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Physicochemical and Microbiological Characteristics of Robusta Coffee Processed Using Wet Fermentation Method with and Without *S. Cerevisiae* Starter Culture

Woro Setiaboma[1,2,*], Dita Kristanti[1,2], Diang Sagita[2], Annisa Dwi Yunniar[2], Lia Ratnawati[2], Yose Rizal Kurniawan[2], Diki Nanang Surahman[2] , Evana Evana[3]

[1] *Research Center of Food Technology and Processing, National Research and Innovation Agency, BRIN, Jl. Raya Jogja-Wonosari Km 31.5, Playen, Yogyakarta, 55861, Indonesia;* [2] *Research Center for Appropriate Technology, National Research and Innovation Agency, BRIN, Jl K.S. Tubun 5, West Java, Subang, 41213, Indonesia;* [3] *Research Center of Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency, BRIN, Jl. Raya Jakarta, Pakansari, Cibinong, Bogor, West Java ,16912Indonesia*

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Abstract

Fermentation is utilized in washed coffee to expedite the degradation of mucilage, thereby altering the coffee's quality. The study investigated the fermentation process of de-pulped Robusta coffee beans with and without *Saccharomyces cerevisiae*. This research purpose was to investigate the effects of fermentation on the physicochemical (moisture, ash, color, total phenolic content, DPPH radical scavenging, and caffeine content) and microbiological (total plate count (TPC), total yeast (TY)) characteristics of de-pulped Robusta coffee produced, using two fermentation conditions (with and without *S. cerevisiae* starter culture) and varying the duration of fermentation (24, 48, and 72 h). The data was analyzed using ANOVA and Duncan' post hoc test, while the relationship between dependent variables and treatments was quantified using principal component analysis (PCA) and hierarchical clustering analysis (HCA). Results showed that the moisture, pH caffein, and total phenolic content of the coffee fermented with and without *S. cerevisiae* starter culture were 1.02-1.32 %; 4.12 to 4.23; 4.03-9.50 ppm; and 350.89-444.08 (GAE mg/100 mL), respectively. The TPC and TY gradually increased with fermentation time, while both parameters were higher in fermentation without *S. cerevisiae*. The PCA revealed that moisture positively correlated with phenolics and caffeine, while negatively correlated with DPPH. The HCA revealed that coffee fermented with and without *S. cerevisiae* starter culture varied significantly.

Keywords : Antioxidant; robusta coffee; spontaneous fermentation; wet process; yeast fermentation

1. Introduction

Coffee has become one of the most common alcohol-free beverages consumed around the globe. The global production of coffee in 2019/2020 was 168.84 million bags. This condition is also supported by a significant transformation of world coffee market as a result of the increasing popularity of "specialty coffee", i.e. coffee with organoleptic characteristics of a particular region (Sunarharum *et al.*, 2014). Coffee grows in many regions in the world such as Africa, South America and Asia. Coffee had a long history in Indonesia and a considerable impact on the country's economic development, with Indonesia ranking as the world's fourth-largest coffee producer in 2019 (Jamil, 2019). It has three dominant species, i.e. *Coffee arabica* (Arabica), *Coffee canephora* (Robusta), and Liberica, where Arabica and Robusta are commonly cultivated in Indonesia.

Both the pre-harvest (genotype, geographical area, climate, and agronomic techniques) and post-harvest (primary processing, drying process, roasting process, and storage conditions) variables influenced the coffee beverage quality (Elhalis *et al.*, 2021). According to Karim *et al.*(2019), cultivation and post-harvest processing contribute 70% of coffee quality. There are three common techniques for converting coffee cherries into beans, called as wet, dry, and semi-dry (Karim *et al.*, 2019; Pereira *et al.*, 2015). The dry process, known as natural process, is a process where cherry coffee is directly dried without de-pulping process. The semi-dry process involves a pulping process, then the coffee is dried in the form of coffee beans with mucilage. The wet process is conducted by pulping the cherry coffee to obtain the coffee beans with mucilage which is ready for fermentation. The wet method, also known as "washed," requires a significant amount of water during fermentation. If done properly, this kind of treatment preserves the interior properties of coffee beans better and results in a more homogenous green coffee, which yields fewer faulty beans (Pereira *et al.*, 2015; Elhalis *et al.*, 2021; Elhalis *et al.*, 2020; Silva, 2014). As a result, wet processed coffee is thought to be of greater quality and is, therefore, sold at a higher price. Wulandari *et al.* (2021) studied the effect of the processing technique on the sensory and chemical characteristic of coffee produces, where the moisture and the caffeine content of

* Corresponding author. e-mail: wrboma@gmail.com/woro002@brin.go.id.

the wet process was lower than dry process, and from the sensory evaluation, both methods were accepted by the panellists. It was reported that the wet process developed the formation of the coffee aroma (Lee *et al*., 2015) as affected by metabolite activity during fermentation the process.

