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Original article

Caffeine and Chlorogenic acids Concentrations and Organoleptic Test of Robusta Beans (*Coffea canephora*) Fermented with Microbial Starters

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Abstract

Robusta beans contain higher caffeine and chlorogenic acid than Arabica beans. Caffeine and chlorogenic acid were responsible for the bitter flavor of brewed coffee. Microbes could modify the caffeine and chlorogenic acid concentrations of coffee beans. The purposes of this study were to determine caffeine and chlorogenic acid concentrations of Robusta beans fermented with commercial microbial starters, i.e., tape, bread, and tempeh starters, and to determine respondents' preference for their Robusta brewed coffee. Tugusari variety of Robusta beans were fermented aerobically with tempeh starters and anaerobically with tape and bread starters at 28 °C for 6 days. Robusta beans from 1-, 2-, 3-,4-, 5-, and 6-days of fermentation and control (0-day of fermentation) were analyzed for caffeine and chlorogenic acid concentrations by UV-Vis spectrophotometer. Brewed coffees from Robusta beans of the 6th day of fermentation of each starter and the control were tested organoleptically, including scores for color, aroma, viscosity, acidity, sweetness, bitterness, and overall flavor. The highest decreases in both caffeine and chlorogenic acid concentrations were obtained from Robusta beans fermented with tempeh starters. Bread starters fermentation on Robusta beans produced the most fragrant aroma of brewed coffee, while tempeh starters fermentation on Robusta beans produced the least bitterness of brewed coffee. The overall flavor of brewed coffee from Robusta beans fermented with bread and tempeh starters was the most preferred by the respondents.

Keywords: caffeine, chlorogenic acid, microbial starters, organoleptic test, robusta beans

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Introduction

Coffee plants are found in Asia, Africa, South America, and Australia. There are more than 100 species of coffee plants. Coffee plants (*Coffea* sp) are including Rubiaceae family. *Coffea* sp produce beans with economic value, i.e., Arabica beans (*C. arabica*), Robusta beans (*C. canephora*), Excelsa beans (*C. excelca*), and Liberica beans (*C. liberica*). However, only two bean varieties dominated global trade, i.e., Arabica and Robusta beans (Farah, 2012; USDA, 2025). The traded coffee beans are green and roasting (International Coffee Organization, 2025). Although the economic value of Arabica beans is higher than that of Robusta beans, the production costs of Robusta beans were cheaper (Ardhiarisca *et al.* 2022).

Coffea arabica and *C. canephora* are simple to cultivate. *Coffea arabica* is grown at elevations above 1,000 m, while *C. canephora* is grown at elevations of 400-900 m (Maryuna *et al.* 2022; Silalahi & Rosyadi, 2024). *Coffea canephora* is more resistant to climate change, diseases, and pest attacks than *C. arabica* (Syaifurrahman & Akbar, 2021). Resistance to pests and diseases resulted in *C. canephora* being widely distributed in Indonesia. Therefore, Indonesian coffee beans are dominated by Robusta beans.

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The mature coffee cherries are yellow or red. Coffee cherries consist of pulp and beans. The pulp consists of exocarp, mesocarp, and endocarp. Coffee beans consist of hard parchment, soft silver skin, and embryo (beans). Coffee beans consist of two cotyledons, but some beans have a cotyledon. The bean of *C. arabica* is oval, large, and has a curved center groove, while the bean of *C. canephora* is round, small, and has a straight center groove.

The Indonesian government, through the Coffee Research Institute, has been developing Arabica and Robusta varieties. These varieties include advantages in production capacities and resistance to pests and diseases. Arabica varieties include Kartika, Abyssinia, Andungsari, Sigarar Utang, Gayo, Kopyol Bali, and Komasti. Robusta varieties include Sintaro, Korolla, Sehasence, BP, and SA (Randriani and Dani, 2018). Furthermore, there are local Robusta varieties, including Sidikalang, Lampung, Flores, Temanggung, Pupuan, Toraja, and Jember (Tugusari/BP534). The Tugusari variety has been grown outside Jember, including in Lampung (Evizal *et al.* 2015) and Temanggung (Erdiansyah & Wahono, 2016).

