

Article

Antioxidant Properties of Fermented Green Coffee Beans with *Wickerhamomyces anomalus* (Strain KNU18Y3)

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Abstract: A few yeast species have been tested frequently to improve the tastes, flavors, and other important quality parameters of coffee. However, continuing evaluations of different yeast species for fermenting green coffee beans will have a significant positive contribution to the coffee industry. This experiment was conducted to evaluate the antioxidant properties, total phenol content (TPC), total flavonoid content (TFC), total tannin content (TTC), and the consumer acceptability of fermented green coffee beans with *Wickerhamomyces anomalus*. The coffee beans were roasted at different roasting conditions (light, medium, and dark). There was no significant ($p > 0.05$) difference between the yeast-fermented and non-fermented coffee with regard to the oxygen radical absorbance capacity (ORAC) values in medium and dark roasted coffee. Similarly, the superoxide dismutase-like (SOD)-like activity did not significantly differ in all roasting conditions. However, the SOD-like activity was significantly different ($p < 0.05$), particularly within light roasted and medium roasted, and between light roasted and dark roasted in both the control and fermented coffee extracts. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power (FRAP) were improved in fermented coffee beans. There was a significant ($p \leq 0.05$) difference between the yeast-fermented and non-fermented coffee with respect to the TPC and TFC in all roasting types and the TTC in the light and dark roasting conditions. The fermentation of green coffee beans with *W. anomalus* increased the TPC and TFC. However, the TTC was lower in the fermented coffee beans compared to the non-fermented coffee beans in medium and dark roasted coffee. In general, fermentation of green coffee beans with *W. anomalus* has the potential to improve the functionality of coffee beans.

Keywords: antioxidant; coffee; fermentation; *W. anomalus*

1. Introduction

Fermentation has the potential to improve the functionality of foods. Fermentation is primarily relied upon during the wet processing of coffee to remove mucilage. However, it also improves coffee's sensory quality attributes [1]. Fermentation has been extensively applied in the food, chemical, and pharmaceutical industries to aid in the manufacturing, extraction, and modification of bioactive compounds [2,3]. Microbial fermentation is an interesting biotechnological processing system that can improve the total phenolic content of foods and herbs by liberating their insolubly bound phenolics and hence boost their nutritional value [4,5]. Numerous research papers provide a comparison of the antioxidant properties of popular drinks such as coffee, tea, and cocoa [6–8]. Phenolic compounds are found in a broad range of regularly consumed plant foods, such as vegetables, fruits, legumes,

and cereals, and in beverages of plant origin, such as coffee, tea, and wine [9,10]. Flavonoids naturally exist in plants with a variable phenolic structure. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by chelating metal ions and scavenging free radicals [11,12]. Flavonoids in food are commonly responsible for the color, the taste, the prevention of fat oxidation, and the protection of enzymes and vitamins [13].

Wickerhamomyces anomalus (formerly *Pichia anomala*) is an ascomycetous heterothallic yeast of the family *Wickerhamomycetaceae* that propagates sexually through the development of hat-shaped ascospores and asexually by budding [14]. *W. anomalus* strains are found in several environments and have been isolated from cereal grains, fruits, maize silage, wine, and high-sugar food products [14]. *W. anomalus* is categorized as a biosafety level-one organism; it is deemed harmless for healthy individuals and can grow under severe environmental stress conditions, such as high and low pH, high osmotic pressure, anaerobic conditions, and low water activity [15]. As mentioned by Comitini et al. [16], *W. anomalus* toxins have been examined as antimicrobial agents against some spoilage yeasts such as *Dekkera/Brettanomyces*. Some researchers have reported that the wild species of *W. anomalus* strains have the potential to use maltose and grow better than other commercial brewing yeasts [17]. However, other studies have referred to its clear inability to metabolize maltose [18]. Considering these findings, *W. anomalus* is preferred as a good starter culture in sequence or inoculated together with other yeasts in winemaking [19]. *p. anomala*-mixed starters have been evaluated to improve the final quality of cider [18]. Due to its positive effect on sensory quality, a mixed starter of *W. anomalus* and *Saccharomyces cerevisiae* has been proposed for making Chinese baijiu [20].

Recently, studies have been conducted to improve the functionality of coffee through green bean fermentation with selected microorganisms. In these studies, the *Saccharomyces* species [21,22], *Saccharomycopsis fibuligera* [22], *Rhizopus oligosporus* [23], and *Yarrowia lipolytica* [24], have been used to ferment green coffee beans. The fermentation of green coffee beans with *R. oligosporus* significantly enhanced the compositions of aroma precursors [23]. As reported by Kwak et al. [21] and Mesfin and Kang [22], the fermentation of green coffee beans improves antioxidant activity, total phenol content (TPC), and total flavonoid content (TFC), and it reduces the total tannin content (TTC), which is mostly responsible for coffee's astringency. *W. anomalus* strain KNU18Y3 has the ability to produce a pectinase enzyme, it was selected as a starter culture for coffee fermentation in the wet processing method [25]. Since this yeast has not been tested previously to ferment green coffee beans, this study was conducted to evaluate the antioxidant activities of fermented coffee beans with *W. anomalus* (strain KNU18Y3).

