7.55 ± 0.33 ^b	7.45 ± 0.41 ^{a,b}	7.9 ± 0.45 ^b	7.75 ± 0.43 ^b
Final Score	78.65 ± 3.75 ^{a,b}	75.2 ± 4.58^a	79.2 ± 3.52 ^b	79.2 ± 3.37 ^b	80.6 ± 3.68 ^b	79.75 ± 3.43 ^b	80.95 ± 1.65 ^b	81.2 ± 2.03 ^b	80 ± 2.37 ^b	80.1 ± 3.90 ^b	80.55 ± 2.38 ^b	79.95 ± 2.37 ^b	82.9 ± 2.46^b	81.8 ± 2.46 ^b

^{a-c} Different lowercase letters on the same row indicate significant differences within each sample ($p < 0.05$). Note: CM (Coffee Mature-untreated; positive control), CI (Coffee Immature-untreated; negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

pH of immature coffee beans after treatment with acetic acid

The pH of coffee beans indicates their acidity. The effect of acetic acid treatment on the pH of immature coffee beans was studied both in green beans and after roasting. As shown on **Table 2**, The pH values of the untreated immature and mature green beans did not differ significantly, which aligns with the findings of Mazzafera [38]. However, after roasting at the same medium level, the pH of the immature coffee beans was higher than that of the mature coffee beans. This suggests that brews from immature coffee beans are less acidic than those from mature beans, as reported by

Vasconcelos *et al.* [39] for immature Arabica coffee beans. Laukalja *et al.* [40] reported that the pH of coffee products is determined by the aliphatic acids formed during roasting. The main precursors of aliphatic acids in roasted coffee are simple carbohydrates and sucrose. During roasting, simple carbohydrates or sucrose are degraded into glucose and fructose, and then further degraded into acetic acid, lactic acid, formic acid, and glycolic acid through heating [14]. The higher simple carbohydrate or sucrose content in mature roasted coffee beans may contribute to the lower pH by facilitating the formation of more aliphatic acid compounds.

Table 2 pH changes of immature coffee green and roasted beans during treatment acetic acid.

Coffee Sample	pH Value	
	Green Bean	Roasted Bean
CM	5.71 ± 0.04 ^{c, A}	5.7 ± 0.09 ^{a, b, A}
CI	5.72 ± 0.12 ^{c, A}	6.11 ± 0.05 ^{c, B}
CI0-30	5.78 ± 0.3 ^{c, A}	5.60 ± 0.18 ^{a, b, A}
CI0-60	5.8 ± 0.26 ^{c, A}	5.77 ± 0.32 ^{b, c, A}
CI0-90	5.73 ± 0.19 ^{c, A}	5.58 ± 0.27 ^{a, b, A}
CI1-30	5.22 ± 0.22 ^{b, A}	5.48 ± 0.21 ^{a, b, A}
CI1-60	4.99 ± 0.24 ^{a, b, A}	5.51 ± 0.31 ^{a, b, B}
CI1-90	4.89 ± 0.33 ^{a, b, A}	5.37 ± 0.16 ^{a, b, B}
CI2-30	4.94 ± 0.34 ^{a, b, A}	5.47 ± 0.17 ^{a, b, B}
CI2-60	4.94 ± 0.40 ^{a, b, A}	5.48 ± 0.20 ^{a, b, B}
CI2-90	4.76 ± 0.28 ^{a, b, A}	5.33 ± 0.17 ^{a, B}
CI3-30	4.80 ± 0.31 ^{a, b, A}	5.39 ± 0.20 ^{a, b, B}
CI3-60	4.81 ± 0.30 ^{a, b, A}	5.38 ± 0.22 ^{a, b, B}
CI3-90	4.57 ± 0.17 ^{a, A}	5.31 ± 0.15 ^{a, B}

^{a-c} Different lowercase letters on the same Colom indicate significant differences within each sample ($p < 0.05$). ^{A-B} Different uppercase letter on the same row indicates significant different within green and roasted bean for each sample ($p < 0.05$). Note: CM (Coffee Mature-untreated; positive control), CI (Coffee Immature-untreated; negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

As shown in **Table 2**, the pH of the green and roasted beans decreased after acid soaking. Longer exposure times and higher acetic acid concentrations led to a decrease in the pH. During soaking, water and acetic acid enter the beans, increasing the acid concentration.