Caffeine and chlorogenic acid are the main phenolic compounds of coffee beans. Caffeine is an alkaloid that is synthesized from xanthosines, a purine nucleotide acid derivative. Caffeine is responsible for the bitter flavor of brewed coffee (Flament, 2002; Poole and Tordoff, 2017). Chlorogenic acid is an ester of hydroxycinnamic acid and quinic acid. The main hydroxycinnamic acids of chlorogenic acid are coumaric, ferulic, caffeic, and sinapic acids. Chlorogenic acid is an astringent that strengthens the bitter flavors of brewed coffee (Purwoko, 2023). Furthermore, sweetness and sour flavors are produced by the

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Maillard reaction between chlorogenic acid and melanoidins. The Maillard reaction is produced by the caramelization of sugars and amino acids (Vignoli *et al.* 2011).

In addition to the processing and roasting methods, the flavors of brewed coffee also depend on the species, variety, and cultivation conditions. Since Arabica beans contain less caffeine and chlorogenic acid, their brewed coffee is sweet and slightly bitter (Agustini *et al.* 2022). According to Dani *et al.* (2013), the differences in brewed coffee flavors are caused by the differences in coffee varieties. The flavors of brewed coffee also depended on the elevations (Purba *et al.* 2020) and the species of screen plants (Sakiroh *et al.* 2013). The physicochemical profile of the soil could produce complex flavors (Supriyadi *et al.* 2016). Besides its acidity, consumers prefer Arabica beans due to their sweetness and slightly bitter flavors.

Microbial fermentation on coffee beans could affect the flavors of brewed coffee. These microbes could originate from the cherries or beans (endogenous) or from the outside (external). According to Junqueira *et al.* (2019), coffee cherries contained various microbial species that could ferment coffee cherries or beans. Endogenous microbial fermentation occurred from the time the coffee cherries ripened until become coffee beans. Significant endogenous microbial fermentation occurs during the wet and semi-dry processing methods of coffee cherries into coffee beans (Evangelista *et al.* 2015). External microbial fermentation can use a single or mixed cultures (Afriliana *et al.* 2019; Purwoko *et al.* 2022).

Microbes synthesize enzymes for the degradation of caffeine and chlorogenic acid, resulting in a lower concentration of caffeine and chlorogenic acid in coffee beans (Tawali *et al.* 2018; Purwoko *et al.* 2022). Furthermore, microbes also produce enzymes to modify caffeine and chlorogenic acid into other organic acids, resulting in flavor characteristics (Bressani *et al.* 2020). A previous study showed that respondents preferred the flavors of brewed coffee from beans fermented with yeast *S. cerevisiae* compared to the unfermented coffee beans (Purwoko, 2023).

Microbial fermentation can use commercial microbial starters. There were several studies that used commercial starters for food fermentation. In bread production, yeast *Saccharomyces cerevisiae* is used to ferment the dough anaerobically. A bread starter consisting of *S. cerevisiae* could be used to ferment cocoa beans anaerobically (Syauqi *et al.* 2022). Tempeh starter was used for an aerobic fermentation of coffee beans (Yusianto & Widyotomo, 2013). Anaerobic fermentation of tape starters applied to cocoa beans (Fitriani *et al.* 2024). Other commercial starters, such as yogurt starters, could be applied to coffee bean fermentation (Tawali *et al.* 2018). Commercial starters fermentation on coffee beans could modify the flavors of brewed coffee (Yusianto & Widyotomo, 2013) and could produce specialty Robusta beans (Khotijah, 2018). Therefore, commercial microbial starters, such as tape, bread, and tempeh starters, can be applied to ferment Robusta beans and modify the flavors of brewed coffee. The objectives of this study were to determine the caffeine and chlorogenic acid concentrations of Robusta beans fermented with tape, bread, and tempeh starters, and to determine respondents' preferences for brewed coffee from Robusta beans fermented with tape, bread, and tempeh starters.

Methods

Materials

The chemicals in this experiment consisted of caffeine, chlorogenic acid, dichloromethane, and oxygen quenchers. These chemicals were obtained from E-Merck Indonesia. Tugusari variety of Robusta beans were obtained from Karangpring, Sukorambi, Jember, East Java, Indonesia. Commercial starters, i.e., tape starters (NKL brand), bread starters (Fermipan brand), and tempeh starters (Raprima brand) were obtained from traditional markets in Surakarta, Central Java, Indonesia.

Tape starters consisted of bacteria, yeasts, and molds (Muhiddin *et al.* 2019). Bacteria in tape starters were Lactic Acid Bacteria (LAB) and *Enterobacteriaceae* (Gultom, 2016). Yeasts in tape starters were *Kluyveromyces* sp, Pichia sp, and *Saccharomyces* sp (Daniati *et al.* 2023). Molds in tape starters were *Rhizopus* sp and *Mucor* sp (Wardani *et al.* 2022). Bread starters consisted of yeast *S. cerevisiae*. Tempeh starters consisted of mold *R. oligosporus* (Yarlina *et al.* 2023).

