Research Article

Effect of Yeast Fermentation of Green Coffee Beans on Antioxidant Activity and Consumer Acceptability

Han Sub Kwak ¹, Yoonhwa Jeong ², and Misook Kim ²

¹Division of Functional Food Research, Korea Food Research Institute, 245 Nongsaeangmyeong-ro, Iseo-myeon, Wanju-gun, Jeollabuk-do 55365, Republic of Korea

²Department of Food Science and Nutrition, Dankook University, 19 Dandae-ro, 119 Dongnam-gu, Cheonan-si, Chungnam 31116, Republic of Korea

Correspondence should be addressed to Misook Kim; mkim5@dankook.ac.kr

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This study assessed the functionality and consumer acceptance of yeast fermented coffee beans. Green coffee beans were fermented for 24 h with three different yeast strains to increase functionality. The yeast fermentation was effective in fortifying the functionality of coffee by significantly increasing antioxidant activity according to the results of ORAC and SOD-like assay ($P < 0.05$). The TPC and TFC contents in the fermented coffee beans were significantly higher than those in the controls ($P < 0.05$). The consumer acceptance for the fermented coffee beans was slightly lower than that of the controls. Fermentation seemed to influence the aroma and flavor of coffee. However, agglomerative hierarchical clustering analysis revealed that approximately 39% of consumers significantly liked one of the fermented coffees (F3) more than the controls ($P < 0.05$). These consumers indicated that the yeast fermentation of green coffee beans did not generate a negative aroma or flavor and can be attractive with high antioxidant activity.

1. Introduction

Coffee is one of the most traded commodities worldwide. It is harvested in tropical regions and mostly exported to developed countries such as the USA, Europe, Russia, Japan, and Korea [1, 2]. Many coffee drinkers consume coffee on a regular basis, similar to tea, and coffee is regarded as a hedonic food. However, coffee contains significant amounts of phenolic compounds such as chlorogenic and hydroxycinnamic acids and antioxidants including caffeine, melanoidins, and other Maillard reaction products and volatile compounds [3–5]. Levels of chlorogenic and hydroxycinnamic acids are also determined on the final aroma and taste of the roasted coffee [6, 7]. Richelle et al. [8] demonstrated that a cup of Robusta and Arabica coffee had two times more antioxidant activity than a cup of green and black tea. A cup of coffee per day was found to reduce the relative risk of diabetes by 7% in meta-analysis [9] and moderate coffee drinking (below 4 cups per day) showed the strongest inverse relation to heart failure [10]. In some European countries, coffee is one of the major sources of antioxidants in the human diet [11–13].

The functionality of food products can be increased by fermentation. Ginseng is one of the best known functional foods. Ginsenoside contents and SOD-like activity after fermentation were significantly increased by solid-microbial fermentation of white ginseng [14, 15]. Increased antioxidant activities and phenolic compounds are also frequently observed in fermented tea products. Jayabalan et al. [16] showed an increase in epicatechin isomers over 18 days of kombucha tea fermentation. DPPH radical scavenging activity and total polyphenol content in Pu-Erh tea were significantly increased by fermentation [17].

The fermentation of coffee is known as coffee cherry fermentation and effectively removes the mucilage layer prior to the drying process for obtaining green coffee beans [18]. Therefore, the primary objective of coffee cherry fermentation is to improve the ease of obtaining green coffee beans rather than to increase the functionality of the coffee beans. Green coffee beans can gain higher functionality with additional processing steps such as soaking in fruit extracts and fermentation. Lim et al. [19] reported higher antioxidant activity, total polyphenol contents, and consumer

acceptability after soaking green coffee beans in mulberry extract. However, the fermentation of green coffee beans has not been widely studied as a second processing step for increasing antioxidant activity and phenolic compounds. As the fermentation of tea products increases their antioxidant activity and the number of phenolic compounds, the antioxidant activity and phenolic compounds in green coffee beans could also be increased by fermentation. The fermented coffee beans can become fortified functional coffee beans. Therefore, the objective of this study was to ferment green coffee beans using commercial yeasts and investigate the resulting physicochemical properties, antioxidant activity, total polyphenol and flavonoid contents, and consumer acceptability with the intention of making fortified functional coffee beans.