Fermentation promotes the breakdown of complex organic compounds into simpler ones through the activity of microbes (Sharma *et al*., 2020). The fermentation process in the wet process of coffee has the purpose to accelerate the degradation of the mucilage (polysaccharides particularly pectin) attached to the coffee beans. The fermentation can be done spontaneously or by adding particular microbes such as yeast, lactic acid bacteria or a combination of both. Elhalis *et al.* (2020) found that the yeast plays a significant part in wet coffee fermentation, producing coffee with higher flavor, fragrance, and overall sensory attribute. The iron and antioxidant levels of Arabica coffee pulp from Mengani, Indonesia, were similar to those found in commercial Brazilian products, as shown by similarity based on multivariate data (Setyobudi *et al.*, 2022)**.** Study on Arabica coffee cherry fermentation using yeast addition revealed that *P. kluyveri* and *S. cerevisiae* were able to create aromatic volatile substances that led to aroma enhancement in roasted coffee (Jimenez *et al.*, 2023; Pereira *et al.*, 2015; Saerens & Swiegers, 2016). Also, Wang *et al.* (2020) performed fermentation on Arabica green coffee beans. They reported that *P. kluyveri* and *S. cerevisiae* were great starter cultures for coffee flavor biological transformation since both improved the fruity component of roasted coffees. Because of its increased sensory quality, high viability, and pectinolytic activity which breaks down mucilage during fermentation and produces volatile compounds *S. cerevisiae* is used as a culture (Elhalis *et al*., 2023). The quality of the coffee produced will be enhanced by the proper duration of fermentation, not over or under fermentation.

However, study on the fermentation of de-pulped Robusta coffee beans is still limited, especially in the wet processing technique which combine with the *S. cerevisiae* addition and with several fermentation time. Therefore, the goal of the research was to explore the influence of fermentation with and without *S. cerevisiae* addition in the wet processing technique on the chemical characteristic of Robusta coffee produced.

2. Methodology

2.1. Materials

The coffee cherry from the Robusta species was collected from Subang, West Java, Indonesia (-6.728272°S, 107.617418°E). The good (fresh, not rotten and non-empty fruit) and red coffee cherries were sorted and used for experiments. Cherries that were submerged were selected and cherries that floated were discarded. The starter culture used was instant commercial *S. cerevisiae* (Merk "Fermipan", Societe Industrielle Lesaffre (SIL), France) purchased from the local market.

2.2. Fermentation of coffee beans

The red coffee cherries were peeled using a pulper machine to remove the cherry skin, then the coffee beans that still have mucilage were produced and ready for fermentation. The coffee beans were put in plastic jars. In *S. cerevisiae* fermentation, 5 mL of *S. cerevisiae* culture stock was added to the jar with a concentration of 9.32 log colony forming unit (CFU)/mL, while in spontaneous fermentation, the distilled water (5 mL) was added. Stock culture was prepared by adding 0.55 g of "Fermipan" (according to the instructions on the package) into 50 mL of BWP then incubated at 37°C for 24 h. Both *S. cerevisiae* and spontaneous fermentation were fermented for 24, 48, and 72 h (at ambient temperature, 28-30°C). After fermentation, the coffee beans were washed several times with running water before being dried in a cabinet dryer at 50°C for 18 h to produce green coffee beans, consist 12% of moisture content. From this experiment, 6 treatments were generated, i.e. A1 = fermented by *S. cerevisiae* for 24 h, A2 = fermented by *S. cerevisiae* for 48 h, A3 = fermented by *S. cerevisiae* for 72 h, B1= fermented by spontaneous for 24 h, B2= fermented by spontaneous for 48 h, B3= fermented by spontaneous for 72 h.