2. Materials and Methods

2.1. Chemicals

Disodium phosphate (Na_2HPO_4), aluminium chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), monosodium phosphate (NaH_2PO_4), sodium carbonate (Na_2CO_3), D-glucose, and sodium nitrite (NaNO_2) were supplied by Dae-Jung Chemicals & Metals Co., Ltd., (Jeongwang-dong, Shiheung-city, Gyeonggi-do, Republic of Korea). Pyrogallol, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), and fluorescein sodium were purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA). NaCl, ethylenediaminetetraacetic acid, yeast peptone dextrose, gallic acid, Folin-Ciocalteu's phenol reagent, quercetin, Trolox, 2,2-diphenyl-1-picrylhydrazyl, potassium ferricyanide ($\text{C}_6\text{N}_6\text{FeK}_3$), trichloroacetic acid ($\text{C}_2\text{HC}_3\text{O}_2$), and ferric chloride (FeCl_3) were purchased from Sigma Aldrich LLC (St. Louis, MO, USA). Methanol was supplied by Merck KGaA (Darmstadt, Germany), and HCl was purchased from Junsei Chemical Co., Ltd. (4-4-16 Nihonbashi-honcho, Chuo-ku, Tokyo, Japan).

2.2. Fermentation of Green Coffee Beans

A freeze-dried *W. anomalus* (strain KNU18Y3) yeast cells were cultivated in a sterile yeast peptone dextrose broth (0.5% *w/v* yeast extract, 2% *w/v* dextrose and 1% *w/v* casein peptone) for 48 h at 30 °C. The pH of the media was calibrated to 5.0 with 1 M HCl. Then, the yeast cells were collected via

centrifugation at 8000× g. Finally, the collected yeast cells were washed twice using the deionized distilled water (ddH₂O) and mixed in a similar volume of 100 mM phosphate-buffered saline solution.

The green coffee beans (*Coffea arabica* L.) that imported from Kenya were used for this study. The coffee beans (600 g) saturated in ddH₂O at 4 °C for 24 h and then steamed at 80 °C for 40 min to kill native yeasts that exist on the coffee beans. Next, the steamed coffee beans then allowed to cool at 20–23 °C. The heat-treated green coffee beans were divided into two lots: fermented through inoculation (1.0 × 10⁴ colony-forming unit (CFU)/g of coffee beans) and non-inoculated (control). Then, each lot was divided into three parts and underwent a different roasting treatment. Fermentation of green coffee beans and roasting were done in three replications. The fermentation was carried out for 24 h at 30 °C. After that, the coffee beans were washed thoroughly using a ddH₂O. The fermented coffee beans were dried using oven at 45 °C until the moisture content reached between 10% and 12%.

The pH of the fermented solution (the water that used to ferment the coffee beans) was recorded using a pH meter at 0 h and 24 h. A sample solution was taken after 24 h fermentation and serially diluted and plated onto yeast peptone dextrose agar (YPDA) plates. The plates were incubated for 24 h at 30 °C to estimate the growth of yeast cells. The pH measurements and microbial counts were made in five replications.

2.3. Coffee Roasting, Grinding, Extraction

The green coffee beans were roasted at 245 °C for 11.5 (light roast), 13.5 (medium roast) and 16 (dark roast) min using a coffee roaster (GeneCafe CR-100 coffee roaster, Genesis, Ansan, Korea) to ensure uniform roasting conditions. Each roasting was done in triplicate. After roasting, the beans were allowed to cool for 30 min and then ground by adjusting in a medium level on a coffee grinder (Latina 600N electric grinder). Hot brew extraction made using a coffee maker (HD7450, Philips, Nanjing, China) by mixing 36 g of coffee powder with 500 mL of water. A filter paper was used during brewing. After each brewing, the coffee machine was cleaned prior to brewing the next sample. The extraction was made in three replications. The analysis of antioxidant activities, TPC, TFC, and TTC were measured from this extract.

2.4. Color Parameters

Chroma Meter (CR-400 Chroma Meter, Tokyo, Japan) was used to estimate the color of the roasted coffee powder. A small coffee powder (5 g) was kept into a Petri dish for reading, and a* (redness), b* (yellowness) and L* (lightness) were assessed. The color reading was made in triplicate.