2. Materials and Methods

2.1. Chemicals. Sodium carbonate (Na_2CO_3), sodium nitrite (NaNO_2), monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), potassium acetate (CH_3COOK), and aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) were purchased from DUKSAN Pure Chemicals (Ansan-si, Korea). HCl, NaCl, ethanol, and methanol were supplied by Samchun Pure Chemicals (Pyeongtaek-si, Korea). D-Glucose was bought from Duchefa Biochemie BV (Haarlem, Netherlands). YPD was purchased from Becton Dickinson (Sparks, MD, USA). Folin-Ciocalteu's phenol reagent, fluorescein sodium, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH), 2,5-dinitrosalicylic acid (DNS), trolox, ethylenediaminetetraacetic acid (EDTA), pyrogallol, quercetin, and gallic acid were supplied by Sigma-Aldrich LLC (St. Louis, MO, USA).

2.2. Coffee Fermentation, Roasting, and Brewing. Green coffee beans (300 g; Brazil Ipanema Euro Natural, Coiners International Ltd., Bucheon-si, Korea) were put into a water (450 mL) and yeast (3.75 mL , $1.0 \times 10^7 \text{ CFU/mL}$) mixture. Three different commercial yeasts (*Saccharomyces* species) were used for fermentation: F1 (Lalvin 71B, Lallemand Inc., Montreal, Canada), F2 (Lalvin Cy3079, Lallemand Inc.), and F3 (BDX, Lallemand Inc.). Fermentation was conducted for 24 h at 30°C . After fermentation, green coffee beans were washed three times using sterile water. The fermented coffee beans were dried in a dry oven at 45°C until their moisture content reached 10%. Two controls were used in this study; one control (C1) was the original green coffee beans and the other was a control (C2) treated with the same procedure as the yeast fermented coffee beans but without yeasts.

Coffee roasting was conducted with an automatic coffee roaster to ensure a similar roasting condition for all green coffee beans (Behmor 1600, Behmor Inc., Incline Village, NV, USA). The green coffee beans were put into the roaster and roasted at maximum heating power (1,600 W) for 16 min. The roasted coffee beans were cooled for 15 min and kept at room temperature for three days. The roasted coffee beans were ground using the medium option on a coffee grinder (KG79, De'Longhi, Milano, Italy). The ground coffee (36 g) was brewed with 600 mL of filtered water using a coffee maker (HD7564, Philips, Netherlands).

2.3. Fermentation Characteristics. The pH of the fermentation solution was measured using an electronic pH meter (Orion 3 star, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 0 and 24 h.

The reducing sugar content in the green coffee beans was determined by the DNS method [20] with some modifications. A sample (200 μL) was mixed with 600 μL DNS solution and heated at 100°C for 5 min. Water (3 mL) was added to the reaction solution and its absorbance was measured at 550 nm. D-Glucose solutions (0.1–2.5 mg/mL) were used for a standard curve ($R = 0.997$).

Yeast-containing solutions for green coffee bean fermentation were serially diluted with 0.85% NaCl solution and spread onto the surface of YPD agar plates. The spread plates were incubated for 24 h at 30°C to determine the total microbial counts.

2.4. Physicochemical Characteristics of Roasted Coffee. Moisture content determination was performed according to the Association of Official Agricultural Chemists [21] guidelines. Approximately 1 g of ground coffee beans was dried at 105°C after roasting until the weight became consistent.

Crude ash content was also measured with the Association of Official Agricultural Chemists [21] guidelines. The roasted coffee beans (1 g) were incinerated at 550°C until the weight became consistent. Crude ash content was determined gravimetrically.

The color of the ground coffee after roasting was measured using a Hunter colorimeter system (JC-801S, Color Techno system Co., Tokyo, Japan). Ground coffee (2 g) was put into a small Petri dish for measurement. Lightness (L), redness (a), and yellowness (b) were measured. L , a , and b values for the standard white color plate were $L = 98.38$, $a = 0.29$, and $b = -0.41$, respectively.

