

Enhancing the Quality of Ready-to-Drink Robusta Coffee through Enzymatic Decaffeination, Cold Brew Techniques, and Sterilization

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ABSTRACT

Cold brew coffee has been reported to possess distinct sensory characteristics compared to hot brew. Therefore, this study aims to enhance the flavor of decaffeinated robusta cold brew coffee as a specialty coffee. The decaffeination process (using bromelain enzyme from pineapple), cold brew (immersion method), and sterilization techniques were used to examine the sensory qualities and physicochemical characteristics (color, pH, TDS, caffeine, chlorogenic acid/CGA, trigonelline, and volatile compounds) of decaffeinated robusta cold brew coffee and ready-to-drink decaffeinated robusta cold brew coffee. Medium-roasted robusta coffee was used in 2 different brewing methods, namely cold brew (T= 15 °C, 20 °C, 25 °C; t= 8, 10, 12 h) and hot brew as a control. The sensory analysis was conducted by trained panelists from Indonesian Coffee and Cocoa Research Institute using cupping technique. The Specialty Coffee American Association (SCAA) method was used to determine the flavor profile of a fine robusta cupping form. The results showed that the highest overall sensory score was obtained by decaffeinated cold brew robusta at 20°C for 8 hours. It contained 0.081 mg/100 g caffeine, 0.024 mg/100 g CGA, and 0.048 mg/100 g trigonelline. Specialty coffee could be defined as ready-to-drink cold brew coffee brewed at 20 °C for 10 hours, 25 °C for 8 hours, and 25 °C for 10 hours. Notes, such as brown sugar, honey, caramel, chocolate, and herbal, were enhanced by sterilization, while hot brew coffee presented a less favorable profile, scoring only 78.25.

Keywords: Robusta, decaffeinated coffee, cold brew, sterilization, specialty coffee

INTRODUCTION

The majority of global coffee production is obtained from *Coffea arabica* and *Coffea canephora* (ICO, 2023), and these varieties are considered the two most significant coffees in the global economy (Gloess et al., 2014; Ludwig et al., 2012). Indonesia is the world's fifth-largest producer of robusta and arabica coffee (USDA, 2023). Data from the Secretary General

Ministry of Agriculture within the previous five years also showed that South Sumatera, Lampung, Bengkulu, East Java, and Central Java are Indonesia's primary centers for robusta coffee production (Secretary General Ministry of Agriculture, 2023). Robusta is often chosen by local customers due to its greater caffeine level than arabica, leading to a stronger, more bitter flavor that is less acidic and more "earthy" or "woody." Due to its caffeine concentration, coffee can be harmful to people

with health issues, such as hypertension (Zhang et al., 2011). Consequently, decaffeination is often carried out to lower the caffeine level in coffee beans through the use of organic solvents, water, enzymes, or supercritical carbon dioxide (Park et al., 2024; Ramalakshmi & Raghavan, 1999).

Decaffeination can be done in various ways, one of which is by enzymatic means using protease enzymes obtained from natural materials such as pineapple fruit. Bromelain, derived from pineapple, is a proteolytic enzyme capable of destroying homogeneous proteins by hydrolyzing and disrupting gelatin gelling. A part of the molecular structure of caffeine is similar to protein because it has amide groups, leading to the ability of bromelain enzymes to reduce coffee caffeine levels (Fitzhugh et al., 2008; Hapsari et al., 2022). A previous study revealed that pineapple pulp can reduce caffeine levels in robusta coffee by up to 42.07% with the best treatment at a concentration of 10% and a fermentation duration of 24 hours (Pramadika, 2016). Decaffeinated coffee, which contains up to 97% less caffeine, is a popular alternative for people with hypertension. Several studies have also proven that decaffeinated coffee reduces the risk of hypertension (Palupi & Fatimah, 2021). Enzymes, such as bromelain, have emerged as a viable alternative to the previous decaffeination methods due to their ability to provide safer, more natural coffee, and preserve coffee flavor without leaving any chemical residue behind. This method aligns with the coffee market's current focus on flavor quality, sustainability, and health, particularly in ready-to-drink (RTD) products, such as cold brew.

According to previous studies, cold brew coffee has a 1:10 ratio of ground coffee to water and is brewed for 8-24 hours at a lower temperature (5-25°C) than hot brewing (Cordoba et al., 2020; Fuller & Rao, 2017; Portela et al., 2021) using direct contact (immersion) or indirect contact (dripping) (Cordoba et al., 2020). It has a unique profile that includes richness, chocolatey, fruity, and floral flavors, as well as moderate bitterness and acidity, because it is extracted under different conditions (Angeloni et al., 2019; Cordoba, 2020; Darmajana et al., 2022).

Cold brew coffee often contains less acidity and caffeine than hot brew coffee (Cordoba, 2020; Portela et al., 2021; Rao & Fuller, 2018). The temperature and time of the extraction process can affect the physicochemical and sensory qualities, producing beverages with various flavor profiles (Cordoba et al., 2019). Cold brew coffee has distinct physicochemical and sensory properties because it is brewed for an extended time at low temperatures. When this type of coffee is

made or frozen, it is usually consumed immediately. Many commercial suppliers have recently invested in cold-brewed coffee that is ready-to-drink and stored for long-term storage (Bellumori et al., 2021).

An effective method to extend shelf life is sterilization, which also enhances the flavor of the beverage, as shown by better sensory scores (Wang et al., 2020). Due to increased awareness of the various qualities and flavors of coffee varieties, specialty coffee has become more popular in recent years. Specialty coffee beans have been grown in a particular and ideal climate and have few defects, a distinct flavor, and taste.