Consequently, the pH of the beans decreased. The pH of roasted coffee beans was higher than that of green coffee beans, which is consistent with the findings of Liu *et al.* [10]. Roasting reduces the moisture and acid in the beans, making them less acidic. Additionally,

compounds formed during roasting have a buffering effect on the acidic conditions [41]. Immature Robusta coffee bean samples treated with 3 % acetic acid for 90 min had the lowest green and roasted bean pH values, reaching 4.57 and 5.31, respectively. These results are closely to those obtained by Liu *et al.* [10], who treated mature Robusta coffee beans with 2 % acetic acid for 2 h, with pH values of 4.47 for green and 5.13 for roasted beans, respectively. According to Mussato *et al.* [42], coffee with a pH below 4.9 has a very sour taste, while coffee with a pH of more than 5.2 has a taste that tends to be flat and bitter. Hence, coffee brewed from immature coffee beans treated with 3 % acetic acid is still consumable. When correlated with the sensory attribute data presented in **Table 1**, it was observed that the lower the pH of the acid-treated coffee sample, the higher was the acidity value tends to increase. A high acidity value indicates that the acidity of the coffee is perceived as pleasant and acceptable to consumers.

Chlorogenic acid, caffeine, and trigonelline content of immature coffee beans after acetic acid treatment

This study also examined the effect of acetic acid treatment on chlorogenic acid, caffeine, and trigonelline in immature coffee beans. These compounds contribute to the flavor and antioxidant properties of coffee. The caffeine, trigonelline, and chlorogenic acid content of control and acid-treated immature Robusta coffee beans samples, showed in **Figure 3**. The results showed that the levels of these compounds were higher in immature beans than in mature beans, both in green and roasted beans. This aligns with previous findings by Mazzafera [38] and Franca *et al.* [43], as well as more recent

research by Wahyuni *et al.* [5] which found that the caffeine, trigonelline, and chlorogenic acid (5-CQA) contents in immature green coffee beans were higher than those in non-defective coffee beans. Bastian *et al.* [21] reported that chlorogenic acid content in immature coffee beans was 6.5 times higher than that of mature coffee beans.

Caffeine levels in both immature and mature Robusta coffee remained unchanged after roasting, whereas trigonelline and chlorogenic acid levels decreased significantly. According to Sualeh *et al.* [44], caffeine content in coffee beans remained stable following medium-level roasting. This is because there was no change in the caffeine compounds into volatile or other chemical compounds by the heating reaction during roasting. However, chlorogenic acid and trigonelline levels decreased significantly after roasting due to their degradation into volatile and nonvolatile compounds [44]. A previous study by Awwad *et al.* [45] reported that caffeine content in coffee beans increased during light and medium roasting levels but then decreased during dark roasting. Hall *et al.* [46] reported that caffeine is a thermostable alkaloid compound and maintains a relatively constant concentration during roasting. Caffeine is more thermostable owing to its high melting point, reaching 238 °C [37]. In contrast, the trigonelline compound is thermolabile alkaloid compound and can degrade into volatile compounds such as pyridine and pyrrole during roasting [19]. Chlorogenic acid compounds are thermolabile phenolic compounds that can be degraded into chlorogenic acid lactones, isomers, and volatile phenolic compounds during roasting [15,21].