Experimental Design

The caffeine and chlorogenic acid analysis was conducted using a factorial design with three replications. Two factors were starter treatments and fermentation day treatments. The starter treatments were tape, bread, and tempeh starters. The fermentation day treatments were 1-, 2-, 3-, 4-, 5-, and 6 days of fermentation; and a control (0 days of fermentation). The organoleptic tests were conducted using a completely randomized design with starter treatments, i.e., tape, bread, and tempeh starters, and a control (without starter fermentation). The organoleptic tests required 20 replications (respondents) to achieve data normality.

Coffee Beans Fermentation

The method of Robusta beans fermentation was carried out according to Pangastuti *et al.* (2024). Dried Robusta beans (2 kg) were soaked in hot sterile water (80 °C) for 1 hour. After draining, 1,890 g of Robusta beans were placed in 63 petri dishes and then sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the samples were divided into 3 groups. The first group was Robusta beans (30g) inoculated with tape starters (300 mg) and their control. The second group was Robusta beans (30g) inoculated with bread starters (300 mg) and their control. The third group was Robusta beans (30g) inoculated with tempeh starters (300 mg) and their control. The groups of tape and roti starters were stacked in anaerobic jars (Himedia LE003, India), except for the control. After the addition of oxygen quenchers, they were placed in an incubator (Memert IN110, Germany). The anaerobic fermentation was carried out at 28 °C for 6 days. The group of tempeh starters was placed in an incubator (Memert IN110, Germany), except for the control, and aerobic fermentation was carried out at 28 °C for 6 days. All samples,

including the controls, were dried (50 °C for 5 days) and then stored in a refrigerator at 4 °C before the extraction.

Coffee Beans Extraction

Extraction of coffee beans was carried out according to Purwoko (2023). Samples (15 g) of dried Robusta beans (1-, 2-, 3-, 4-, 5-, and 6-days of each starter's fermentation) and the control (0 days of fermentation) were ground using a blender (Philips, Indonesia). The coffee grounds were poured into an Erlenmeyer flask and extracted with 60 mL of sterile distilled water for 2 hours at 80 °C. After filtering, the filtrates were partitioned with 100 mL of dichloromethane in a separation funnel. The aqueous and dichloromethane phase samples were separated and placed in the bottles, and then stored in a refrigerator at 4 °C before analysis of caffeine and chlorogenic acid concentrations.

Analysis of Caffeine and Chlorogenic Acid Concentrations

Analysis of caffeine and chlorogenic acid concentrations of Robusta beans was carried out according to Navarra *et al*. (2017) using a UV-Vis spectrophotometer (Hitachi UH5300, Japan). Aqueous phase samples (3 mL) were analyzed for caffeine concentration at 275 nm (OD_{275}), while dichloromethane phase samples (3 mL) were analyzed for chlorogenic acid concentration at 324 nm (OD_{324}). The OD_{275} and OD_{324} values were converted into caffeine and chlorogenic acid concentrations based on standard curves of caffeine and chlorogenic acid.

Coffee Grounds Preparation

Coffee grounds preparation was carried out according to Tyas (2022). Samples (100 g) of dry Robusta beans of 6-days of each starter's fermentation and control (without starters) were roasted with a coffee roaster (Rockwood, China) at 220°C until the beans exposed their first crack and their color turned dark brown, resulting in medium-dark roast coffee beans. Samples of roasted coffee beans were ground with a coffee grinder (Latina T60, China) to obtain coarse ground coffee. The ground coffee beans were stored in the bottles at room temperature (±30 °C).

Organoleptic Tests of Brewed Coffees

Brewed coffees were prepared using the French Press method (Tamara, 2025). Ground coffee beans (83 g) were brewed with 1 L of sterile water for 5 minutes at 95 °C. The brewed coffees were filtered and poured into 20 cups, then subjected to organoleptic tests by 20 respondents. The organoleptic tests of the brewed coffees included color, viscosity, aroma, acidity, sweetness, bitterness, and overall flavor. The respondents were 20-25 years old and consumed a cup of brewed coffee a day. After tasting a cup of brewed coffee, the respondents rinsed their mouths with water and then tasted another brewed coffee. Respondents expressed their preferences for the organoleptic tests with a 5-point score (1 = dislike; 2 = somewhat like; 3 = like; 4 = like very much; 5 = like extremely).