Coffee beans are deemed "specialty" when of excellent quality and receive at least an 80 out of 100 rating from professional tasters known as Q-graders (SCAA, 2015). The flavor and aroma of specialty coffees are found to be the most common attributes, contributing to their high drink quality. Several studies have shown that the most common compounds are caffeine and nicotinic acid (Alcantara et al., 2021; Servín-Juárez et al., 2021). Compared to regular coffee, specialty coffee offers a unique experience due to its unique origin, growing practices, and sensory qualities (Lingle & Menon, 2017; Ramírez-Correa et al., 2020). High-quality components with flowery, fruity, citrusy, and occasionally fermented aromas distinguish specialty coffee. The specialty arabica market has been well-established for many years, despite the recent interest in *Canephora*.

Post-harvest processing and cultivation improvements have enhanced *Canephora* coffee quality in Brazil (Baqueta et al., 2024). Fermenting robusta coffee beans with kefir starter is another method to enhance their quality (Afriliana et al., 2018). Therefore, this aims to determine the effects of cold extraction temperature and duration on the physicochemical quality (color, pH, TDS, caffeine, CGA, trigonelline, and volatile compounds) and sensory characteristics of robusta coffee.

METHODS

Materials

Robusta coffee (*Coffea canephora*) samples were obtained from Temanggung (Central Java, Indonesia). A roasting machine was used to roast 2 kg of coffee beans, beginning at 60 °C and finishing at 210 °C after 15 minutes (medium roast). Furthermore, a chromameter was used to test the roasted beans' lightness value (L*), a*, b*, and ΔE. HPLC-grade acetic acid and methanol (Sigma-Aldrich, USA), as well as trigonelline, caffeine, and chlorogenic acid (CGA), were used as standards (TCI Chemical Industry, Japan).

Methods

Preparation of Decaffeinated Bean

A 50 g quantity of robusta coffee beans was incubated with the solution (1:3 w/v). The bromelain enzyme used had an enzyme activity of 20 U/g, which was used in its crude form, and the decaffeination process of robusta coffee beans was incubated using bromelain enzyme at 50 °C for 6 hours by a waterbath shaker (Mettmert WNB7L4). A cabinet dryer was then used to dry the material for 24 hours at 50 °C. The robusta beans were set aside to rest for 24 hours at 25±2 °C following a 15-minute medium-level roasting at 210 °C using a roaster (Cafemasy Coffee Roaster Electric, China), and all samples were in triplicate.

Coffee Preparation

Coffee beans were ground using an automatic grinder (Latina 600N, Taiwan) and then sieved to obtain 40-mesh grounds. A 50 g of coffee was soaked in 500 mL of filtered water with a 1:10 (w/w) coffee-to-water ratio in a glass jar at 15, 20, and 25 °C for 8, 10, and 12 hours to make cold brew coffee, then covered and shaken every 2 hours. The cold brew extract was brewed and then filtered using a coffee filter. Hot brew coffee was made in a glass jar with a 1:15 (w/w) coffee-to-water ratio. Furthermore, the coffee was brewed for approximately 5 minutes while the water was heated to 90 °C. Every sample was kept between 5 and 10 °C in a refrigerator, and was made 3 times.

Sterilization

The pilot plant retort was made of stainless steel that functioned on steam at 110 °C. In this study, the glass jar was filled by pouring the decaffeinated Robusta cold brew coffee extract into a 100 mL glass jar ($d = 5.35$ cm, $t = 7.18$ cm), leaving a headspace of about 1 cm at the top. The glass jar was placed vertically on a tray for full sterilization and heated for 40 minutes. Exhausting was carried out by heating the glass jar that had been pasteurized at 70 °C for 15 minutes, containing the product without being closed, at 100 °C for 15 minutes. This ensured that the air inside the jar was pushed out, then the jar was closed, and oxygen was removed to create a vacuum space in the headspace glass jar. Furthermore, this process could reduce the oxygen content in the headspace (Kusumaningrum et al., 2017). The jar was closed correctly to prevent leakage, which caused decay. To exhaust oxygen and produce a vacuum gap in the glass jar, the cold brew robusta decaffeinated extract was transferred to a 100 mL glass jar and heated to 100 °C for 10 minutes, then

sterilized in a modified horizontal retort at 110 °C for 40 minutes (Suryaningsih et al., 2022). Sterilized cold brew robusta decaffeinated coffee, ready-to-drink, was then stored between 0 °C and 5 °C after naturally cooling to room temperature.

Total Dissolved Solids (TDS) and pH

The TDS was analyzed by drying a 10 mL sample at 105 °C in an oven (Mettmert UN55, Germany) until its weight remained constant. Milligrams per 100 milliliters of coffee brew was the unit of measurement used to express the results. The TDS concentrations in equilibrium (obtained during the kinetic investigation) could be used to determine the extraction yield, taking into consideration the weight of the roasted and ground coffee necessary for preparation (Portela et al., 2021). A pH meter (Mettler Toledo FP20, Switzerland) was used to measure the pH. Furthermore, the pH of cold brew, sterile cold brew, and hot brew coffee was analyzed by dissolving 5 grams of ground coffee in 50 mL of distilled water for coffee beans or 50 mL of coffee drink sample at room temperature (25±2 °C). When the pH meter showed a constant value (pH=7), the electrode was dipped into the sample after calibrating the device with a buffer.