2.5. Antioxidant Activity

2.5.1. Oxygen Radical Absorbance Capacity

The oxygen radical absorbance capacity (ORAC) was estimated with some modifications using the protocol described by Ou et al. [26]. Fluorescein powder was dissolved using a phosphate buffer (NaH₂PO₄-Na₂HPO₄, 10 mM, pH 7.0). The coffee extract (50 µL) was mixed with 25 mM of the fluorescein solution (150 µL) and incubated in a dark environment for about 10 min. Then, 25 µL of 120 mM AAPH solution was mixed to the coffee extract and fluorescein mixture. Meanwhile, 10 mM phosphate buffer was substituted instead of coffee extract and used as a control. The absorbance was measured using a UV/visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). The readings were recorded at one minute interval for 30 min (excitation wavelength: 485 nm; emission wavelength: 535 nm). The absorbance readings for ORAC estimation were made in triplicate and expressed as a µM Trolox equivalent/mL of coffee (µM TE/mL). The ORAC estimated using the formula stated below;

$$\text{ORAC } (\mu\text{M TE/g}) = (\text{C}_{\text{Trolox}} \times (\text{AUC}_{\text{Sample}} - \text{AUC}_{\text{Blank}}) \times k) / (\text{AUC}_{\text{Trolox}} - \text{AUC}_{\text{Blank}}) \quad (1)$$

where C_{Trolox} , k , and AUC were the concentrations of Trolox (5 μM), the sample dilution factor, and the area under the curve, respectively. AUC was computed according to the formula presented below;

$$\text{AUC} = 1 + \sum_{n=1}^{30} \frac{f_n}{f_0} \quad (2)$$

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

2.5.2. Superoxide Dismutase-Like Activity

The protocol suggested by Marklund and Marklund [27] used with some modifications to estimate the SOD-like activity. Coffee extract (400 μL), Tris-HCl buffer (600 μL , 50 mM tris (hydroxymethyl) aminomethane and 10 mM ethylenediaminetetraacetic acid, pH 8.0), and 7.2 mM pyrogallol (40 μL) were mixed together and kept at 25 $^{\circ}\text{C}$ for 10 min. The reaction was terminated by mixing 0.1N HCl (20 μL). Absorbance readings were done at 420 nm using a UV/visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). The absorbance readings for the estimation of the SOD-like activity were performed in triplicate. SOD-like activity was computed with the formula presented below:

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

where A indicates the sample absorbance and B indicates the absorbance of the blank (control).

2.5.3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH activity was measured using the protocol described by Pataro et al. [28], with some modifications. DPPH was dissolved in methanol (0.1 mM) and 3.9 mL of it transferred into a cuvette, then immediately the absorbance was measured at 515 nm (used as a control). The coffee extracts (800 μL) from each sample were mixed with 3.2 mL of methanol-DPPH solution and placed in a dark condition for 30 min. Then the absorbance was recorded. Methanol was used as a blank. The DPPH assay was done in three replications. The DPPH inhibition percentage was calculated using the following formula;

$$\text{DPPH inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (4)$$

where A_{control} is the absorbance of control reaction (DPPH and Methanol), and A_{sample} is the absorbance in the presence of coffee extract.

2.5.4. Ferric Reducing Antioxidant Power (FRAP)

FRAP of the coffee extract was assessed using the method described by Oyaizu [29]. A 2.5 mL of coffee extract was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) 2.5 mL of 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 $^{\circ}\text{C}$ following 2.5 mL of 10% trichloroacetic acid was added. Then, this mixture was centrifuged (2000 \times g for 10 min) and 5 mL of the supernatant was taken and mixed with 5 mL of water and finally added 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan) and the FRAP was subsequently determined using ascorbic acid as standard. The FRAP analysis was done in three replications.

2.6. Total Phenol Content, Flavonoid Content, and Tannin Content

The total phenol content of the coffee extract was measured using a protocol described by Singleton's method [30], with some adjustments. The coffee extract (20 μL) was diluted with 1580 μL ddH₂O. Diluted coffee extract (160 μL) and Ciocalteu's phenol reagent (10 μL) were mixed and kept for 8 min. Then, 30 μL of 20% Na₂CO₃ solution was mixed and incubated in a dark environment

for 2 h. The coffee extract was substituted with distilled water and used as a blank. The absorbance readings were determined at 765 nm using a UV/visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). Gallic acid solutions (0–1 mg/mL) were used to generate a standard curve ($r^2 = 0.997$). The estimation of the TPC was done in triplicate. Results were expressed as mg gallic acid equivalent/mL (mg GAE/mL) of coffee extract.

A protocol stated by Dewanto [31], applied with some modification to estimate the total flavonoid content of the coffee extract. The mixture was prepared using 250 μ L coffee extract, 1 mL of distilled water and 75 μ L of 5% NaNO₂. The 5 min later, 10% AlCl₃ 6H₂O solution (150 μ L) was mixed and incubated for 6 min. Finally, 1N NaOH (500 μ L) was added and incubated for 11 min. The ddH₂O used as a blank sample. The absorbance was measured at 510 nm using a UV/visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). The standard solution was prepared using a quercetin solution (0–1 mg/mL) to make a standard curve ($r^2 = 0.999$). The estimation of the TFC was done in triplicate. The total flavonoids in the coffee were expressed as mg quercetin equivalent/mL of coffee extract.