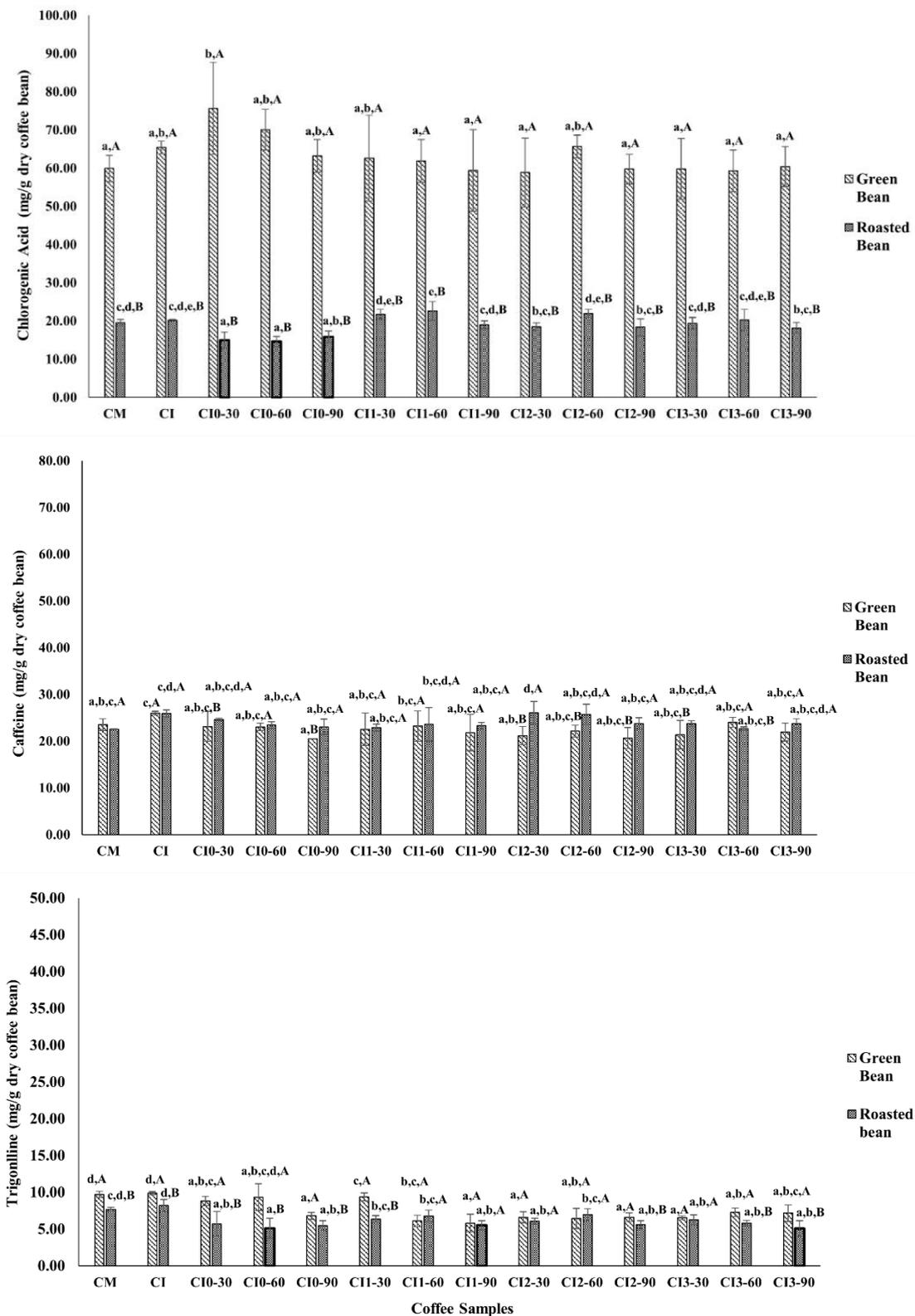


Figure 3 Chlorogenic Acid, Caffeine, and Trigonelline Contents of Immature Robusta Coffee Beans.

^{a-f} Different lowercase letters on the same Colom indicate significant differences within each sample ($p < 0.05$). ^{A-B} Different uppercase letters on the same row indicate significant different within green and roasted bean for each sample ($p < 0.05$). Note: CM (Coffee Mature-positive control), CI (Coffee Immature-negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

Furthermore, chlorogenic acid content between untreated and acetic acid-treated immature green coffee beans were not significantly different. It has been reported that chlorogenic acid is unstable at alkaline pH conditions ($\text{pH} > 6$) and is more stable at acidic pH. Friedman and Kuniyo [47] in their study reported that at $\text{pH} 7 - 11$ there was a change in the spectrum of chlorogenic acid and at $\text{pH} 10$ a new spectral peak began to appear. This shows that there is a change or degradation in chlorogenic acid at alkaline pH conditions. This is also supported by the previous research by Narita *et al.* [48], which shows that chlorogenic acid which is incubated at a temperature of $37\text{ }^\circ\text{C}$ and $\text{pH} > 6$ can be degraded more quickly and forms their isomeric compounds, whereas at pH conditions of 5 and 5.5 the chlorogenic acid content is more stable and isomeric compounds of chlorogenic acid are not formed. Therefore, in this study, the chlorogenic acid content in immature coffee green beans did not experience significant changes after being treated with acetic acid at various concentrations and incubation times.

Roasting significantly reduced chlorogenic acid content in each sample, but there was no significant difference between untreated and acid-treated immature roasted coffee beans. This could be caused by the chlorogenic acid content in untreated and acid-treated immature green coffee bean, which also did not show any significant difference. Meanwhile, the decrease in chlorogenic acid levels in each sample after roasting was due to chlorogenic acid being degraded by heat during roasting.