Data Analysis

Caffeine and chlorogenic acid data were statistically analyzed using factorial ANOVA (SPSS version 18; p=0.05), while organoleptic score data of brewed coffees (color, viscosity, aroma, acidity, sweetness, bitterness, and overall flavor) were analyzed using one-way ANOVA (SPSS version 18; p=0.05). Further analysis was conducted when the analyses showed significant differences (p<005). The post hoc analysis of factorial ANOVA was conducted conservatively (pairwise) using the Tukey-HSD test. One-way ANOVA was conducted non-conservatively (multi-range) using the Duncan post hoc test (Agbangba *et al.* 2024).

Results and Discussion

The amounts of all microbial starters in our experiment were 1% of Robusta beans. Ninsix (2013), Banoet *et al.* (2024), and Yulia *et al*. (2019) reported that 1% of tape, bread, and tempeh starters produced a good tape, bread dough, and tempeh. Therefore, our study applied 1% microbial starters for Robusta bean fermentation. The total microbial populations in the tape, bread, and tempeh starters were $1.6x10^8$, $7.5x10^8$, and $5.7x10^7$ cfu/mg. The difference between populations of microbial starters did not affect the visualization of Robusta bean fermentation. Brand selection of microbial starters based on the brand's availability in the traditional market. Therefore, brands of NKL, Fermipan, and Raprima were used in our experiment.

Spectrophotometry analysis for caffeine and Chlorogenic acid corresponded with high-performance liquid chromatography (HPLC) analysis. According to Navarra *et al*. (2017), the spectrophotometry method was not significantly different from the HPLC method for analyzing caffeine and chlorogenic acid concentrations. Therefore, they suggested a spectrophotometer method for rapid, accurate, and economical analysis of caffeine and chlorogenic acid concentrations.

Caffeine Concentrations of Robusta Beans

The caffeine concentrations of Robusta beans fermented with each microbial starter and on each day of fermentation are shown in Table 1. The caffeine concentrations of the Tugusari variety of Robusta beans (control) were 19.90–20.40 mg/g. This result corresponded to the caffeine concentrations reported for Robusta beans by Navarra *et al.* (2017), i.e., 17–30 mg/g. The caffeine concentrations of the Robusta beans fermented with all microbial starters decreased with increasing fermentation days. After 6 days of fermentation, the caffeine concentrations of Robusta beans fermented with tape, bread, and tempeh starters decreased to 16.13±1.09, 16.24±0.95, and 13.83±1.31 mg/g, respectively. These due to the caffeine degradation activities of microbes in the starters. Bacteria and yeasts grew well under anaerobic conditions, and molds grew well under aerobic conditions (Hasseltine et al. 1985). Therefore, Robusta beans fermented with tape and bread starters were under anaerobic conditions, and Robusta beans fermented with tempeh starters were under aerobic conditions. The caffeine concentrations of Robusta beans fermented with tempeh starters for 6 days of

Table 1. Caffeine concentrations of Robusta beans fermented with microbial starters

No	Fermentation days	Tape starters fermentation (mg/g)	Bread starters fermentation (mg/g)	Tempeh starters fermentation (mg/g)
1.	Control	19.90±1.69^{a,x}	20.40±0.69^{a,x}	20.24±0.59^{a,y}
2.	1-day	19.57±1.04^{a,x}	20.41±0.83^{a,x}	19.44±0.71^{a,y}
3.	2-days	19.19±0.93^{a,x}	19.57±0.81^{a,x}	17.92±1.35^{a,y}
4.	3-days	18.70±1.82^{b,x}	19.01±1.75^{b,x}	17.04±1.71^{b,y}
5.	4-days	17.74±2.11^{b,x}	17.71±1.42^{b,x}	15.77±1.66^{b,y}
6.	5-days	17.04±0.45^{c,x}	16.83±0.27^{c,x}	14.23±1.51^{c,y}
7.	6-days	16.13±1.09^{c,x}	16.24±0.95^{c,x}	13.83±1.31^{c,y}

Note: $^{a-c}$: Different letters showed significantly different results in each column ($p<0.05$)
$^{x-y}$: Different letters showed significantly different results in each row ($p<0.05$)

fermentation were lower than those with tape and bread starters. This is due to the degradation of caffeine activity by molds in tempeh starters, which was greater than that by bacteria, yeasts, and molds in tape and bread starters. According to Nonthakaew *et al*. (2015), caffeine was toxic to microbes. Therefore, caffeine in Robusta beans did not affect microbial growth in the microbial starters. Microbes in tape, bread, and tempeh starters degraded caffeine by 19%, 20%, and 32%, respectively.