The total tannin content (TTC) was measured using the Folin-Ciocalteu method, with some modifications [32]. About 100 μ L of the coffee extract was added to a tube (10 mL) containing 7500 μ L of distilled water, 500 μ L of Folin-Ciocalteu phenol reagent, and 1000 μ L of 35% sodium carbonate solution and was then diluted to 10,000 μ L with distilled water. The mixture was well-mixed and placed at room temperature for 30 min. A set of standard solutions of tannic acid (20, 40, 60, 80, 100 μ g/mL) were made as a reference. Absorbance for the test and standard solutions was measured with a UV/Visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan) against the blank (distilled water) at 700 nm. The estimation of the TTC was done in triplicate. The tannin content was expressed in mg/mL of tannic acid in the coffee extracts.

2.7. Sensory Evaluation

The consumer responses of the coffee made from fermented and non-fermented coffee beans were evaluated with 50 people (students and staff members of the horticulture department) at Kangwon National University, Republic of Korea. The medium roasted coffee beans were selected for testing the consumer acceptability since the Korean people widely used the medium roasted coffee. The coffee was brewed using a coffee maker (HD7450, Philips, Nanjing, China) using 36 g of coffee powder with 500 mL of water. The prepared coffee was poured into a thermos to maintain the temperature. Each consumer was provided with coffee sample (20 mL, 60 \pm 2 $^{\circ}$ C) in a paper cup (150 mL) and a glass of water (24 \pm 2 $^{\circ}$ C) for cleansing palate after each taste. The scale was 1 to 7 and labeled as 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like very much.

2.8. Statistical Analysis

The results were compiled using Microsoft Excel 2013. Analysis of variance (ANOVA) was performed using SAS 9.4 software (SAS Institute, 100 Campus Drive, Cary, Raleigh, North Carolina, USA), to classify significant variations among samples.

3. Results and Discussion

3.1. pH and Colony Count (log CFU/mL)

The pH of the solution (the water that was used to ferment the coffee beans) was measured right after the inoculation of the yeast (0 h) and after 24 h of fermentation. A pH reduction was observed in both the yeast-inoculated and non-yeast-inoculated (control) treatments. However, the pH reduction rate was higher in the yeast-inoculated sample. The pH in the control treatment was 6.02 at 0 h and 5.78 after 24 h (Figure 1A). In the fermented treatment, the pH was reduced from 6.05 to 5.46 within 24 h of fermentation (Figure 1A). The pH of the yeast-inoculated solution showed a higher reduction

compared to the non-yeast-inoculated solution. The alkalinity and acidity of the solution are expressed based on the pH values. The pH continues to decrease while fermentation takes place for various reasons. Fermentation is responsible for the production of organic acids and the absorption of basic amino acids, which substantially reduces the pH [33]. However, a small pH reduction was found in the non-yeast-inoculated treatment. This reduction was expected because the water-soluble organic acids leaked from the coffee beans into the water [21,34] and/or because of bacteria that naturally exist on the coffee beans [21]. The yeast populations were measured; initially, they were 7.66 log CFU/mL, and after 24 h, they reached 9.62 log CFU/mL (Figure 1B).

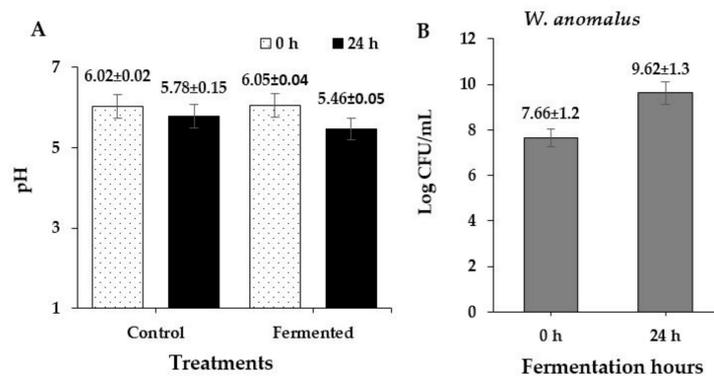


Figure 1. The pH in yeast inoculated and non-inoculated treatments (A) and yeast population (B).

3.2. Colorimeter Data

The fermented and non-fermented ground coffee after roasting is shown in Figure 2. A significant ($p < 0.05$) differences were observed between the yeast-fermented and non-fermented treatments of ground roasted (light roasting level) coffee with respect to the colorimetric parameter (L^* and a^*) (Figure 3A,B). The L^* and a^* of ground roasted coffee did not significantly differ between the fermented and control treatment in both medium and dark roasting condition. However, we observed that the L^* value increased and the a^* value decreased as the roasting time increased. The b^* value was significantly higher in the fermented treatment at the light, medium and dark roasting level (Figure 3C). Supporting reports showed that fermentation of green coffee beans with *S. cerevisiae* strains [21,22], and *S. fibuligera* [22] resulted in high L^* , a^* , and b^* values compared to non-fermented coffee beans. During cocoa fermentation, gradually, a color change was observed as a result of a high amount of phenolic compounds and flavonoids, which act as a substrate of polyphenol oxidase in the presence of oxygen [35].