Caffeine and Trigonelline are the main alkaloid compounds in coffee beans, which contribute to coffee flavor and aroma [49]. Effect of acetic acid treatment on immature green coffee bean also studied for these 2 compounds. The caffeine content in untreated and acid-treated immature green beans was not significantly different, suggesting that acetic acid treatment did not affect their caffeine content. A study by Sulistyningtyas *et al.* [50], showed that robusta green beans' caffeine content remained unchanged after soaking in water for 8 h. After roasting at a medium level, the caffeine content of the acid-treated immature coffee beans remained constant. Since caffeine is a

thermostable alkaloid compound, and its concentration is relatively constant during roasting.

Data on **Figure 3** showed that trigonelline levels in immature coffee green beans significantly decreased after being treated with acetic acid solution for 90 min at each variation of acetic acid concentration. Trigonelline has a high solubility in water [51]. Considering that trigonelline is highly soluble in water, probably during the soaking treatment, acetic acid solutions are imbibed into the coffee bean cell matrix, causing trigonelline to be dissolved in acetic acid solution. These findings align with the study conducted by Hyunbeen *et al.* [52], which demonstrated that trigonelline levels in green beans subjected to soaking in the decaffeination process were lower than those in regular green beans, attributable to the high solubility of the trigonelline compound.

Trigonelline levels in untreated and acetic acid-treated immature coffee beans decreased significantly after the medium level roasting process. The decrease in trigonelline levels is related to the thermolabile nature of the trigonelline compound [53]. Then, the trigonelline levels in roasted coffee from acid-treated immature beans were slightly lower than those in roasted coffee from untreated immature beans. However, trigonelline levels showed no significant differences among the acid-treated roasted immature coffee samples. This is in line with the results of trigonelline levels in acid-treated immature green beans, which also showed no significant differences among the acid-treated green beans. This indicates that differences in acetic acid concentration (0 - 3 %) and treatment duration (30 - 90 min) do not have a significant effect on the trigonelline content of immature green and roasted coffee beans. These results also showed that the decrease in trigonelline levels in coffee was mainly caused by the soaking process during treatment and heat during the roasting process.

The change in total soluble phenolic and antioxidant activity of immature robusta coffee beans after acetic acid treatment

Total soluble phenolic content

Coffee bioactive compounds can be grouped into 3 primary categories: phenolic compounds, flavonoids, and alkaloids [54]. Phenolic compounds contribute to

the flavor and antioxidant properties of coffee. The total aqueous soluble phenolic content in immature green and roasted coffee beans was higher than that in mature green and roasted coffee beans (Table 3). According to a study by Mazzafera [38], immature green coffee beans have a higher total phenolic content than mature coffee

beans. These results align with the chlorogenic acid data presented in Figure 3. Chlorogenic acid is the most abundant phenolic acid in coffee beans. Therefore, immature coffee beans have higher total aqueous soluble phenolic content than in mature beans.

Table 3 Soluble phenolic total and antioxidant activity in water extract of liwa robusta coffee beans.

Coffee Samples	Phenolic Total (mg GAE/g dry coffee)		Antioxidant Activity (% DPPH Scavenging Activity)	
	Green Beans	Roasted Beans	Green Beans	Roasted Beans
CM	58.91 ± 1.33 ^{b, A}	54.63 ± 1.22 ^{c, B}	86.46 ± 0.59 ^{a, A}	83.80 ± 0.82 ^{b, A}
CI	65.70 ± 1.55 ^{c, A}	61.58 ± 2.99 ^{d, B}	85.46 ± 0.47 ^{a, A}	84.52 ± 0.14 ^{b, A}
0: 30	53.65 ± 1.02 ^{a, b, A}	49.16 ± 0.21 ^{a, b, c, B}	82.49 ± 4.24 ^{a, A}	84.54 ± 0.25 ^{b, A}
0: 60	54.98 ± 0.43 ^{a, b, A}	47.32 ± 1.91 ^{a, b, B}	84.41 ± 6.65 ^{a, A}	78.64 ± 6.89 ^{b, B}
0: 90	54.15 ± 0.76 ^{a, b, A}	48.07 ± 0.35 ^{a, b, B}	82.69 ± 4.64 ^{a, A}	81.19 ± 0.26 ^{a, b, A}
1: 30	53.48 ± 3.28 ^{a, b, A}	46.95 ± 1.94 ^{a, b, B}	84.71 ± 4.06 ^{a, A}	85.17 ± 0.23 ^{b, A}
1: 60	56.53 ± 2.89 ^{a, b, A}	48.27 ± 2.92 ^{a, b, B}	86.50 ± 3.02 ^{a, A}	85.49 ± 0.46 ^{b, A}
1: 90	52.72 ± 0.38 ^{a, A}	46.43 ± 1.23 ^{a, B}	87.39 ± 3.67 ^{a, A}	84.87 ± 0.34 ^{b, A}
2: 30	53.66 ± 0.89 ^{a, b, A}	45.26 ± 0.92 ^{a, B}	86.99 ± 4.29 ^{a, A}	84.74 ± 0.52 ^{b, A}
2: 60	57.76 ± 4.69 ^{b, A}	48.58 ± 0.45 ^{a, b, B}	87.54 ± 3.36 ^{a, A}	84.88 ± 0.21 ^{b, A}
2: 90	51.76 ± 2.61 ^{a, A}	46.26 ± 1.38 ^{a, B}	86.13 ± 4.09 ^{a, A}	85.65 ± 1.33 ^{b, A}
3: 30	52.78 ± 1.47 ^{a, A}	46.68 ± 2.17 ^{a, b, B}	85.39 ± 3.90 ^{a, A}	84.87 ± 0.50 ^{b, A}
3: 60	56.36 ± 0.22 ^{a, b, A}	52.18 ± 6.13 ^{b, c, B}	87.43 ± 3.42 ^{a, A}	84.97 ± 0.32 ^{b, A}
3: 90	53.50 ± 0.62 ^{a, b, A}	48.85 ± 0.57 ^{a, b, c, B}	85.82 ± 0.43 ^{a, A}	77.11 ± 3.62 ^{a, B}