Determination of Color Attributes

A chromameter (Minolta CR-400, Japan) was used to measure color changes in cold brew coffee samples that were placed in a petri dish. The results were presented using the CIEL*a*b* technique, with a viewing angle of 10° and illuminant D65. In this study, the following variables had been identified, namely a* (negative values are green, positive values are red), b* (negative values are blue, positive values are yellow), and L* (lightness, L* = 0 [black], L* = 100 [white]). Color difference (ΔE) between hot and cold brew samples, and the ΔE equation could determine the difference between treated and control samples (Liu et al., 2019).

Measurement of Caffeine, Chlorogenic Acid, and Trigonelline

Quantification of trigonelline, caffeine, and chlorogenic acids (CGA) was performed using high-performance liquid chromatography (HPLC) equipped with an LC-20AD system (Shimadzu Corporation, Kyoto, Japan). The analytical procedure was adapted with minor modifications from a previously reported method (Córdoba et al., 2020). Detection was carried out using a UV-visible diode array detector at a wavelength of 278 nm. The concentrations of caffeine, CGA, and trigonelline were determined based on external calibration curves constructed from five standard concentrations of each

compound, with peak areas plotted against corresponding concentrations to generate linear regression equations. Quantitative analysis was conducted using regression equations derived from HPLC standard peak areas of trigonelline (purity $\geq 98\%$), CGA (purity $\geq 98\%$), and caffeine (purity $\geq 99\%$) obtained from Sigma-Aldrich (St. Louis, USA).

Standard solution and coffee extract analytical methods were adapted from TCI Chemical Industry, Japan.—Each analysis was conducted at 25 °C with 50% mobile phase A and 50% mobile phase B (A: 93% Aqua Pro Injection, 5% Methanol HPLC Grade (Merck, Darmstadt, Germany), and 2% Acetic Acid; B: 88% Methanol HPLC Grade (Merck-Hitachi, Darmstadt/Tokyo, Germany/Japan). The mobile phase mixture was attached to a Shim-pack GIST C-18, 5 μm column (15 cm \times 4.6 cm) (Shimadzu, Shim-pack, HPLC Packed Column) with a C-18 guard column. Furthermore, the mobile phase flow rate was 1.5 mL/minute, with an injection volume of 10 μL . Before injecting a 10 mL coffee sample into chromatography, the supernatant was filtered through a 0.22 μm filter membrane, and the sample was centrifuged at 10,000 \times 10g for 10 minutes. The levels of trigonelline, CGA, and caffeine were determined in milligrams per 100 milliliters of coffee.

Analysis of Sensory

The Ahmad Dahlan University Ethics Committee granted ethical approval for the sensory analysis (1041/IT3.KEPMSM-IPB/SK/2023) approach used in this investigation. Using the robusta cupping form based on the Specialty Coffee Association of America Coffee Cupping Form and cupping test method, 5 skilled panelists participated in sensory analysis of 9 sterile cold brew robusta decaffeinated and hot brew robusta decaffeinated beverages. The evaluation was conducted by the Indonesian Coffee and Cocoa Research Institute (ICCRI) in Jember, Indonesia. Furthermore, the coffee beans were brewed for approximately 5 minutes at a coffee-to-water ratio of 8.75g per 150 mL of water (hot brew).

Before conducting a sensory exam (cupping test), the panelists must sign an informed consent form. Cold brew robusta decaffeinated sterile was tested by opening the glass jar and testing it when it had reached room temperature. Hot brew robusta decaffeinated was also tested after brewing, when it reached room temperature. Subsequently, a sample was periodically given to the trained panellist. Before tasting the sample, panelists must complete the coffee brew attribute on the assessment form. Participants described or confirmed the sample's sensory characteristics on the assessment form (Dooley et al., 2010). A total of 8

sensory attributes, such as taste, aftertaste, mouthfeel, balance, bitter/sweet, salt/acid, fragrance/aroma, and overall, were included in the assessment form.

Analysis of Volatile Compounds

Conditions for aroma sampling were selected according to Liu et al. (2019). Trace 8890 Gas Chromatography apparatus and a Single-quadrupole Mass Spectrometer (Agilent, Switzerland) were used for the analysis. A 1.5 g sample was placed in a 20 mL GC headspace container (Sigma Aldrich, UK) measuring 22.5 mm \times 75.5 mm and capable of holding 3 samples. The samples were incubated at 40 °C for 5 minutes. An 80 μm DVB/CWR/PDMS SPME fiber (Agilent, Switzerland) and manual SPME were applied to extract volatile compounds. After 5 minutes of sample exposure, the fiber was thermally desorbed for 2 minutes at 200°C in splitless mode with a constant carrier pressure of 18 psi.

Separation was carried out using GC-MS5 using a 30 m HP-5MS capillary column (0.25 mm internal diameter, 0.25 μm film thickness, Agilent, Switzerland). After 5 minutes at 40 °C, the temperature program increased to 180 °C at a rate of 3 °C per minute, then increased to 240 °C and stayed there for 2 minutes at a rate of 8 °C per minute. Mass spectra in full scan mode were obtained in the 20 to 300 m/z mass range.

Data Analysis

All experiments were performed in triplicate. Data were analyzed using ANOVA followed by Duncan's multiple range test at a significance level of $p < 0.05$. Data's mean and standard deviation were shown, and all statistical studies, including principal component analysis (PCA), were conducted using Excel XLSTAT (Version 2017) or IBM® SPSS® Statistics version 25.0.