Statistical analysis showed that the caffeine concentrations of Robusta beans fermented with microbial starters for 5 and 6 days of fermentation were significantly different from those of the 3 and 4 days of fermentation ($p<0.05$), and 3 and 4 days of fermentation were significantly different from those of the 0, 1, and 3 days of fermentation ($p<0.05$). This indicated that microbes in the starters significantly degraded caffeine every 2 days of fermentation. Statistical analysis also showed that the caffeine concentrations of Robusta beans fermented with tempeh starters were significantly different from those of the tape and bread starters ($p<0.05$). This indicated that molds in tempeh starters degraded caffeine more than bacteria, yeasts, and molds in tape and bread starters.

The daily degradation of caffeine by tempeh starters was high at 2 and 5 days of fermentation, and by tape and bread starters was high at 4 days of fermentation (Figure 1). This showed that caffeine degradation activity by molds in tempeh starters reached maximum at 2 days of fermentation. Bacteria, yeasts, and molds in tape and bread starters reached maximum at 4 days of fermentation. The caffeine degradation activity is due to the activity of caffeine-degrading enzyme, i.e., caffeine demethylase (Mills *et al.* 2021). This enzyme degrades caffeine by removing the methyl group (Kim *et al*. 2019). Microbes in tape, bread, and tempeh starters might synthesize caffeine demethylase for caffeine degradation.

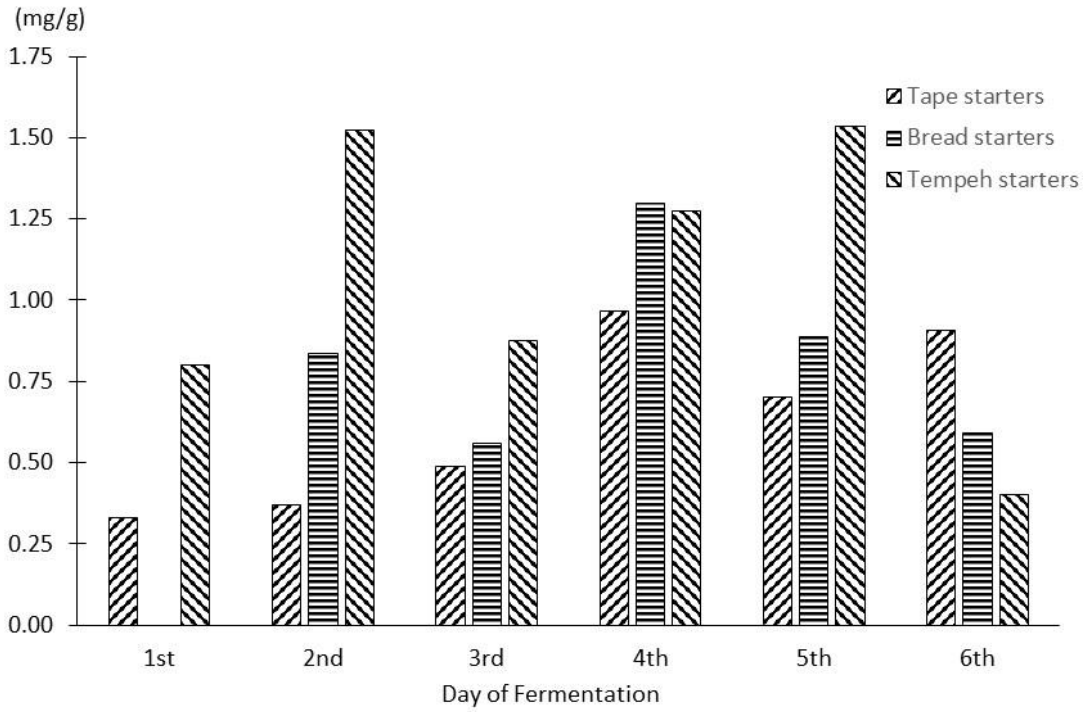

Fig. 1. Daily caffeine degradation by microbial starters during fermentation of Robusta beans