2.3. Coffee roasting, grinding, and brewing

The dried green bean was roasted to a medium-roasted level using a drum-type coffee roaster developed by Hidayat *et al.* (2020). The initial feeding temperature was 160°C, and the roasting duration was 7 min. After a week, the roasted coffee bean was ground using grinder (Philips HR73010, China). Brewing of ground coffee was conducted by SCAA (2015). A coffee powder (8.25 g) was placed in ceramic glass and added 150 mL hot water. A filter paper was used to remove the spent coffee ground; thus, the liquid coffee extract was obtained. The liquid coffee extract was then used for analysis which consisted of color, pH, phenolic compounds, total tannins, antioxidant activity, and caffeine. The coffee powder was also used and analysed for moisture content and color.

2.4. Total plate counts and total yeast

Total plate count (TPC) and Total Yeast (TY) were determined according to the SNI.19-2897-92 (BSN, 1992). A 10 mL of the liquid fermentation medium was dissolved in 90 mL of Buffered Peptone Water (BPW, Merck, Germany) then homogenized and diluted until 10-6. Then, 1 mL was taken from dilution and placed in a petri dish. A Plate Count Agar media was poured for Total Plate Count analysis, while Potato Dextrose Agar (PDA, Merck, Germany) was poured for total yeast analysis. Incubation was carried out for 24-48 h at 37°C using incubator (BD400, Binder, Germany).

2.5. Moisture content and pH

Gravimetry method was used to determine the moisture content of the roasted coffee powder (AOAC, 2000) in triplicate on all samples. A pH meter (Lab 865, SI Analytics, Germany) was used to determine the pH of the liquid coffee extract.

2.6. Color parameters

The color of the coffee powder and the brewing coffee were measured according to Bicho et al.(2012) using CIE method (Chromameter, NH310, 3NH, China). The color was described according to L (lightness), a*(redness, red-green), and b* (yellowness, yellow-blue).

2.7. Analysis of total phenolic content, antioxidant activity, and caffeine

2.7.1. Total phenolic content

The total phenolic content of coffee extract was determined using Folin-Ciocalteu's method (Haile & Kang, 2020). About 100 µL of sample was diluted with distilled water in a vial. The diluted coffee sample was put in a dark vial, mixed with Folin-Ciocalteu's (Merck, Germany) phenol reagent (100 µL), and incubated for 8 min. After adding 300 µL of 20% Na2CO3 (Merck, Germany), the mixture was incubated for 2 h. Spectrophotometer (UV-Vis 1900, Shimadzu, Japan) was used to detect the absorbance at 765 nm. A plotting curved (Y=0.077X-0.031, R2= 0.996) was created using gallic acid (Sigma Aldrich, USA) solutions with serial concentrations of 0, 10, 20, 30, 40, and 50 mg/L. The result was performed as mg Gallic Acid Equivalent per gram of sample (GAE)/ 100 mL.

2.7.2. Antioxidant activity DPPH radical scavenging

The antioxidant activity (DPPH radical scavenging) in the liquid coffee extract was determined according to Haile and Kang (2020). The sample (2 mL) was diluted to distilled water (10 mL). A sample of 800 µL was mixed with 3.2 mL of a 0.1 mM methanol (Merck, Germany)-DPPH (Sigma Aldrich, USA) solution. The mixture was kept in a dark area at room temperature for 30 mins. The sample's absorbance was measured at 515 nm with a spectrophotometer (UV-Vis 1900, Shimadzu, Japan). Ascorbic acid (Merck, Germany) solutions were utilized as the standard curve (y=1.1341x-1.0471, R2 = 0.999). The antioxidant activity was quantified in mg/100 mL as Ascorbic acid (AAE).

2.7.3. Caffeine content

The caffeine content analysis was conducted according to Hainil *et al.* (2019). Amount of 2 g of coffee grounds and 150 mL of hot water were put into Erlenmeyer (250 mL) and stirred. Then, the hot coffee was filtrated with filter paper to obtain a liquid coffee extract and was added with 1.5 g of calcium carbonate (CaCO3, Merck, Germany) which was inserted into the separated funnel. The mixture was extracted using 25mL of chloroform (Merck, Germany) by three times, then the chloroform phase was evaporated to obtain the extract caffeine. The sample was added with 1 mL of distilled water and was measured using spectrophotometer UV-Vis 1900 (Shimadzu, Japan) at 200-400 nm. The plotting curve for the caffeine (Sigma Aldrich, USA) solution (y=0.0502x+0.0014, R2=0.99) was used, with result expressed in ppm.