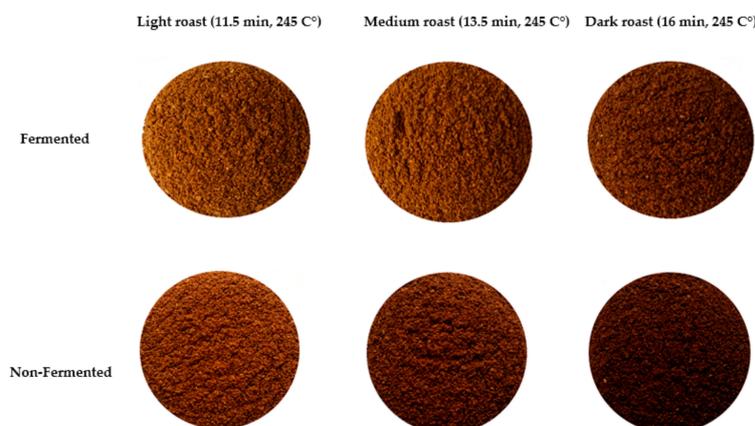


Figure 2. The fermented and non-fermented ground coffee that roasted at a different level.

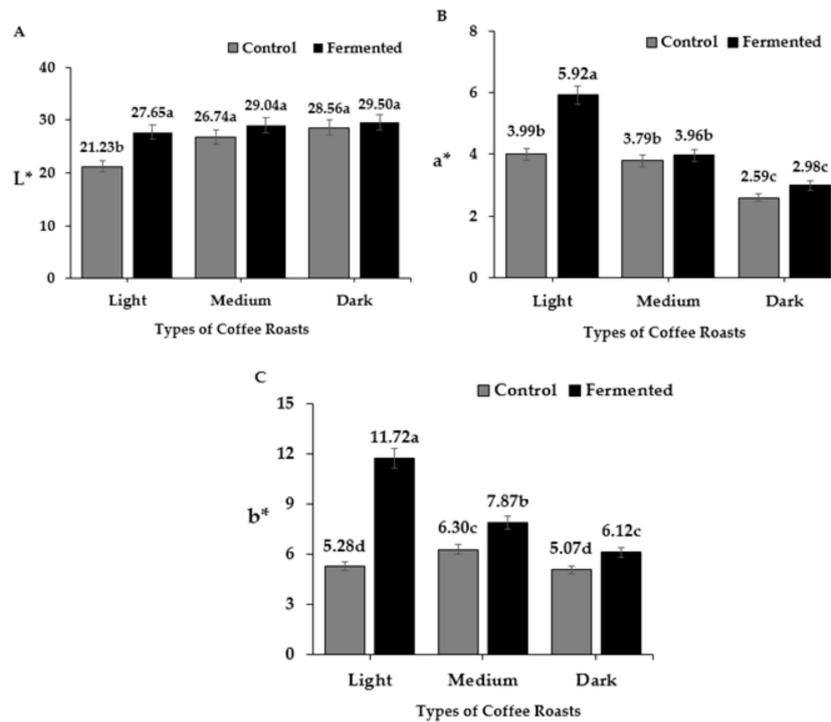


Figure 3. Colorimeter (L^* , a^* and b^*) values of fermented roasted and ground coffee (A–C). Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

3.3. Antioxidant Activity

3.3.1. SOD-Like and ORAC

The antioxidants of fermented and non-fermented coffee extract were evaluated in terms of the oxygen radical absorbance capacity (ORAC) and superoxide dismutase (SOD)-like activity. The peroxy radical scavenging potentials of aqueous soluble components present in the coffee could be estimated more accurately with a modified ORAC assay [36,37]. There was no significant ($p > 0.05$) difference between the yeast-fermented and non-fermented coffee extracts with respect to the ORAC value in medium (Figure 4A) and dark roasted coffee. The SOD-like activity was not significantly differed between fermented and non-fermented coffee in all roasting conditions (Figure 4B). However, both ORAC and SOD-like activity were significantly lower in both treatments in the light roasting condition. The means of ORAC value for the yeast-fermented coffee extracts was 28.33, 46.08, and 47.82 μ MTE/mL in the light, medium and dark roasted coffee, respectively (Figure 4A). The means of the SOD-like activity of yeast-fermented coffee extracts was 59.56, 87.31, and 89.05% at the light, medium and dark roasting levels, respectively (Figure 4B). The ORAC value was higher by 3.96 μ MTE/mL in fermented coffee compared to non-fermented coffee in the light roasting condition (Figure 4A). We found a significant improvement in the ORAC value and SOD-like activity in the medium and dark conditions compared to the light roasted coffee regardless of the fermentation. The medium and dark roasting conditions improved the ORAC by 21.93 and 23.94 μ MTE/mL in the control treatment. However, it was increased by 17.75 and 19.49 μ MTE/mL in the fermented coffee extracts compared to light roasted coffee, respectively. This is in agreement with previous reports on the influence of roasting on antioxidant activity. Nicoli et al. [38] reported higher antioxidant activity for medium and dark roast brews. Similarly, the elevated antioxidant activity for higher degrees of roasting has been confirmed in studies [39–41]. As the roasting temperature increased the antioxidant activities of the kernel, cashew nut and testa extracts increased [42]. Several publications have described variations in coffee antioxidant contents as a cause of roasting conditions [43,44]. Del Castillo et al. [43] noticed a higher antioxidant activity in the medium roasted coffee brew. However, Perrone et al. [45]