Chlorogenic Acid Concentrations of Robusta Beans

The chlorogenic acid concentrations of Robusta beans fermented with each microbial starter and on each day of fermentation are shown in Table 2. The chlorogenic acid concentrations of the Tugusari variety of Robusta beans (control) were 74.91–75.56 mg/g. These chlorogenic acid concentrations corresponded to those reported by Navarra *et al*. (2017), i.e., 59–151 mg/g. The chlorogenic acid concentrations of the Robusta beans fermented with all microbial starters decreased with increasing fermentation days. After 6 days of fermentation, chlorogenic acid concentrations of Robusta beans fermented with tape, bread, and tempeh starters were 55.16±3.93, 53.42±3.00, and 46.31±1.95 mg/g, respectively. These due to the chlorogenic acid degradation activities of microbes in the starters. The chlorogenic acid concentrations of Robusta beans fermented with tempeh starters for 6 days of fermentation were lower than those with tape and bread starters. This is due to the degradation of chlorogenic acid activity by molds in tempeh starters, which was greater than that by bacteria, yeasts, and molds in tape and bread starters. A previous study reported that chlorogenic acid affected pathogens (Lou *et al*, 2011). However, our study used non-pathogenic microbes. Therefore, chlorogenic acid did not affect their growth, and the microbes degraded chlorogenic acid in the Robusta beans. Microbes in tape, bread, and tempeh starters degraded caffeine by 27%, 29%, and 38%, respectively.

Statistical analysis showed that the chlorogenic acid concentrations of Robusta beans fermented with microbial starters for 5 and 6 days of fermentation were significantly different from those of the 3 and 4 days of fermentation ($p<0.05$), and 3 and 4 days of fermentation were significantly different from those of the 0, 1, and 3 days of fermentation ($p<0.05$). This indicated that microbes in the starters significantly degraded chlorogenic acid every 2 days of fermentation. Statistical analysis also showed that the chlorogenic acid concentrations of Robusta beans fermented with tempeh starters were significantly different from those of the tape and bread starters ($p<0.05$). This indicated that molds in tempeh starters degraded chlorogenic acid more than bacteria, yeasts, and molds in tape and bread starters. A previous study showed that the mold *R. oryzae* degraded more caffeine than yeast *S. cerevisiae* and Lactic Acid Bacteria (Purwoko *et al.* 2022). The daily degradation of chlorogenic acid by tape, bread, and tempeh starters was high at 4 days of fermentation (Figure 2). This showed that caffeine degradation activity by microbes in the starters reached maximum at 4 days of fermentation. The chlorogenic acid degradation activity is due to the activity of chlorogenic acid-degrading enzyme, i.e., cinnamoyl esterase (Bel-Rhlid *et al.* 2013). This enzyme degrades chlorogenic acid into quinic acid and caffeic acid (Collombel *et al*. 2019). Microbes in tape, bread, and tempeh starters might synthesize cinnamoyl esterase for chlorogenic acid degradation.

Table 2. Chlorogenic acid concentrations of Robusta beans fermented with microbial starters

No	Fermentation days	Tape starters fermentation (mg/g)	Bread starters fermentation (mg/g)	Tempeh starters fermentation (mg/g)
1.	0-day (control)	$75.10\pm10.77^{a,x}$	$74.91\pm6.31^{a,x}$	$75.11\pm10.09^{a,y}$
2.	1-day	$73.85\pm 9.58^{a,x}$	$72.42\pm6.88^{a,x}$	$69.92\pm 7.43^{a,y}$
3.	2-days	$71.17\pm 9.88^{a,x}$	$68.67\pm6.80^{a,x}$	$64.27\pm 6.03^{a,y}$
4.	3-days	$66.66\pm11.74^{b,x}$	$65.86\pm7.35^{b,x}$	$57.24\pm 2.70^{b,y}$
5.	4-days	$57.96\pm 5.91^{b,x}$	$58.10\pm6.10^{b,x}$	$52.07\pm 3.78^{b,y}$
6.	5-days	$56.35\pm 6.63^{c,x}$	$55.70\pm4.53^{c,x}$	$48.43\pm 1.09^{c,y}$
7.	6-days	$55.16\pm 3.93^{c,x}$	$53.42\pm3.00^{c,x}$	$46,31\pm 1.95^{c,y}$

Note: $^{a-c}$ Different letters showed significantly different results in each column ($p<0.05$)
$^{x-y}$ Different letters showed significantly different results in each row ($p<0.05$)