2.8. Data analysis

Analysis of Variance (ANOVA) and Duncan's Means Test (IBM SPSS Statistics 26) at a 95% confidence level were used to analyze the data. The PCA was employed to evaluate the relationship between dependent parameters derived in all treatments. The PCA analysis were performed in R-statistical software. Furthermore, interrelationships pattern between variable, and cluster of treatments were described by hierarchical clustering analysis (HCA) using R-statistical. The dependent variable data were standardized (range 0 to 1).

3. Result and discussion

3.1. Microorganisms of wet coffee fermentation

Figure 1 show the total microbes and total yeast during *S. cerevisiae* and spontaneous fermentation. The initial TPC and TY were about 5.86 log CFU/mL, which increased in 48 h to reach 7.87 and 7.96 log CFU/mL, and then dropped to 7.65 and 7.53 log CFU/mL, respectively, at the end of *S. cerevisiae* fermentation. Meanwhile, in spontaneous fermentation, the total microbes reached to 8.28 log CFU/mL at the end of fermentation. The population of yeast was similar to that of *S. cerevisiae* fermentation at the beginning, which reached to 8.24 log CFU/mL in 24 h, but subsequently declined significantly for the rest of the period of fermentation. In this study, the results were slightly different from previous studies which stated that the highest yeast population during spontaneous wet fermentation was 36 and 40 h *(*Elhalis *et al.,* 2020*;* Pereira *et al*., 2014). This might be due to differences in coffee varieties and also fermentation conditions such as temperature and pH.

Total microbes and total yeast in spontaneous fermentation was higher than *S. cerevisiae* fermentation. This occurs due to in spontaneous fermentation, microbial growth occurs naturally and uncontrollably according to the indigenous microorganisms present in coffee beans. Velmourougane (2013) stated that in the freshly pulped Robusta beans, the population of yeast is dominant followed by bacteria and fungi. Meanwhile, in *S. cerevisiae* fermentation, the addition of *S. cerevisiae* inoculum may inhibit the growth of bacteria, other types of yeast and fungi due to substrate competition.

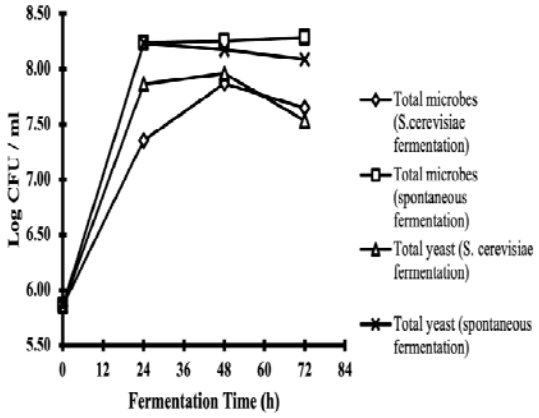

Figure 1. The growth of yeast during *S. cerevisiae* and spontaneous fermentation of the coffee fermented.

Table 1. Total plate counts, total yeast, and final pH of the coffee fermented for Subang.

Sample	TPC (log CFU/mL)	TY (log CFU/mL)	pH
A1	7.35±0.02^{c}	7.86±0.07^{c}	4.18±0.008^{b}
A2	7.87±0.22^{b}	7.96±0.22^{bc}	4.17±0.07^{b}
A3	7.64±0.04^{b}	7.53±0.05^{d}	4.17±0.08^{b}
B1	8.24±0.16^{a}	8.24±0.06^{a}	4.12±0.02^{b}
B2	8.25±0.06^{a}	8.17±0.12^{a}	4.23±0.01^{b}
B3	8.28±0.21^{a}	8.09±0.06^{ab}	4.43±0.1^{a}

The results are presented as mean ± standard deviation. Means in columns with different subscript showed significant differences ($p < 0.05$). A1-fermented by *S. cerevisiae* (24 h), A2-fermented by *S. cerevisiae* (48 h), A3-fermented by *S. cerevisiae* (72 h), B1-fermented by spontaneous (24 h), B2-fermented by spontaneous (48 h), B3-fermented by spontaneous (72 h).