2.5. Antioxidant Activity. Oxygen radical absorbance capacity (ORAC) was measured using the method described by Ou et al. [22] with some modifications for *in vitro* antioxidant activity. Phosphate (NaH_2PO_4 - Na_2HPO_4) buffer (10 mM, pH 7.0) was used to dissolve fluorescein powder. Coffee extract (50 μL) and 25 mM fluorescein solution (150 μL) were mixed and then incubated in a dark room for 10 min. A volume of 25 μL of 120 mM AAPH solution was added to the coffee extract and fluorescein mixture. The control was 10 mM phosphate buffer instead of coffee extract. Fluorescence was measured by the microplate reader (Spectra max M2, Molecular Devices, LLC., Sunnyvale, CA, USA). Measurements were taken every minute for 90 min (excitation wavelength: 485 nm; emission wavelength: 535 nm). The ORAC values were calculated by the following formula and presented as μM trolox equivalent/mL of coffee ($\mu\text{M TE/mL}$):

$$\text{ORAC } (\mu\text{M TE/g}) = \frac{C_{\text{Trolox}} \times (AUC_{\text{Sample}} - AUC_{\text{Blank}}) \times k}{(AUC_{\text{Trolox}} - AUC_{\text{Blank}})}, \quad (1)$$

where C_{Trolox} , k , and AUC were the concentration of trolox (5 μM), the sample dilution factor, and the area under

TABLE 1: Change of pH, reducing sugar, and viable cells before and after fermentation.

Sample	pH		Reducing sugar (mg/mL)		Viable cell count (log CFU/mL)	
	0 h	24 h	0 h	24 h	0 h	24 h
C2	5.98	5.13	0.68 ± 0.01 ^{c(1)}	2.57 ± 0.13 ^a	<1.16 ± 0.53	7.78 ± 0.70 ^a
F1	5.79	4.92	1.12 ± 0.02 ^b	2.51 ± 0.12 ^a	6.50 ± 5.83	9.31 ± 0.85 ^a
F2	5.93	4.52	1.28 ± 0.03 ^a	2.47 ± 0.09 ^a	6.41 ± 5.62	9.25 ± 0.86 ^a
F3	5.78	5.04	1.12 ± 0.01 ^b	2.67 ± 0.09 ^a	6.30 ± 5.30	9.03 ± 0.81 ^b

⁽¹⁾ Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

the curve, respectively. AUC was calculated based on the following formula:

$$\text{AUC} = 1 + \sum_{n=1}^{90} \frac{f_n}{f_0}, \quad (2)$$

where f_n is the fluorescence at time n (min).

Superoxide dismutase-like (SOD-like) activity was determined using the method described by S. Marklund and G. Marklund [23] with some modifications. Coffee extract (400 μL), Tris-HCl buffer (600 μL , 50 mM tris(hydroxymethyl)aminomethane and 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0), and 7.2 mM pyrogallol (40 μL) were mixed together and kept at 25°C for 10 min. The reaction was stopped by adding 0.1 N HCl (20 μL). The absorbance was measured at 420 nm using a UV/visible spectrophotometer (Ultrospec 2100 Pro, Biochrom Ltd., Cambridge, UK). SOD-like activity was calculated based on the following equation:

$$\text{SOD-like activity (\%)} = \left(1 - \frac{A}{B}\right) \times 100, \quad (3)$$

where A is absorbance of the sample and B is absorbance of the control.

2.6. Total Polyphenol and Flavonoid Contents. The total polyphenol content (TPC) of brewed coffee was measured using Singleton's method [24] with some modifications. Coffee extract (20 μL) was diluted with 1,580 μL of distilled water. Diluted coffee (160 μL) was mixed with Folin-Ciocalteu's phenol reagent (10 μL) and allowed to sit for 8 min. A volume of 30 μL of 20% Na_2CO_3 solution was added and the mixture was incubated in a dark room for 2 h. Distilled water instead of coffee extract was used as a control. The absorbance was measured at 765 nm by a microplate reader (Spectra max M2, Molecular Devices, LLC.). Gallic acid solutions (0–1 mg/mL) were used to generate a standard curve ($R^2 = 0.997$). The results were presented as mg gallic acid equivalent/mL of coffee extract (mg GAE/mL).