^{a-c} Different lowercase letters on the same Colom indicate significant differences within each sample ($p < 0.05$). ^{A-B} Different uppercase letters on the same row indicates significant different within green and roasted bean for each sample ($p < 0.05$). Note: CM (Coffee Mature-positive control), CI (Coffee Immature-negative control), 0 - 30 to 3 - 90 (Acetic acid concentration - treatment duration).

Additionally, the total aqueous soluble phenolic content of the immature and mature coffee beans decreases significantly after roasting at medium level. During the roasting process, chemical reactions occur which cause degradation of phenolic compounds and the formation of other compounds. The previous study of Laukalja *et al.* [40] and Liao *et al.* [55] showed that in light, medium and dark roasting, the concentration of chlorogenic acid compounds decreased, while the concentration of 4-hydroxybenzoic acid, gallic acid, caffeic acid, sinapic acid and ferulic acid compounds increased. The increase in the content of phenolic acid compounds can compensate for the decrease in the content of chlorogenic acid. While another study by

Alnsour *et al.* [56] and Liao *et al.* [55] showed that the total phenolics of coffee beans increased at light and medium roasting levels and further decreased with increasing roasting temperature and time.

This study investigated the total phenolic content, antioxidant activity, and alterations following acetic acid treatment of immature Robusta coffee beans (Table 3). The data presented in Table 3 demonstrate that the total soluble phenolic content in all acid-treated immature coffee bean samples (CI0-30 to CI3-90) was significantly lower than that in untreated immature coffee bean samples (CI). This observation indicated that the total soluble phenolic content of immature coffee beans decreased significantly after acetic acid

treatment. Previous investigations by Xu *et al.* [57] and Thepthanee *et al.* [58] similarly reported a significant reduction in the total phenolic content of certain legumes and sunflower seeds following soaking. The reduction in total phenolic content observed in immature coffee beans following acetic acid treatment may be attributed to softening of the coffee bean texture during the soaking process, potentially resulting in the dissolution of certain phenolic compounds in the soaking solution

Furthermore, the total soluble phenolic content in all acid-treated immature coffee bean samples showed no significant differences ($p > 0.05$). This observation suggests that varying acetic acid treatment did not significantly affect the soluble phenolic content of immature green beans. This finding aligns with the data on chlorogenic acid levels presented in **Figure 3**, which shows no significant change in chlorogenic acid levels among acid-treated immature green beans. These results are related to the properties of several phenolic compounds in coffee beans, such as chlorogenic acid, caffeic acid, and gallic acid, which are more stable in acidic conditions. Another study by Pasquet *et al.* [59] reported that total phenolic levels decreased significantly under alkaline pH conditions (pH 13.5), whereas at acidic pH (pH 2) total phenolic levels remained relatively stable. Kanan *et al.* [60] reported an increase in total phenolic content in rice after soaking in water for 24 h, due to the breakdown of complex bonds in bound phenolic compounds, resulting in the formation of free phenolic compounds. Consequently, soaking immature green coffee beans in an acid solution for a shorter duration did not significantly affect the total soluble phenolic content.