The final pH of the coffee ranged from 4.12 to 4.23 (Table 1). The pH was decreased during the fermentation, where the initial value was 5.22. A similar observation by Velmourougane (2013) had been reported that the final pH of Robusta coffee was 4.05. A decline in pH occurred due to microbial activity which degraded complex organic compound like polysaccharide in mucilage into simpler sugar and acid components, promoting the growth of microorganisms that produce acids and enhancing coffee flavour. Vaz *et al.*(2022) stated that a long time of fermentation gave a pH decrease, and a combination of appropriate time and temperature of fermentation provided a higher pH decrease. The treatments using *S. cerevisiae* present a major pH reduction (compared to the initial pH), which was proven by the increase of total microbes and total yeast. Thus, the coffee flavour's richness enhances with the increasing diversity of microorganisms during fermentation, as indicated by the gradual decrease in pH values over time (Velmourougane, 2013; Wamuyu *et al.*, 2017).

A larger consortiums microorganism accelerates drying of layer the surface of coffee beans, causing rapid sugar leaching that affects coffee aroma, taste, and color. The fermentation produces acetic and lactic acid, which increase the acidity value in coffee. Additionally, the fermentation process produces alcohol and secondary metabolite like volatile compounds like isoamyl alcohol, phenylethyl alcohol, and aldehydes, which give sweet flavour and fruity aromas. These secondary metabolite are more abundant in combination fermentation compared to single fermentations (Elhalis *et al.*, 2021; Mahingsapun *et al.*, 2022; Zafar *et al.*, 2021).

3.2. Chemical properties of coffee fermentation

The moisture content of coffee was not significantly different in all treatments (ranging from 1.02 to 1.32%), as shown in Table 2. Fermentation has been reported not to affect the moisture content change in coffee bean (Kwak *et al.*, 2012), green coffee bean (Haile & Kang, 2019b), fermented coffee (Kwak *et al.*, 2018) and cascara (Kristanti *et al.*, 2022). This finding is consistent with an investigation performed by (Kristanti and Ratnawati, 2022) which stated that fermentation in the different type of microorganism was not caused the different in moisture content. This occurred due to the drying process performed after fermentation. The moisture content of coffee powder was 1.29-1.80 % (w/w) (Pittia *et al.*, 2007).

The color of the wet coffee powder is shown in Table 3. The lightness of the coffee powder ranged from 29.26-33.16 while the redness value ranged from 1.19-1.96. The duration of fermentation affected the color, where the longer duration produced lower color value both *S. cerevisiae* and spontaneous. This result is in line with (Haile and Kang, 2020) which stated that color properties such as L, a*, and b* values of fermented coffee was lower than non-fermented coffee. This evidence also happened in coffee fermented with different types of *Saccharomyces* in the study by Kwak *et al.* (2018). The color changed during fermentation due to hydrolysis of phenolic compounds (Afoakwa *et al.*, 2008).

Table 2. Moisture content and color profile of wet coffee powder of fermentation *S. cerevisiae* and spontaneous.

Samples	Moisture content(%)	L	a*	b*
A1	1.32±^{a}	33.16±0.13^{a}	1.96±0.16^{a}	3.92±0.22^{a}
A2	1.13±^{a}	32.86±0.30^{ab}	1.49±0.06^{b}	3.66±0.06^{ab}
A3	1.02±^{a}	32.71±0.34^{b}	1.33±0.22^{b}	3.52±0.04^{ab}
B1	1.29±^{a}	29.26±0.21^{d}	1.30±0.24^{b}	3.39±0.32^{b}
B2	1.15±^{a}	31.92±0.05^{c}	1.19±0.36^{b}	3.37±0.37^{b}
B3	1.15±^{a}	31.93±0.28^{c}	1.22±0.09^{b}	3.34±0.26^{b}

The results are presented as mean ± standard deviation. Means in columns with different subscript showed significant differences ($p < 0.05$). A1-fermented by *S. cerevisiae* (24 h), A2-fermented by *S. cerevisiae* (48 h), A3-fermented by *S. cerevisiae* (72 h), B1-fermented by spontaneous (24 h), B2-fermented by spontaneous (48 h), B3-fermented by spontaneous (72 h).