The total flavonoid content (TFC) for each coffee extract was evaluated according to the method described by Dewanto study with some modifications [25]. Coffee extract (250 μL), distilled water (1 mL), and 75 μL of 5% NaNO_2 were mixed together. After 5 min, 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution (150 μL) was added and incubated for 6 min. 1 N NaOH (500 μL) was injected, and the mixture was incubated for 11 min. A blank sample was substituted for the diluted coffee extract with distilled water. The absorbance of the sample was measured

at 510 nm against the blank sample using a microplate reader (Spectra max M2, Molecular Devices, LLC). The difference in absorbance between the sample and the blank was compared to the absorbance of the quercetin solution used as the positive control ($R^2 = 0.999$). The amount of flavonoids in the coffee was presented as mg quercetin equivalent/mL of coffee extract.

2.7. Consumer Acceptance Test. The consumer acceptance test for the yeast fermented coffee was performed with 74 subjects (24 males and 50 females, ages 19–30) who were students and staff at Dankook University (Yongin-si, Korea). Subjects were coffee drinkers that consumed Americano coffee (espresso coffee extract + hot water) at least once a week. Consumer testing was conducted in an open area under incandescent lighting. Subjects were assigned to tables and were prohibited from talking and using cellphones during the test. Coffee (20 mL) was served in 60 mL paper cups using a completed balanced design [26] in order to minimize carry-over effects. The temperature of the coffee was approximately 60°C. Acceptance of overall quality, coffee aroma, bitter taste, astringent taste, and smooth mouthfeel was rated using a nine-point hedonic scale on paper ballots. Each category on the scale was labeled with numbers and descriptors in order to give a clear understanding of the scale. Starting from the left side, the scale was labeled as 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = extremely like. A cup of filtered water was served as a palate cleanser. Subjects were monetarily compensated after the test (approximately \$8.5).

2.8. Statistical Analyses. Results were analyzed using Excel integrated statistical software (XLSTAT version 2012, Addinsoft, Paris, France) in this study. Analysis of variance (ANOVA) was performed to identify significant differences across samples. Post hoc analysis was carried out using Fisher's least significant test at $P < 0.05$ when the significance was observed by ANOVA. Agglomerative hierarchical clustering (AHC) analysis was conducted to generate consumer segment groups based on their overall quality ratings.

3. Results and Discussion

3.1. Fermentation Characteristics. Measurements of pH values, reducing sugar contents, and viable cell counts were conducted to investigate the effect of yeast fermentation on green coffee beans (Table 1). Reducing sugar contents for

TABLE 2: Moisture content, crude ash, and color of ground roasted coffee beans after 24 h of yeast fermentation.

Sample	Moisture content (%)	Crude ash (%)	Color			
			<i>L</i>	<i>a</i>	<i>b</i>	ΔE
C1	1.50 $\pm$ 0.09 ^{a(1)}	4.73 $\pm$ 0.05 ^a	31.52 $\pm$ 1.01 ^a	7.91 $\pm$ 0.44 ^a	16.78 $\pm$ 1.92 ^a	69.60 $\pm$ 1.27 ^b
C2	1.30 $\pm$ 0.05 ^b	4.08 $\pm$ 0.06 ^{cd}	25.24 $\pm$ 0.62 ^c	4.90 $\pm$ 0.25 ^b	8.70 $\pm$ 0.89 ^c	74.00 $\pm$ 0.62 ^a
F1	1.27 $\pm$ 0.17 ^b	4.05 $\pm$ 0.06 ^d	30.62 $\pm$ 0.81 ^{ab}	7.11 $\pm$ 0.94 ^a	14.74 $\pm$ 0.80 ^{ab}	69.91 $\pm$ 0.52 ^b
F2	1.23 $\pm$ 0.06 ^b	4.15 $\pm$ 0.05 ^c	25.17 $\pm$ 0.68 ^c	4.43 $\pm$ 0.26 ^b	7.63 $\pm$ 0.53 ^c	73.90 $\pm$ 0.61 ^a
F3	1.31 $\pm$ 0.10 ^b	4.40 $\pm$ 0.05 ^b	29.78 $\pm$ 0.97 ^b	6.97 $\pm$ 0.54 ^a	13.75 $\pm$ 1.08 ^b	70.51 $\pm$ 0.70 ^b