found a greater antioxidant activity for light roast brews. The results from various investigations regarding the antioxidant activity of coffee have been considerably diverse. The causes of such variable research results can be associated with the development of melanoidin compounds, which increase the antioxidant capacity despite the reductions in the polyphenol content. Nevertheless, other studies have not detected any significant effects of roasting on antioxidant activity [46], nor any indications that roasting for a longer duration at high temperature reduce antioxidant activity [44,47,48] when polyphenol degradation is not compensated by melanoidin formation [49,50]. However, amongst other coffees, this phenomenon was noticed for dark roasted Robusta coffee [40].

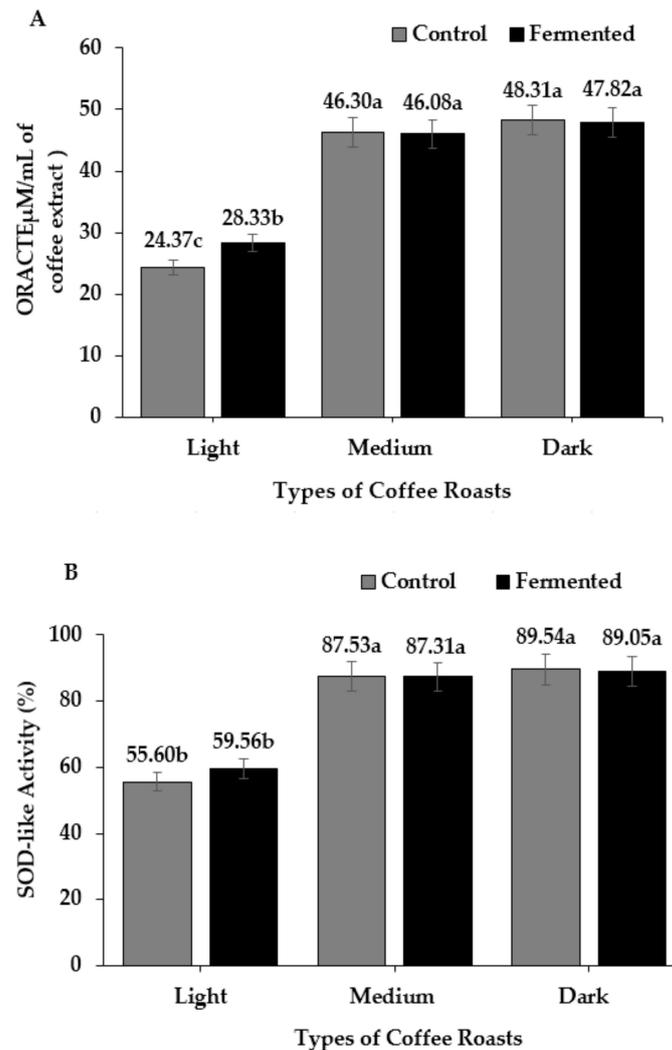


Figure 4. The oxygen radical absorbance capacity (ORAC) and superoxide dismutase-like (SOD)-like activity of fermented coffee at different degrees of roast (A and B, respectively). Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

3.3.2. DPPH and FRAP

DPPH and FRAP are among the various methods of evaluating the antioxidant properties of foods and drinks. The percentage of DPPH inhibition was higher in the fermented coffee than the non-fermented coffee in all roasting conditions (Figure 5A). Like the DPPH, the FRAP was improved in the fermented coffee in the light, medium, and dark roast types (Figure 5B). The maximum DPPH inhibition resulted from the fermented treatment in the light roasting condition (46.31%). The highest FRAP was found in the fermented coffee in the dark roasting condition (176.11 AAE µg/mL of coffee extract). In general, fermentation of green coffee beans using *W. anomalus* improved the DPPH and

FRAP. Adetuyi and Ibrahim [51] reported that fermentation enhanced the DPPH radical-scavenging ability and FRAP of okra seeds. The increase in DPPH capacity after the fermentation process shows that fermentation likely has great potential in generating some metabolites with superior radical scavenging activity [52]. The percentage of DPPH inhibition dropped as the roasting time increased (from the light to dark roasting condition). The result is in agreement with Jung et al. [53], who reported that lightly roasted coffee extract has the highest antioxidant activity with a low roasting temperature and that the DPPH decreases in the dark roasted extract. However, the FRAP improved as the roasting time increased. A supporting result published by Song et al. [54] showed that as the roasting time increased from 11 to 13 min (medium-light to medium-dark) the FRAP increased as well.