Table 3 presents the pH and color of brewed coffee, where the pH and color parameters show significantly different results. In both types of fermentation, the pH of brewed coffee showed a decrease in value (significantly different) as the fermentation time increased. Fermentation reduced pH because fermentation converts complex sugars into simple sugars and acidic components. The Millard reaction takes place during roasting when sugar interacts with amino acid in the absence of an enzyme. However, sugar plays a crucial role in the formation of aliphatic acid, which include formic, acetic, glycolic, and lactic acid (Wei and Tanokura, 2015). Research by Bressani *et al.* (2021) showed that fermentation with *S. cerevisiae* produces pyrazine, lactone, furan, and furanone compounds through the Maillard reaction. Fermented natural coffee and pulped natural fermented coffee have acidity, medium to high sweetness, with malic acid dominating in natural coffee and citric acid in pulped coffee. This supports the finding that fermentation has an impact on acid in coffee brew

The lightness value (L*) of brewed coffee produced by spontaneous fermentation was significantly higher than fermented by *S. cerevisiae* (Table 3). The increase in fermentation period caused the decline of lightness values of brewing coffee fermented by both spontaneous and *S. cerevisiae*. These results indicated that the brewed coffee fermented by spontaneously color was brighter than fermented by *S. cerevisiae*. Moreover, the longer fermentation time caused the darker brewed coffee in both fermentation types. The redness (a*) and yellowness (b*) values of coffee fermented by *S. cerevisiae* were significantly higher than those fermented spontaneously.

This indicated that the *S. cerevisiae* fermentation treatment produced a redder and yellower color of coffee. A similar study conducted by (Kwak *et al.*, 2012) and (Kristanti *et al.,* 2022) found that the yeast fermentation had a higher redness and yellowness values than spontaneous fermentation. The redness and yellowness values of coffee powder fermented by spontaneously from 48 to 72 h was increased. This phenomenon indicates that the fermentation time caused the redder and yellowness. According to Kristanti *et al.* (2022), the increasing of fermentation time affected the increase of lightness, redness, and yellowness values in Robusta cascara. The fermentation affected the increase of yellowness value in green coffee bean with medium roasted treatment (Haile and Kang, 2020).

Table 3. The pH value and color profile of brewed coffee fermented by *S. cerevisiae* and spontaneously.

Sample	pH	L	a*	b*
A1	5.66±0.09 ^{ab}	15.13±0.03^{c}	2.40±0.12^{a}	4.94±0.02^{a}
A2	5.58±0.02 ^{b}	14.09±0.01^{d}	1.79±0.11^{c}	4.92±0.02^{a}
A3	5.58±0.05 ^{b}	14.08±0.05^{d}	1.64±0.07^{d}	4.89±0.03^{a}
B1	5.75±0.07 ^{a}	25.60±0.01^{a}	1.37±0.02^{e}	3.24±0.02^{c}
B2	5.59±0.003 ^{b}	25.60±0.008^{a}	1.35±0.05^{e}	3.26±0.03^{c}
B3	5.64±0.03^{b}	22.65±0.02^{b}	2.15±0.01^{b}	3.81±0.08^{b}

The results are presented as mean ± standard deviation. Means in columns with different subscript showed significant differences ($p < 0.05$). A1-fermented by *S. cerevisiae* (24 h), A2-fermented by *S. cerevisiae* (48 h), A3-fermented by *S. cerevisiae* (72 h), B1-fermented by spontaneous (24 h), B2-fermented by spontaneous (48 h), B3-fermented by spontaneous (72 h).

3.3. The total phenolic content, DPPH radical scavenging, and caffeine content

In general, total phenolic content (TPC) was significantly different ($p<0.05$), and the highest TPC value was achieved through spontaneous fermentation, but both 48-h fermentation treatments (A2 and B2) resulted in TPC valued exceeding 390 GAE mg/100mL. Duration of fermentation, both spontaneous and *S. cerevisiae* fermentation, showed an increase in phenolic content, consistent with Tan et al. (2023) research that phenolic compounds are released and form chlorogenic acid, primary phenolic component in roasted coffee beans. Polyphenol are bound to polysaccharide or protein complexes through glycosidic or peptide bonds, respectively. During fermentation, microorganisms break these bond, breaking down phenolic components into smaller compounds, releasing phenolics from the food complex and increasing free phenolic (Wamuyu *et al.*, 2017; Yang *et al.*, 2023). Coffee contains phenolic compounds that are primarily chlorogenic acids with 5-O-caffeoyl-quinic acid, such as caffein, ferulic, p-coumaric, caffeoylquinic acid, 3-feruloylquinic acid, di-caffeoyl-quinic acid, and proanthocyanidin (Parras *et al.*, 2007). However, duration of fermentation lead to a decrease in phenolic content (B3). This finding was consistent with earlier investigations on green coffee bean (Haile and Kang, 2019a), and Robusta cascara (Kristanti *et al.*, 2022). It was reported that the phenolic content decreased as yeast fermentation time increased. The phenolic compounds may degrade into other component due to the fermentation process.