RESULT AND DISCUSSION

Sterilization

Exhausting was used to increase sterilization efficiency by making sure that hot steam reached all surfaces, removing trapped air that could obstruct heat transfer and impact the sterilization process and adjusting the retort's temperature and pressure to match the parameters required to kill microorganisms (Suryaningsih et al., 2022). The glass jar packaging was closed, and the samples were moved into a vertical, static, stainless steel laboratory retort with steam. Furthermore, the typical sterilizing temperature range was 110 to 121 °C, as a result, the retort condition was set at 110 °C for 40 minutes (Heinz & Hautzinger, 2007). Jars were sealed with special lids as packaging

material to sterilize foodstuffs at temperatures that were not as high as canning materials and without sudden temperature fluctuations (Deák, 2014), and the cooling process was carried out.

According to the acquired emulsion, the canned milk coffee beverages were sterilized using a retort sterilizer for 40 minutes at 110 °C, and the *Bacillus steraothermophilus* D value was 34.6 minutes at 110 °C (Ogawa & Cho, 2015). Following sterilization, sterile cold brew robusta decaffeinated was either naturally chilled to room temperature or cooled by air before being stored between 0 and 5 °C. An aseptic system combined several packaging methods that created a sterile environment by filling a sterilized container with a cooled, presterilized product and capping it with a sterile cover. Presently, the most used method of food preservation is thermal sterilization. Due to public health concerns about *Clostridium botulinum*, a spore-forming bacterium that produced a toxin that was lethal to humans even at extremely low concentrations, government organizations had imposed stringent regulations on thermal processing of low-acid canned foods (Ghani et al., 2001).

One of the greatest ways to preserve food was to sterilize it, which kept ready-to-eat meals healthy and safe for a long time at room temperature (Teixeira, 2014). In order to ensure the beverage's edible safety and extend its shelf life, sterilization was a crucial step in the production process (Wang, 2020). In order to be thermally inactivated, spore-forming bacteria that could thrive in low-acid diets needed temperatures significantly higher than the ambient boiling point of water. Furthermore, equipment systems that work under pressure, such as pressurized retorts used in canning for in-container sterilization, must be used to prepare low-acid foods (Teixeira, 2014). The nutritional content of food was lost during thermal sterilization, and the finished items' color, flavor, and texture were altered (Deák, 2014; Teixeira, 2014). Based on a previous study, the sterilizing and preserving of coconut milk affected sensory, specifically the generally sweet and nutty flavor of coconut milk, and had a favorable impact on it (Wang, 2020).

Color

Using the conventional method, the extracted decaffeinated cold brew robusta with longer brewing time and higher brewing temperature decreased the L* value while the a* and b* values increased (Table 1). This occurred due to more compounds, including tannins, pigments, and other components, dissolving in the liquid as brewing time increased, giving the beverage a darker, more intense brown color that

reduces brightness and raises red and yellow color values, increasing a* and b* values (Cai et al., 2022). While hot-brewed coffee (92 °C) yielded a deeper color, cold-brewed robusta decaffeinated coffee (22 °C) tended to be redder (Yeager et al., 2022). The differences could be attributed to various factors, including the type of compounds extracted during the process. This occurred due to melanoidin compounds that contributed to the brown color resulting from the heating process, including roasting and sucrose caramelization, which produced a brown-colored solution (Farah, 2012; Moreira et al., 2012).

pH Analysis

While the pH of hot brew without sterilization (control) was 5.21, the pH of cold brew robusta decaffeinated before sterilization ranged from 5.40 to 5.75 and after sterilization from 5.06 to 5.12 (Table 2). The pH of cold brew robusta ranged from 5.32 to 5.38 (Portela et al., 2021). Sterilization significantly affected the pH of cold brew robusta decaffeinated ($p < 0.05$). Almost all the samples showed that pHs significantly differed between the treatment before and after sterilization. However, the pH of cold brew robusta decaffeinated before sterilization was significantly different in 20 °C and 25 °C treatments for 8 hours. Although the pH range of 5 to 6 was generally steady during cold brewing, this discrepancy was a result of differing sample preparation and extraction techniques (Liang et al., 2024). Smoke chemicals evaporated, and acid characteristics decreased as a result of laborious, high-temperature disposal and sterilization procedures, and due to the breakdown of CGA. This was because of the thermal process that occurred during sterilization, leading to the hydrolysis of CGA into quinic and caffeic acids. A decrease in pH value could be caused by aliphatic acid compounds such as citric acid, malic acid, and formic acid, which were formed as a result of carbohydrate degradation during the roasting process and heat treatment (Fibrianto et al., 2018), including the breakdown of CGA into quinic acid and caffeic acid. This acid was produced when heating caused CGA to hydrolyze (Dawidowicz & Typek, 2017; Murda et al., 2016; Santanatoglia et al., 2024).