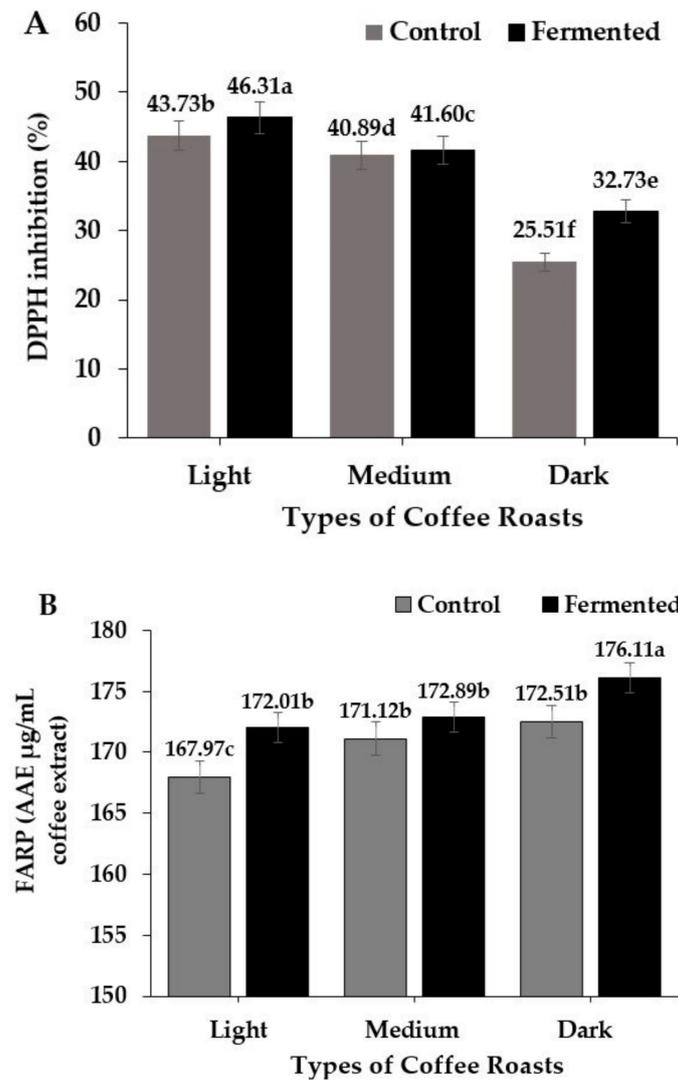


Figure 5. The DPPH inhibition (%) and Ferric reducing antioxidant power (AAE μg/mL of coffee extract) of fermented coffee. (A and B, respectively). Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

3.4. Total Phenol, Flavonoid and Tannin Content

The TPC of yeast-fermented coffee extracts had significant differences ($p < 0.05$) compared to the non-fermented coffee in all roasting types (Figure 6). During the medium roasting condition, the highest TPC difference was observed between the fermented and non-fermented coffee. The TPC in the control treatment showed a decreasing trend as the roasting time increased (light to medium to dark). The TPC in the fermented treatment was 0.98, 1.29 and 0.91 GAEmg/mL of coffee extract

in the light, medium and dark roasting condition, respectively (Figure 6). The fermentation of green coffee beans with *W. anomalous* increased the TPC of the coffee extract by 0.09, 0.46, and 0.21 GAE mg/mL of coffee extract compared to non-fermented coffee at each degree of roasting (light, medium and dark roast, respectively) (Figure 6). Generally, fermentation improved the TPC compared to non-fermented coffee. These results are in agreement with several reports on fermented seeds, where fermentation induced an improvement in the phenolic content of seeds, such as legumes [55–59], okra seeds [51], soybeans [60], and coffee [21,22]. Similarly, fermentation elevated the phenolic and flavonoid contents and improved the antioxidant properties of the following plant seeds: chestnut, adlay, walnut, and lotus seed [61]. This might be associated with, proteolytic enzymes from the starter organism that hydrolyze the complexes of phenolics into simple, soluble-free phenols and biologically more active forms during fermentation, which are readily absorbed by organisms [56,62]. However, other researchers' findings indicate the proteolytic activities alone did not show a significant TPC increase; rather, when it was combined with a pectinase enzyme, the olive oil polyphenol content showed a significantly high increment [63]. These findings lead us to conclude *W. anomalous*'s ability to produce pectinase enzymes linked with the increased TPC content in this experiment. In our experiment, the TPC was decreased as the roasting time increased, except the fermented coffee in the medium roast condition which significantly increased the TPC, regardless of the fermentation. A supporting result was published by Odžaković, B., et al. [64], the TPC decreased as the roasting temperature increased. During coffee roasting the degradation of polyphenols [36,44–46], which are sensitive to heat, are affected by roasting temperature. The reduction in the polyphenol content which is linked to the extended roasting is due to the heat-sensitive nature of such compounds and the lengthened roasting duration, as well as the high processing temperature. Phenol reductions or losses during the processing steps are undesirable because of their profound effects on human health.