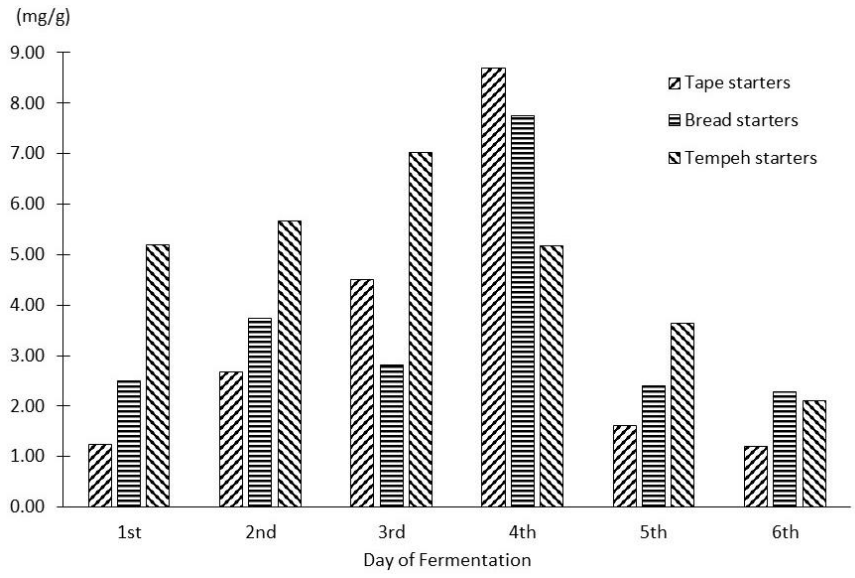

Fig. 2. Daily chlorogenic acid degradation by microbial starters during fermentation of Robusta beans

Organoleptic Scores of Brewed Coffees

The organoleptic scores were obtained from respondents' preference scores for the color, thickness, aroma, acidity, sweetness, bitterness, and overall flavor of Robusta-brewed coffees. The organoleptic scores for brewed coffee from Robusta beans fermented with tape, bread, and tempeh starters and without starters are shown in Table 3. The flavor of Robusta-brewed coffee was predominantly bitter. This could affect respondents' sensitivity to the flavors of Robusta-brewed coffee. Therefore, brewed coffee was prepared using the French Press method to obtain unconcentrated brewed coffee. Therefore, the respondents were able to discriminate the sweetness, acidity, and bitterness of brewed coffee. The French Press method was similar to the *Tubruk* method. The French Press method used coarse-ground beans, whereas the *Tubruk* method used fine-, medium-, and coarse-ground beans.

Table 3. Organoleptic test scores of brewed coffees fermented with microbial starters

Fermented with	Color	Thickness	Aroma	Acidity	Sweetness	Bitterness	Overall flavor
Control (without starter)	3.75±0.44	3.20±0.41	3.00±0.00^{a}	3.00±0.00^{b}	1.00±0.00^{a}	2.50±0.51^{a}	3.00±0.00^{a}
Tape starters	3.75±0.44	3.15±0.37	3.00±0.47^{a}	3.70±0.47^{c}	1.00±0.00^{a}	2.75±0.44^{a}	3.20±0.41^{a}
Bread starters	3.75±0.44	3.20±0.41	4.30±0.00^{b}	3.70±0.47^{c}	1.00±0.00^{a}	2.75±0.44^{a}	3.50±0.51^{b}
Tempeh starters	3.75±0.44	3.15±0.37	3.00±0.00^{a}	2.40±0.50^{a}	1.40±0.50^{b}	3.50±0.51^{b}	3.35±0.49^{b}

Note: $^{a-c}$ Different letters showed significantly different results in each column ($p<0.05$)

There are three levels of coffee bean roasting, i.e., light, medium, and dark (Afriliana 2018). However, Coriate (2023) details it into eight levels of coffee bean roasting, i.e., cinnamon, light, light to medium, full medium, medium dark, dark, French, and Italian. Our study used medium dark, that characterized by dark brown color in roasted coffee beans. Therefore, respondents gave a score of 3.75 for the color of all brewed coffees. This indicated that microbial starters' fermentation did not change the color of the roasting level of Robusta beans, so the color of brewed coffee from starters' fermented beans was not different from that of brewed coffee without starters' fermented beans.