The total soluble phenolic content of untreated and treated immature coffee beans decreased after roasting because phenolic compounds are generally unstable at high temperatures. However, statistically the total soluble phenolic content between all samples of the acid-treated immature roasted coffee beans was no significant difference. This was influenced by the total phenolic content in the green bean sample, which was also not significantly different after the application of acetic acid treatment with variations in acid concentration and treatment time. Therefore, acetic acid treatment applied in this study to immature coffee beans

did not have a significant effect on the total soluble phenolic content of immature green and roasted beans.

Antioxidant activity

This study evaluated the antioxidant activities of immature and mature coffee beans and found no significant differences between them. The DPPH radical scavenging activities of the aqueous extracts of immature and mature robusta green coffee beans were 85.46 and 86.46 %, respectively. These results consistent with previous research by Kulapichitr *et al.* [61], who also reported that there was no significant difference in DPPH radical-scavenging activity on immature and mature Arabica green coffee beans. The natural antioxidant activity contained in green coffee beans depends on the content of bioactive compounds which are influenced by the conditions of the cultivation conditions and the post-harvest processes applied.

Furthermore, the DPPH radical scavenging activity of acid-treated immature green coffee beans showed no significant difference ($p > 0.05$), which aligns with the previous findings on the total soluble phenolic content (**Table 3**). After the roasting process, The DPPH free radical-scavenging activity of the aqueous extracts of untreated and acid-treated coffee beans, not significantly decreased statistically. The roasting process not only breaks down polyphenolic compounds through heat exposure but also generates new bioactive substances with antioxidant properties. These newly formed compounds include melanoidin, hydroxymethylfurfural, and chlorogenic acid derivatives [55]. The formation of new bioactive compounds may compensate for the degradation of polyphenolic compounds during roasting. Another phenomenon showed that the antioxidant activity of samples CI0-60 and CI3-90 decreased significantly after roasting by 78.64 and 77.11 %, respectively. Previous studies by Sunarharum *et al.* [62] and Alnsour *et al.* [56], showed a decrease in antioxidant activity in coffee beans after roasting at medium and dark levels. Meanwhile, other studies by Liao *et al.* [55] showed that the antioxidant activity of coffee beans increases after medium roasting and begins to decrease in dark roasting. Another result showed that the CI3-90 roasted coffee sample had the lowest significant DPPH free radical scavenging activity compared to the control and acid-treated roasted coffee

samples. This might be due to the use of a higher acetic acid concentration and longer treatment duration, so that the cell matrix of coffee beans is more exposed, and more bound polyphenol compounds are degraded into free polyphenol compounds. As a result, after roasting, the CI3-90 treatment sample experienced a more significant decrease in DPPH free radical scavenging activity owing to the more degraded polyphenolic compounds. According to Varady *et al.* [63], the value of the antioxidant activity of coffee beans depends on the content of antioxidant compounds and their balance in the formation and degradation during the roasting process. Therefore, further research is needed to examine the bioactive compound profile in untreated and acid-treated immature Robusta coffee beans to clarify the changes in total phenolic content and antioxidant activity.

Conclusions

This study revealed differences in the physicochemical characteristics between immature and mature Robusta coffee beans for example, immature beans tend to roast to a yellowish-brown color and have higher levels of chlorogenic acid, caffeine, and total soluble phenolics compared to mature beans. Acetic acid treatment can improve the cupping score and color of roasted immature coffee beans. Treating immature coffee beans with 3 % acetic acid for 90 min resulted in roasted bean colors that are close to the roasted color of mature coffee beans. In addition, immature coffee beans treated with 3 % acetic acid for 90 min had the highest cupping score. This indicates that there is an improvement in the color and flavor of immature coffee beans. Consequently, treatment 3 % acetic acid for 90 min was considered the best among the acetic acid treatments. Acetic acid lowered the pH of both green and roasted immature coffee beans. The lowest pH achieved by samples treated with 3 % acetic acid for 90 min, with a pH value for green beans of 4.57 and roasted beans of 5.31. Acetic acid treatment can lower trigonelline levels, but did not significantly impact caffeine and chlorogenic acid levels in green or roasted coffee beans. Additionally, it did not affect on total soluble phenolic content and antioxidant activity in immature green coffee beans. However, after roasting, total soluble phenolic content slightly decreased. Roasted immature

coffee beans treated with 3 % acetic acid for 90 min had the lowest antioxidant activity.

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