The DPPH values in Table 4 showed significant differences ($p<0.05$). The DPPH inhibition of coffee increased in *S. cerevisiae* and spontaneous fermentation treatments for 48 h but decreased after 72 h of fermentation. Generally, the DPPH values for spontaneous fermentation were higher than those for *S. cerevisiae* fermentation. This revelated that 48 h of fermentation was the optimal time to the increase antioxidant activity of coffee processed under wet processing method. This study found was in accordance with Haile and Kang (2020), where the DPPH inhibitor of medium roasted coffee was increased after fermentation for 24 h. According to Zofia *et al.* (2020), increasing the fermentation period has an influence on the increasing of DPPH inhibitor green coffee kombucha fermented.

Table 4. Total phenolic content, DPPH radical scavenging, and caffeine content of fermented coffee.

Sample	Total phenolic content (GAE mg/100 mL)	DPPH radical scavenging (AAE mg/100 mL)	Caffeine content(ppm)
A1	360.25±9.47^{bc}	2241.80±19.49^{d}	9.50±0.05^{a}
A2	394.29±16.26^{b}	2341.78±35.75^{b}	6.00±0.02^{d}
A3	365.40±25.67^{bc}	2295.39±6.38^{c}	4.03±0.18^{e}
B1	350.89±1.28^{c}	2295.71±11.37^{c}	7.89±0.08^{b}
B2	463.32±5.51^{a}	2480.41±10.14^{a}	6.30±0.11^{c}
B3	444.08±26.91^{a}	2291.49±9.36^{c}	6.10±0.05^{bc}

The results are presented as mean ± standard deviation. Means in columns with different subscript showed significant differences ($p < 0.05$). A1-fermented by *S. cerevisiae* (24 h), A2-fermented by *S. cerevisiae* (48 h), A3-fermented by *S. cerevisiae* (72 h), B1-fermented by spontaneous (24 h), B2-fermented by spontaneous (48 h), B3-fermented by spontaneous (72 h).

The caffeine content of the extract fermented coffee is shown in Table 4, and the caffeine ranged from 4.03-9.50 ppm. The highest value was A1 while the lowest was A3. Furthermore, A1 and B1 (fermented for 24 h) produced the highest caffeine compared to the others. The treatments showed that the caffeine content significantly decreased with the increase in fermentation period, where the lowest was obtained in A3 treatment (with *S. cerevisiae* for 72h). Caffeine, as the fermentation time increases, can be degraded by fungi, bacteria, or yeast into uric acid, 7-methylxanthine, and xanthine or dimethyl xanthine (Lakshmi *et al.*, 2013; Mardhatilah *et al.*, 2023; Oktavianawati *et al.*, 2020). This result in this study is in line with a previous study conducted by Mardhatilah *et al.* (2023) which reported that fermentation of Arabica coffee during 30 h using yeast starter reduced caffein content. Due to its weak mannosidic base, caffeine readily breaks down in acidic environments, where it will produce unstable salts. Caffeine content, according to the Food Drug Administration (FDA), should not be more than 0.1% by weight (Health, 2003). The caffeine of Robusta green bean was 1.7-4 %, which is higher than the caffeine of Arabica green bean (0.8-1.4%) (Belitz *et al.*, 2009).

3.4. Principal component analyses and hierarchical clustering analyses

The PCA of the physicochemical of fermented coffee can be seen in Figure 2. Relationship of dependent variables was visualized by either PC1 and PC2 that had

total of 82.48 % in which PCA 1 and PCA2 were 44.14 % and 38.34 %, respectively. The a* value, moisture, total phenolic content, and caffeine content were negatively correlated with TPC and DPPH radical scavenging. Furthermore, the total yeast (TY), lightness (L value), and pH had no correlation with other parameters except the b* value, where the b* value negatively correlated with those parameters.