Total Dissolved Solids (TDS) Analysis

The results of TDS in this study were shown in Table 2. A notable variation in TDS occurred before and after sterilization, except for the extraction at 25 °C for 8 to 12 hours, with considerably different results ($p < 0.05$). The longer the samples were brewed and the higher the brewing temperature, the greater the TDS (Cordoba et al., 2020). This was consistent with the

Table 1. Color cold brew robusta decaffeinated before and after sterilization

Sample	8 hours				10 hours				12 hours				
	L*	a*	b*	ΔE	L*	a*	b*	ΔE	L*	a*	b*	ΔE	
Hot brew (control)	L: 17.06 ± 0.01 ⁱ												
	a*: 7.96 ± 0.03 ^j												
	b*: -0.54 ± 0.04 ^k												
	ΔE : 0												
15°C	Before sterili-zation	15.64 ± 0.21 ^{fg}	4.41 ± 0.12 ^{abcde}	0.03 ± 0.01 ^b	3.87 ± 0.13 ^{bc}	15.46 ± 0.02 ^{defg}	4.50 ± 0.17 ^{bcdef}	0.11 ± 0.03 ^{bc}	3.87 ± 0.17 ^{bc}	15.33 ± 0.05 ^{defg}	4.61 ± 0.03 ^{cdefg}	0.15 ± 0.06 ^{bc}	3.83 ± 0.08 ^{bc}
	After Sterili-zation	16.08 ± 0.12 ^h	4.17 ± 0.36 ^a	0.67 ± 0.12 ^{de}	4.18 ± 0.05 ^{de}	16.07 ± 0.21 ^h	4.20 ± 0.17 ^{ab}	0.70 ± 0.02 ^{de}	4.12 ± 0.05 ^{cde}	16.07 ± 0.55 ^h	4.24 ± 0.15 ^{ab}	0.86 ± 0.10 ^{ef}	4.13 ± 0.05 ^{cde}
20°C	Before sterili-zation	15.32 ± 0.09 ^{defg}	4.70 ± 0.08 ^{efg}	0.22 ± 0.10 ^{bcd}	3.77 ± 0.05 ^b	15.16 ± 0.03 ^{bcd}	4.71 ± 0.12 ^{efg}	0.23 ± 0.01 ^{bcd}	3.84 ± 0.06 ^{bc}	14.84 ± 0.01 ^{abc}	4.76 ± 0.06 ^{fg}	0.37 ± 0.01 ^{bcd}	4.00 ± 0.06 ^{bcd}
	After Sterili-zation	15.76 ± 0.14 ^{gh}	4.25 ± 0.12 ^{ab}	1.27 ± 0.26 ^{fg}	4.36 ± 0.05 ^{ef}	15.57 ± 0.22 ^{efg}	4.31 ± 0.21 ^{abc}	1.47 ± 0.41 ^{gh}	4.46 ± 0.05 ^{fg}	15.49 ± 0.08 ^{efg}	4.32 ± 0.08 ^{abcd}	1.66 ± 0.23 ^{gh}	4.56 ± 0.05 ^c
25°C	Before sterili-zation	14.79 ± 0.06 ^{abc}	4.79 ± 0.01 ^{fg}	0.44 ± 0.03 ^{bcd}	4.02 ± 0.05 ^{bcd}	14.76 ± 0.03 ^{ab}	4.82 ± 0.08 ^g	0.45 ± 0.08 ^{bcd}	4.02 ± 0.07 ^{bcd}	14.72 ± 0.02 ^a	5.23 ± 0.07 ^h	0.54 ± 0.05 ^{cde}	3.70 ± 0.05 ^b
	After Sterili-zation	15.21 ± 0.28 ^{cdef}	4.57 ± 0.34 ^{cdefg}	1.69 ± 0.63 ^d	4.52 ± 0.05 ^{fg}	15.21 ± 0.46 ^{cdef}	4.60 ± 0.17 ^{cdefg}	1.84 ± 0.53 ^h	4.57 ± 0.05 ^{fg}	15.03 ± 0.49 ^{abcd}	4.63 ± 0.06 ^{defg}	1.47 ± 0.03 ^{gh}	4.65 ± 0.05 ^g

Notes: a* (negative values = green and positive values = red), b* (b* negative values = blueness and b* positive values = yellowness), and L* (lightness, L* = 0 [black] and L* = 100 [white]).

Table 2. pH and total dissolved solids analysis

Sample	8 hours				10 hours		12 hours	
	pH*	TDS**	pH**	TDS**	pH**	TDS**	pH**	TDS**
Hot brew (control)	5.21 ± 0.01 ^e	0.213 ± 0.007 ^{hi}	-	-	-	-	-	-
15°C	Before sterilization		5.69 ± 0.01 ^j	0.180 ± 0.010 ^a	5.69 ± 0.01 ^k	0.186 ± 0.006 ^{ab}	5.70 ± 0.01 ^j	0.196 ± 0.006 ^{abc}
	After sterilization		5.08 ± 0.01 ^{abc}	0.203 ± 0.006 ^{bcd}	5.08 ± 0.01 ^{bc}	0.203 ± 0.003 ^{bcd}	5.08 ± 0.01 ^{abc}	0.210 ± 0.012 ^{cde}
20°C	Before sterilization		5.46 ± 0.01 ^g	0.213 ± 0.007 ^{cdef}	5.46 ± 0.01 ^j	0.216 ± 0.006 ^{defg}	5.63 ± 0.01 ⁱ	0.226 ± 0.021 ^{efghi}
	After sterilization		5.08 ± 0.01 ^g	0.223 ± 0.003 ^{efgh}	5.08 ± 0.01 ^{cd}	0.226 ± 0.005 ^{efghi}	5.06 ± 0.01 ^a	0.230 ± 0.008 ^{fghi}
25°C	Before sterilization		5.52 ± 0.01 ^h	0.233 ± 0.026 ^{ghi}	5.52 ± 0.01 ^h	0.240 ± 0.026 ^{hi}	5.40 ± 0.01 ^f	0.243 ± 0.006 ⁱ
	After sterilization		5.10 ± 0.01 ^d	0.236 ± 0.004 ^{hi}	5.11 ± 0.01 ^d	0.240 ± 0.002 ^{hi}	5.11 ± 0.01 ^d	0.243 ± 0.008 ⁱ