⁽¹⁾ Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

FIGURE 1: Antioxidant activities of yeast fermented coffee extracts by oxygen radical absorbance capacity (a) and superoxide dismutase-like (SOD-like) activity (b) assays. Different letters meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

C2, F1, F2, and F3 were 2.567, 2.507, 2.469, and 2.673 mg. pH decreased after 24 h of fermentation. The pH for C2 also decreased. This decrease would be due to the soluble organic acids released from green coffee beans [27] and produced by the fermentation of naturally occurring lactic acid bacteria in coffee beans. After 24 h of fermentation, there was no significant difference in reducing sugar content in the fermented solution ($P > 0.05$). Viable cell counts for C2, F1, F2, and F3 showed that fermentation was conducted by yeasts (Table 1). The initial number of viable microbial cells was less than 1.16 log CFU/mL. The number of microbial cells, naturally present in green coffee beans, increased to 7.78 CFU/mL after 24 h fermentation. Solutions from F1, F2, and F3, fermented by starter culture, showed approximately 2.5 higher log CFU/mL of solution than that from C2. This provided supporting evidence for the proliferation of yeasts during fermentation and the role of yeasts in the fermentation. Throughout the 24 h of fermentation, there was no drastic change in pH and reducing sugar content; hence yeast fermentation occurred across all samples.

3.2. Quality Characteristics of Fermented Coffee Beans. The moisture content, crude ash, and color of fermented coffee beans after roasting are presented in Table 2. No significant difference across fermented coffee beans and C2 was observed. This indicates that the drying and roasting of the fermented green coffee beans were performed identically. The moisture content of C1 was 1.50% and was significantly higher

than those of C2 and the fermented coffee beans ($P < 0.05$). This was due to slight overdrying prior to roasting. The ash content was also significantly higher in C1 at 4.73%, while the others ranged from 4.05 to 4.40%. Moisture content and crude ash showed a strong negative correlation ($R = 0.894$, $P = 0.041$). The color of the ground roasted coffee beans showed differences across the samples. C1, F1, and F3 had higher *L*, *a*, and *b* values and lower ΔE values than C2 and F2. The color of the ground coffee beans showed a different pattern in comparison with the moisture content and crude ash. Therefore, the different colors of the roasted coffee beans likely partially originated from the fermentation as well as the difference in moisture contents.

3.3. Antioxidant Activity. The antioxidant activities of the coffee extracts in ORAC and SOD-like assays are shown in Figure 1. ORAC for the yeast fermented coffee extracts were 34.95, 37.44, and 34.09 μ M TE/mL for F1, F2, and F3, respectively (Figure 1(a)). ORAC for C1 and C2 were 20.25 and 14.11 μ M TE/mL, respectively. There was no significant difference in ORAC values after soaking the green coffee beans for 24 h ($P > 0.05$); however, a slight decrease in the ORAC value was observed in C2. It seemed that the soluble antioxidants in green coffee beans might be eluted into water. The ORAC values of the yeast fermented coffee extracts (F1, F2, and F3) were significantly higher than those from the controls (C1 and C2) ($P < 0.05$). There was no significant difference across the fermented coffee beans

FIGURE 2: Total polyphenol (a) and flavonoid (b) contents of yeast fermented coffee extracts. Different letters meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

($P < 0.05$). In SOD-like activity, F1, F2, and F3 showed 82.76, 79.79, and 69.58%, respectively (Figure 1(b)). On the other hand, C1 and C2 showed 33.67 and 29.63% SOD-like activity, respectively, and were not significantly different ($P > 0.05$). An increase of more than two times the SOD-like activity was observed after 24 h of yeast fermentation. Among the fermented samples, F1 showed the highest activity and the activity was significantly higher than F3 ($P < 0.05$). Soaking green coffee beans in water (C2) did not significantly influence the antioxidant activity of the coffee extracts ($P > 0.05$) although the ORAC value of C2 was slightly lower than that of C1. Fermentation for 24 h was effective in increasing the antioxidant activity significantly. The increase of antioxidant activity by fermentation was similar to other fermented materials such as ginseng, garlic, tea, and soy [15, 16, 28, 29].