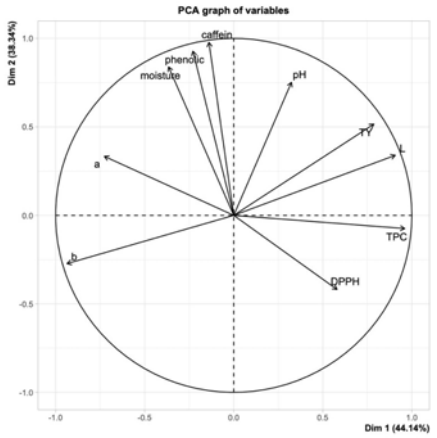

Figure 2. PCA biplot of dependent variables of coffee wet process fermented.

The hierarchical cluster analysis (HCA) was visualised in Figure 3. The HCA found that the fermented coffee was clearly clustered by the type of fermentation (*S. cerevisiae* and spontaneous). The first group was B1 and B2 even thought B3 was solo clustered. The TPC, TY, and L of B1 and B2 was scored of 1 while phenol and moisture were identified score of 0.4 ≥ or ≥ 0.5. In the second group, the samples were clustered with A2 and A3, but A1 was clustered alone due to its the total phenolic, moisture, caffeine, and a* value were higher (score :1). In this case, the HCA interpreted the result of dependent variables that was similar with PCA result. Cluster I contained TPC, TY, L, and DPPH, while Cluster II contained total phenolic, moisture, caffeine, a* value, and pH; and Cluster III was b* value.

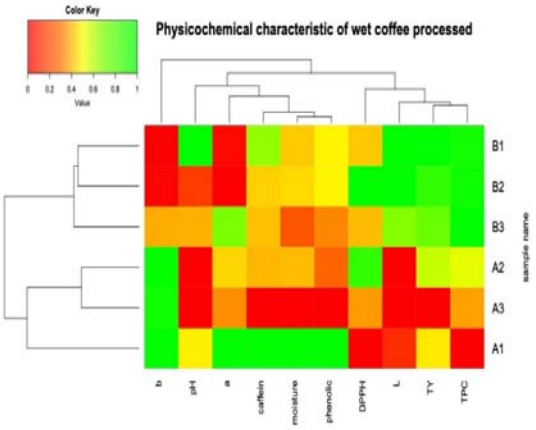

Figure 3. Hierarchical cluster analysis (HCA) of physicochemical of fermented coffee. The color of the tile indicated the strength of the correlations. The green color indicated major while the red indicated minor.

4. Conclusion

Robusta coffee which was fermented using *S. cerevisiae* and spontaneously in the wet processing method had a moisture content of 1.02-1.32%. Total microbes and total yeast in spontaneous fermentation were greater than *S. cerevisiae* fermentation. The caffeine content of the coffee dropped during the fermentation, and the fermentation treatment using *S. cerevisiae* for 42 h produced the lowest caffeine (ppm). The phenolic content during fermentation of coffee declined though the DPPH increased. Although the fermentation treatments reduced bioactive compounds (total phenolic content, DPPH radical scavenging, and caffeine content), the bioactive compounds of spontaneous fermentation were not remarkably as declined as fermented by *S. cerevisiae*. In addition, the fermentation time increased the total microbes and total yeast, while the color of the product became yellowish. Based on the PCA and HCA, the coffee fermented by *S. cerevisiae* and spontaneous was disparate to total plate count, total yeast, b* value, lightness, total phenolic content, and caffeine content.

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Conflict of Interests

The authors declare that they have no conflicting interests.

Highlights:

- The fermentation of de-pulped Robusta coffee beans using with and without *S. cerevisiae* change of coffee quality.
- The fermentation treatments reduced bioactive compounds (total phenolic content, DPPH radical scavenging, and caffeine content).
- The antioxidants of spontaneous treatments were not remarkably as decline as the fermented *S. cerevisiae* treatment had.
- Based on the PCA, the coffee wet process fermented by *S. cerevisiae* and spontaneous were disparate to total plate count, total yeast, b* value, lightness, total phenolic content, and caffeine content.

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