Notes: *pH: a logarithmic scale used to show how basic/acidic water solutions are; bases have a pH >7 and are statistically different (p<0.05), while acids have a pH <7. **Total Dissolved Solids (TDS) and significantly different (p<0.05)

Table 3. Cupping test, sterile cold brew robusta decaffeinated

Sample sterile cold brew	Final score	Notes
15 °C at 8 hours	78.40	Brown Sugar, caramelly, honeyed, sweet, chocolaty, cereally (corn), medium intense, herbal
15 °C at 10 hours	79.40	Brown Sugar, caramelly, sweet, chocolaty, cereally (corn), high intense, acidity, herbal
15 °C at 12 hours	77.20	Chocolaty, cereally, medium intense, acidity, smoky, harsh, baggy, strawy, medicinal oily, charcoal
20 °C at 8 hours	81.80	Brown sugar, caramelly, honeyed, sweet chocolaty, high intense, herbal, acidity
20 °C at 10 hours	81.45	Brown sugar, caramelly, sweet, chocolaty, medium intense, acidity
20 °C at 12 hours	77.35	Brown sugar, sweet, chocolaty, medium intense, acidity, bitter aftertaste, baggy, harsh, mushroom, strawy
25 °C at 8 hours	80.05	Brown sugar, honeyed, caramelly, chocolaty, cereally, low intense, acidity, herbal, strawy, baggy
25 °C at 10 hours	80.70	Brown sugar, chocolaty, caramelly, medium intense, acidity, herbal, strawy, baggy
25 °C at 12 hours	79.05	Caramelly, chocolaty, cereally, medium intense, low body, bitter and astringent aftertaste, strawy, baggy, herbal, oily
Hot brew	78.25	Brown sugar, chocolaty, caramelly, sweet corn, cereally, spicy, rubbery, herbal

expectation that TDS levels could increase with brewing time. In heat sterilization, the water in the sample evaporated faster, which increased the concentration of dissolved solids. Higher TDS values also affected the sensory character of brewed beverages as it positively related to bitterness, sourness, and astringency (Liang et al., 2024). A higher temperature influenced the saturation vapor pressure of fragrance chemicals and enhanced their solubility (Angeloni et al., 2019). The TDS content of cold brew robusta was 0.192-0.21 mg/100 mL (Portela et al., 2021).

Cold brew robusta extraction was impacted by brewing temperature and time conditions. Higher temperatures allowed for larger TDS extraction (Portela et al., 2021). The increased extraction efficiency was reflected in the nonconventional approach's higher TDS content compared to the standard method. Because nonconventional methods disturbed the cell wall and tissue, more water could enter the cell. Therefore, more dissolved substances could be able to flow through the cell membrane. The TDS value of cold brew extraction at 4 °C was larger than that of 93 °C since increasing the brewing temperature improved the extraction yield and solubility of all contained components as well as increased viscosity and body (Wang & Lim, 2021). Some chemicals or volatile compounds were decomposed or lost during sterilization, which caused variations in dissolved solids content. In other sterilization methods,

such as with chemicals, new compounds added to the solution, thus increasing TDS.

Sensory Analysis

The cupping test's PCA biplot separated the trained panelists' opinions of the brewed coffee samples' sensory profile (Figure 1). PCA showed clear differences between sterile cold brew robusta decaffeinated and explained 95.56% of the variability on 2 principal components (PCs). Principal component 1 (PC1) correlated positively with aroma, flavor, aftertaste, salt/acid, bitter/sweet, mouthfeel, balance, overall, and final score (87.34% variation). Meanwhile, samples at 15 °C for 8 hours, 15 °C for 12 hours, and 20 °C for 12 hours showed a negative correlation. The sample brewed at 15 °C for 10 hours showed a positive association with principal component 2 (PC2), accounting for 8.23% of the total variability, whereas the samples brewed at 25 °C for 12 hours and the hot brew (control) exhibited a negative correlation with PC2. This approach affected the sensory character values of the panelists. The coffee brew was made using Robusta Temanggung's sterilized cold brew and hot brew. While aftertaste was linked to smell, scent, and flavor, PCA data showed that overall characteristics and balance were significantly associated with the final score. Taste, aftertaste, salt/acid, bitter/sweet, mouthfeel, body, and balance were the sensory attributes of cold brew robusta decaffeinated with

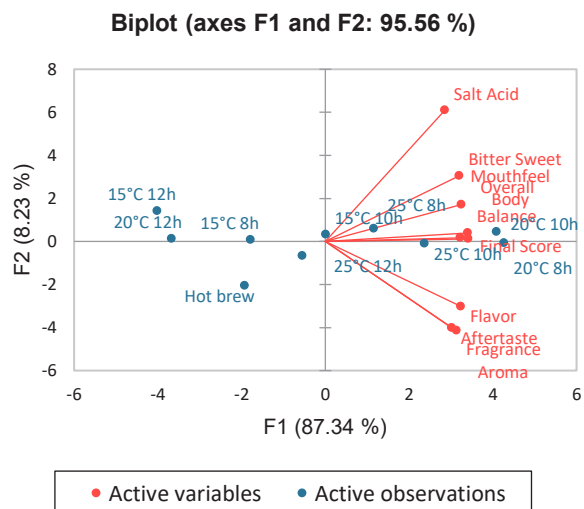


Figure 1. Sensory sterile robusta decaffeinated coffee in both cold brew and hot brew forms using PCA