3.4. Total Polyphenol and Flavonoid Contents. The TPC and TFC of the yeast fermented coffee extracts are presented in Figure 2. The yeast fermented coffee extracts (F1, F2, and F3) had 1.11, 1.18, and 1.30 GAE mg/mL of coffee extract, respectively (Figure 2(a)). F3 had significantly higher TPC than F1 ($P < 0.05$). Control samples (C1 and C2) had significantly lower amounts of TPC than the yeast fermented coffee extracts ($P < 0.05$). C2 had the lowest TPC at 0.72 GAE mg/mL. This was due to the elution of soluble phenolic compounds in the green coffee beans during the soaking period (24 h). A similar finding was observed after soaking green coffee beans in mulberry extract [19]. In Lim's study, the TPC of the coffee extracts decreased after 6 h of soaking. Despite the decrease in TPC due to the soaking process in this study, fermentation positively influenced TPC in the coffee extracts. During the fermentation process, strongly bound phenolic compounds in the cell wall may become weakened [30], making them easier to extract after roasting. Phenolic compounds in coffee are mostly in the forms of chlorogenic acids with 5-O-caffeoyl-quinic acid such as caffeic, ferulic, p-coumaric, and caffeoylquinic acid [3]. Feruloylquinic acid, di-caffeoyl-quinic acid, and proanthocyanidins are also detected in coffee [3].

In TFC, the yeast fermented coffee extracts (F1, F2, and F3) had 1.01, 1.04, and 1.21 QE mg/mL of coffee extract, respectively, and F3 had significantly higher ($P < 0.05$) TFC than F1 and F2 (Figure 2(b)). C1 and C2 had lower amounts of TFC than the yeast fermented coffee extracts. Yeast fermentation was effective in increasing the number of flavonoids in the coffee extracts. The increase in TFC might be due to the conversion of insoluble phenolic compounds into soluble flavonoids during fermentation [31]. The ratio of TFC to TPC in C1 and C2 was 1.03 and 1.07, while F1, F2, and F3 had ratios of 0.91, 0.88, and 0.93, respectively. The ratio was lower in the yeast fermented coffee extracts. Fermentation was more effective in producing soluble phenolic compounds than flavonoids. This result contrasted with those for fermented Columbian coffee [32]. However, Columbian coffee fermentation was conducted with broken green beans, which might elute more soluble phenolic compounds easily over seven days of fermentation. This would also influence the roasting process.

3.5. Consumer Acceptance Test. Consumer acceptance for the fermented and control coffee extracts is shown in Table 3. Mean overall quality rating was the highest in C1, followed by F3. F1 and F2 had significantly lower overall quality ratings than C1 at 4.35 and 4.43, respectively ($P < 0.05$). C2 also received slightly lower overall quality ratings than C1. Therefore, the soaking process does appear to slightly influence the coffee quality. Color acceptance was not significantly different ($P < 0.05$), which meant that there was no difference in the appearance of the coffee extract. Aroma acceptance ratings for the fermented coffee samples (F1, F2, and F3) were significantly lower than those for the controls (C1 and C2) ($P < 0.05$). The consumer acceptability was within the range of the previous acceptance study using various roasting conditions [33]. Fermentation might negatively influence the aroma acceptance of coffee. Across the fermented coffee samples (F1, F2, and F3) the differences in consumer acceptability might be related to the presence of different volatile compounds in coffee beans after fermentation. Different yeasts showed different flavor profiles

TABLE 3: Overall quality and acceptances of color, aroma, sourness, bitterness, astringency, and mouthfeel of yeast fermented coffee extract by 74 consumers.

Sample	Overall quality	Color	Aroma	Sourness	Bitterness	Astringency	Mouthfeel
C1	5.09 ± 1.72 ^{a(1)}	5.59 ± 1.76 ^a	5.62 ± 1.63 ^a	5.12 ± 1.86 ^a	4.51 ± 1.90 ^a	4.81 ± 1.80 ^a	5.80 ± 1.75 ^a
C2	4.69 ± 1.65 ^{ab}	5.30 ± 1.75 ^a	5.12 ± 1.70 ^a	5.07 ± 1.49 ^a	4.12 ± 1.94 ^{ab}	4.57 ± 1.82 ^{ab}	5.35 ± 1.68 ^{ab}
F1	4.35 ± 1.70 ^b	5.18 ± 1.48 ^a	4.08 ± 1.85 ^b	4.78 ± 1.50 ^a	3.84 ± 1.76 ^b	4.20 ± 1.76 ^b	4.99 ± 1.72 ^b
F2	4.43 ± 1.46 ^b	5.23 ± 1.44 ^a	4.51 ± 1.51 ^b	4.19 ± 1.58 ^b	4.01 ± 1.85 ^{ab}	4.03 ± 1.79 ^b	4.85 ± 1.66 ^b
F3	4.84 ± 1.67 ^{ab}	5.54 ± 1.60 ^a	4.45 ± 1.95 ^b	4.84 ± 1.47 ^a	4.41 ± 1.89 ^{ab}	4.36 ± 1.58 ^{ab}	5.12 ± 1.57 ^b