The respondents evaluated the thickness of brewed coffee based on visual and sensory thickness (mouthfeel). The highest thickness of brewed coffee was obtained from dark-roasting level coffee beans (Bahrumi *et al.* 2022). In our study, Robusta beans were roasted at a medium dark level and prepared in the French Press method. The respondents gave scores 3.15—3.20 for the thickness of brewed coffee samples from all brewed coffees. These indicated that respondents were not very likely to perceive the thickness of brewed coffee samples. These were caused by brewed coffee prepared using the French Press method, which did not produce thick brewed coffee.

The respondents gave a score of 3.0 for the aroma of brewed coffees from Robusta beans fermented with tape and tempeh starters and without a starter. However, respondents gave a score of 4.30 for the aroma of brewed coffee from Robusta beans fermented with bread starters. Yeast *S. cerevisiae* in bread and tape starters fermented sugar anaerobically into alcohols. Alcohol was a volatile compound that evaporates easily and gave an accepted aroma for respondents. Although tape starters contained yeast *S. cerevisiae*, they produced a small amount of alcohol. Alcohol aroma mixed with other aromas produced by bacteria and molds; respondents did not recognize the aroma of alcohol. Statistical analysis showed that the score for the aroma of brewed coffee from Robusta beans fermented with bread starters was significantly different ($p<0.05$) compared to the other samples. This indicates that the respondents preferred the aroma of brewed coffee from Robusta beans fermented with bread starters. Previous studies reported that coffee beans fermented with yeast produced a good-accepted aroma (Panji *et al.* 2018; Afriliana *et al.* 2019).

The respondents accepted the acidity flavor of brewed coffee from Robusta beans fermented without a starter and gave it a score of 3.00. The respondents also preferred the acidity flavor of Robusta beans fermented with tape and bread starters, and gave them both a score of 3.70. However, they disliked the acidity of Robusta beans fermented with tempeh starters, giving a score of 2.40. Statistical analysis showed that these scores were significantly different ($p<0.05$). Therefore, the respondents very much like the acidity of brewed coffee from Robusta beans fermented with tape and bread starters. This is due to the ability of Lactic acid bacteria and yeasts in tape and bread starters to produce organic acids (Maicas, 2020). However, molds in tempeh starters did not produce organic acid.

Maligan *et al.* (2022) and Bahtiar *et al.* (2023) stated that sweetness and bitterness flavors are a package of organoleptic test parameters for brewed coffee. The respondents gave scores 1.00—1.40 and 2.50—3.50 for the sweetness and bitterness of brewed coffees. The respondents gave a score of 1.40 and 3.50 for the sweetness and bitterness of brewed coffee from Robusta beans fermented with tempeh starters. These scores were higher than the others. Statistical analysis showed that the sweetness and bitterness of brewed coffees from Robusta beans fermented with tempeh starters were significantly different ($p<0.05$) from those of the others. The bitterness of brewed coffee corresponded with caffeine and chlorogenic acid concentrations of coffee beans. The both concentrations of caffeine and chlorogenic acid of the Robusta beans fermented with tempeh starters were lower than the others; therefore, its scores for sweetness and bitterness score were higher than others.

The respondents gave overall flavor scores 3.50 and 3.35 for brewed coffees from Robusta beans fermented with bread and tempeh starters. Respondents gave overall flavor scores of 3.20 and 3.00 for brewed coffees from Robusta beans fermented with tape starters and without a starter, and gave scores of 3.35 and 3.5 for brewed coffees from Robusta beans fermented with bread and tempeh starters. Statistical analysis showed that the overall flavor of brewed coffees from Robusta beans fermented with

bread and tempeh starters was significantly different ($p<0.05$) from those from Robusta beans fermented with tape starters and without a starter. The respondents preferred brewed coffees from Robusta beans fermented with bread and tempeh starters for their aroma and sweetness-bitterness, respectively.

Conclusion

Microbial starters were able to degrade caffeine and chlorogenic acid, thus decreasing the caffeine and chlorogenic acid concentrations of Robusta beans. Tempeh starters degraded caffeine and chlorogenic acid more than other microbial starters. Therefore, caffeine and chlorogenic acid concentrations of Robusta beans fermented with tempeh starters were lower than those fermented with bread and tape starters.

Caffeine and chlorogenic acid concentration could modify the aroma and acidity, sweetness, and bitterness, and overall flavor of brewed coffee. The respondents preferred the overall flavor of brewed coffees from Robusta beans fermented with bread and tempeh starters. The respondents preferred the aroma of brewed coffee from Robusta beans fermented with yeasts in bread starters and the sweetness-bitterness of brewed coffee from Robusta beans fermented with tempeh starters.

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