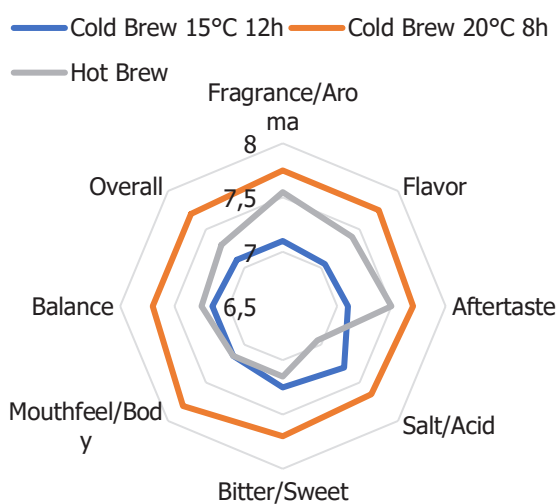


Figure 2. Spider web based on cupping test results on cold brew, sterile, and hot brew robusta decaffeinated coffee.

treatments of 20 °C for 8 hours and 10 hours and 25 °C for 8 hours and 10 hours, according to PCA analysis. Based on the Specialty Coffee Association of America Coffee, coffee with a final score of more than 80 could be categorized as specialty coffee because these sensory qualities were essentially the same throughout.

The sample treated at 20 °C for 8 hours received the highest final score (Figure 1 and Figure 2), with notes of brown sugar, honeyed, sweet, caramelly, chocolatey, acidic, herbal, and high intensity. Furthermore, sterilization had been shown to increase the final score when compared to the control (hot brew), which only obtained a score of 78.25 and had notes of caramelly,

sweet corn, chocolatey, cereally, brown sugar, and less desirable qualities like peppery, rubbery, and herbal (Table 3). Body, which was related to texture and viscosity, is the feeling of thickness, richness, and heaviness (SCAA, 2015). Even when it contained less desirable notes like herbs with relatively poor aftertaste, balance, flavor, mouthfeel, body, and overall values, the final ratings for the 10-hour treatment at 15 °C were over 80. The treatment at 25 °C for 12 hours provided the same sensory attributes as the hot brew (control), including the same smell, scent value, and overall flavor with notes of chocolate, cereal, caramel, and herbal. Samples from 15 °C 8 hours, 12 hours, and 20 °C 12 hours had lower aftertaste, mouthfeel, body, balance, fragrance, aroma, salt, acid, bitter, sweet, flavor, overall, and final score than the other samples. According to sensory tests, the 15 °C 12-hour exhibited less favored characteristics, including strawy, baggy, and abrasive, as well as medium-intense notes of chocolate, cereally, sweet, and brown sugar (Table 3).

Analysis of Trigonelline, Caffeine, and Chlorogenic Acid (CGA)

Due to a lower extraction temperature that removed less of these components, cold brew robusta decaffeinated had lower levels of caffeine, CGA, and trigonelline than hot brew. Roasted bean caffeine was the highest compared to green bean and cold brew coffee because roasting treatment increased caffeine content (Acquatucci et al., 2023; Baggenstoss et al., 2008; J. B. Park et al., 2023). Green beans exhibited the largest level of CGA, which then dropped after roasting and sterile cold brew robusta decaffeinated (Figure 3). This was due to CGA and trigonelline content in green beans, which decomposed after heat treatment (Awwad et al., 2021; Badmos et al., 2019). The beverage's nutty aroma was from the breakdown of trigonelline during roasting, which

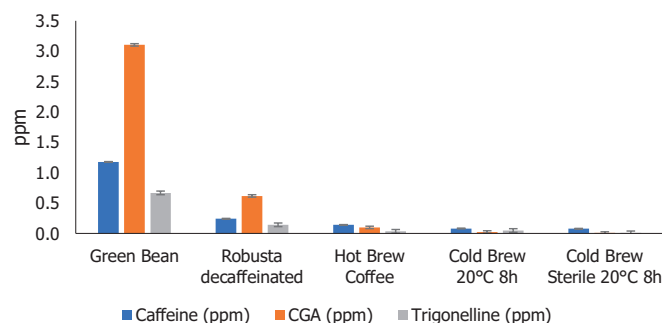


Figure 3. Green beans, hot brew robusta decaffeinated coffee, cold brew robusta decaffeinated coffee, and sterile cold brew robusta decaffeinated coffee all include caffeine, trigonelline, and CGA

released nicotinic acid and other volatile substances such as pyridine and pyrrole (Kalaska et al., 2014).

Examination of Volatile Substances

SPME-GC-MS was the most frequently used method to analyze the decaffeinated sterile cold brew robusta's flavor profile (Caporaso et al., 2022). According to the results of the GC-MS study of volatile chemicals (Figure 4), alcohols, carboxylic acids, and pyridines were the most prevalent compounds in green beans. Aromatics were the most prevalent compound groups in hot-brewed robusta decaffeinated coffee, aldehydes, pyrazines, and pyrroles were the most prevalent groups in sterile cold brew robusta decaffeinated coffee (20 °C for 8 hours), and ketones and furans were the most prevalent groups in hot-brewed robusta decaffeinated coffee.