⁽¹⁾ Different superscripts within a column meant significant difference at $P < 0.05$ by Fisher's least significant difference test.

TABLE 4: Agglomerative hierarchical clustering (AHC) analysis of overall quality.

Class	Subject ⁽¹⁾	C1	C2	F1	F2	F3
1	28	3.79 ± 1.20 ^{b(2)} _{B(3)}	3.82 ± 1.61 ^b _B	3.57 ± 1.48 ^b _B	4.89 ± 1.66 ^a _A	5.32 ± 1.61 ^a _A
2	43	5.95 ± 1.53 ^a _A	5.23 ± 1.49 ^b _A	4.81 ± 1.72 ^{bc} _A	4.09 ± 1.29 ^d _B	4.51 ± 1.71 ^{cd} _B

⁽¹⁾ Three subjects were removed from AHC analysis because they marked same ratings for entire samples. ⁽²⁾ Different superscripts within a row meant significant difference at $P < 0.05$ by Fisher's least significant difference test. ⁽³⁾ Different subscripts within a column meant significant difference at $P < 0.05$.

in rice distilled liquor [34] and cachaça [35]. Acceptance ratings for sourness, bitterness, astringency, and mouthfeel were lower in the yeast fermented coffee samples than in C1 and C2.

In order to segment consumers into a small number of groups, AHC analysis was performed using the overall quality ratings for all except three consumers that selected the same acceptance ratings for the entire sample (Table 4). Consumers were divided into two clusters generated by AHC analysis. Cluster 1 was composed of 39.4% of the total consumers. These were the consumers who preferred fermented coffee (F2 and F3), while disliking the controls (C1 and C2) ($P < 0.05$). On the other hand, cluster 2 was composed of 60.6% of consumers. These consumers had higher overall quality ratings for the controls (C1 and C2). They also rated F1 as an average of 4.81 and had the least acceptance for F2 at 4.09. Approximately 40% of consumers preferred the fermented coffee extracts (F2 and F3) although fermented coffee had lower mean overall quality ratings for the entire group of consumers. Therefore, the fermented coffee samples (F2 and F3) did not have lower acceptance ratings for all consumers; they were acceptable and showed higher acceptability for 39.4% of consumers. This result provides supporting evidence that fermentation does not have a negative influence on the consumer acceptance of coffee. Consumers are therefore segmented and another coffee product can be created for approximately 40% of coffee consumers. In addition, the high antioxidant activity and phenolic compounds in fermented coffee can be attractive to those consumers with moderate acceptance for fermented coffee. The consumer acceptance would also increase with awareness of the high antioxidant activity and phenolic compounds content as shown in a previous blind and informed consumer acceptance test for blueberry functional beverages [36].

4. Conclusion

Yeast fermentation of green coffee beans for 24 h was effective in fortifying the functionality of coffee by inducing

a significant increase in antioxidant activity, TPC, and TFC. Yeast fermentation of green coffee beans causes bound phenolic compounds to be released after roasting. The consumer acceptance for the fermented coffee beans was slightly lower than for the controls. Fermentation might negatively influence the aroma and flavor of coffee extracts. However, the consumer segmentation revealed that approximately 39.4% of consumers preferred one of the fermented coffees (F3) more than the controls. Therefore, it can be concluded that yeast fermentation did not always generate a negative aroma and flavor for consumers. If fermentation was carried out with properly selected yeasts, fermented coffee can be attractive to coffee consumers, and coffee manufacturers can diversify their products with higher functionality.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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