The flavor was a multimodal experience with many closely integrated and mutually reinforcing sensory aspects (mostly tastes and smells) (Chiralertpong et al., 2008). Esters were byproducts of the interaction between alcohols and carboxylic acids, and the Strecker reaction and fat oxidation could produce alcohols (Cui et al., 2020). A total of 19 chemicals, comprising 6 aldehydes, 1 alcohol, 2 pyrazines, 1 aromatic, 3 ketones, 1 pyridine, 2 pyrroles, 1 furan, and 1 carboxylic acid, were identified and measured in the HS-SPME study of volatile ingredients in decaffeinated sterile cold brew. Compared to robusta green bean and roasted bean, there was an increase in several compounds in sterile cold brew robusta decaffeinated, such as aldehydes, pyrazines, ketones, and pyrroles. In cold brew coffee, odor-active substances were discovered, including furans, pyrazines, ketones, pyrroles, aldehydes, and esters (Cordoba et al., 2019).

Aldehyde compounds such as 2-methylbutanal, 3-methylbutanal, and benzaldehyde contributed sweet, bitter, almond, and malty flavors, while hexanal contributed vegetable and green flavor (Cordoba et

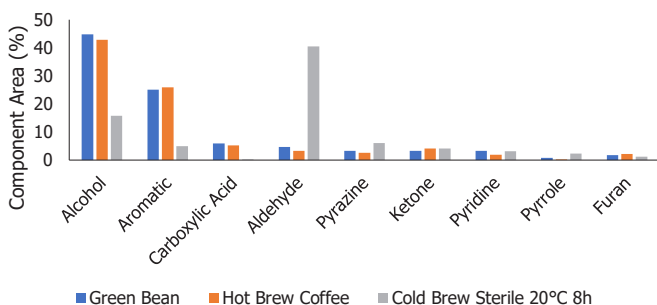


Figure 4. GC-MS analysis of green bean, hot brew robusta decaffeinated coffee, and sterile cold brew robusta decaffeinated coffee

al., 2019; Bellumori et al., 2021; Narváez et al., 2024). Pyrazine, including pyrazine and methyl pyrazine, was a contributor to nutty, roasty, sweet corn, chocolate, spicy, and green aromas (Cordoba et al., 2019; Bellumori et al., 2021; Narváez et al., 2024). This was consistent with earlier studies that found furans and pyrazines to be the most prevalent volatile ingredients in coffee beverages. The high concentration of these volatile component groups in the samples explained the panelists' impressions of the hot and cold brew coffee's sweetness, caramelly, fruity, roasted, and earthy scent and taste (Córdoba et al., 2021).

Ketones and aldehydes were the major volatile compounds found in cold brew. Ketones such as ethanone,1-(2-furanyl), 2-3-Pentanedione, and acetone contribute to sweet, cocoa, buttery, caramel-like, and fruity odors (Bressanello et al., 2017; Bellumori et al., 2021; Narváez et al., 2024). Pyridine added smells of burnt coffee, smoky coffee, and sour coffee (Heo et al., 2020). Coffee drinks also contained pyrroles, a class of volatile compounds that included 1H-Pyrrole-2-carboxaldehyde 1-methyl. Coffee, musty, smoky, spicy, and phenolic smells were caused by these pyrroles (Heo et al., 2020; Narváez et al., 2024). Pyridine and pyrrole were produced from the degradation of trigonelline and the Maillard reaction, which had a very weak bitter taste (Heo et al., 2020; Narváez et al., 2024). Compounds that decreased in sterile cold brew robusta decaffeinated, such as alcohols, aromatics, furans, and carboxylic acids. 2-Furanmethanol contributes to sweet and caramel flavor (Bellumori et al., 2021). Furan contributed to phenolic, malty, and sweet aromas (Cordoba et al., 2019; Cai et al., 2022). The polarity of chemical components and the extraction rate were strongly correlated. Chemicals with stronger polarity, such as alcohols and carboxylic acids, percolated more in hot coffee beverages because of their shorter carbon chains. These chemicals quickly diffused in the nasal canal, increasing the strength and intensity of the coffee fragrance in hot liquids (Cordoba et al., 2019). Carboxylic acids such as acetic acid contributed to the sharp, pungent, sour, and vinegar flavor (Narváez et al., 2024).

CONCLUSION

In conclusion, this study demonstrated that temperature and extraction time play a significant role in shaping the physicochemical and sensory attributes of decaffeinated Robusta cold brew coffee. As both brewing temperature increases (15 °C to 25 °C) and extraction time lengthens (8 to 12 hours), notable changes are observed in color parameters (a decrease in lightness (L^*) and an increase in a^* and b^* values),

pH (which decreased), and TDS (which increased). These changes show more intense color and higher solute extraction at higher temperatures and longer durations. The levels of bioactive compounds, such as CGA, and trigonelline, were affected by both brewing parameters and the sterilization process. Extended extraction time and higher temperature lead to a more pronounced reduction in these compounds, particularly after sterilization. From a sensory perspective, the most favorable results were achieved at 20 °C and 25 °C for 8 to 10 hours, with cupping scores exceeding 80 points, qualifying it as specialty coffee. These samples feature rich sensory notes such as brown sugar, honey, caramel, chocolate, and herbal flavors, with improved overall balance, mouthfeel, and aftertaste compared to the hot brew control. The present study confirms that optimizing cold brew parameters, specifically extraction temperature and time, combined with enzymatic decaffeination and sterilization, can significantly enhance the quality and market potential of ready-to-drink decaffeinated robusta coffee.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this manuscript.

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