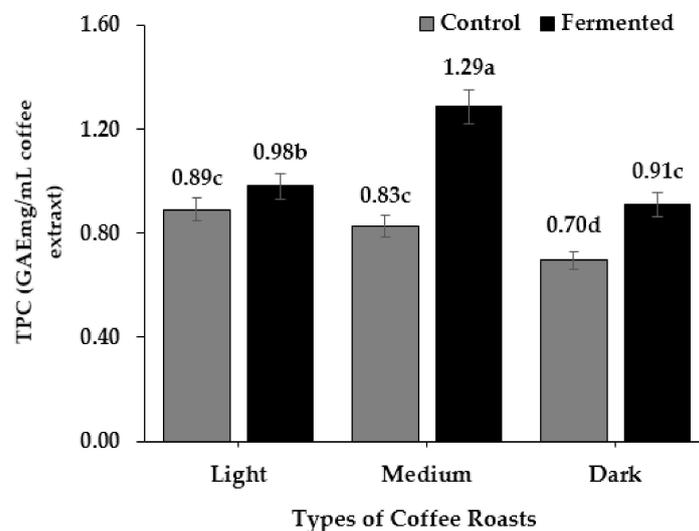


Figure 6. Total phenol contents (GAE mg/mL of coffee extract) of fermented coffee. Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

Fermentation of green coffee beans with *W. anomalous* significantly ($p < 0.05$) increased the TFC compared to non-fermented coffee in all roasting conditions (light, medium and dark) (Figure 7). TFC was approximately consistent in the control treatment in all roasting conditions. However, in the fermented coffee, the TFC was significantly higher in the light roasting compared to medium and dark roasting conditions. As shown in Figure 7, the TFC in the control treatment was 0.64, 0.67, and 0.64. The TFC in the fermented coffee at different roasting levels was 0.86 (light), 0.74 (medium), and 0.72 (dark) QE mg/mL of coffee extract. Fermentation improves the TFC content by 0.22, 0.07, and 0.08 QE mg/mL of coffee extract in the light, medium, and dark roasting conditions, respectively (Figure 7). These findings agree with several published papers on different fermented seeds, where

fermentation significantly enhanced the TFC compared to the TFC in unfermented seeds, such as in soybeans [60], legumes [45,55,56], coffee beans [21,22], and okra seeds [51]. The increase in the flavonoid content might be linked to the rise in acidic values during fermentation, which releases bound flavonoid components and makes them more bioavailable [51].

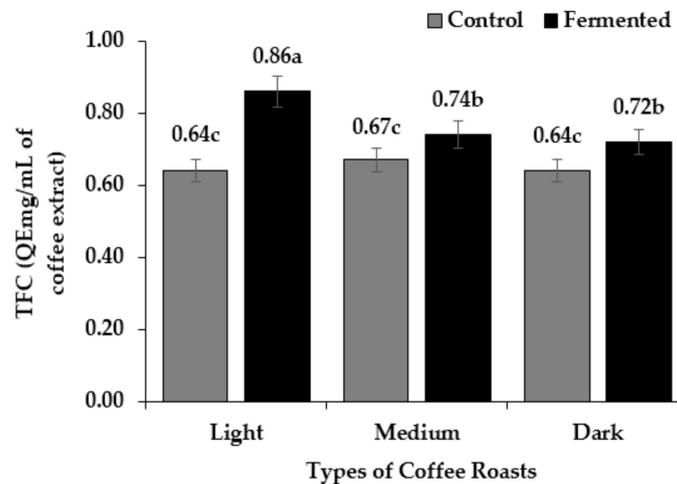


Figure 7. Total flavonoid contents (QE mg/mL of coffee extract) of fermented coffee. Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

Regardless of the fermentation, the average TFC from both treatments at each roasting condition was 0.75 ± 0.11 , 0.71 ± 0.03 , and 0.68 ± 0.04 QE mg/mL of coffee extract at the light, medium, and dark roasting levels, respectively. The average TFC value showed a decreasing trend as the roasting time increased, regardless of the fermentation. The result in our study coincides with Tiwari, B. K., et al. [65], who found that the TFC was reduced significantly as the roasting temperature increased from 80 °C to 120 °C and roasting duration proceeded from 20 min to 40 min. The cause for the reduction in the TFC at a higher temperature might be associated with the degradation of flavonoids. The preparation and processing of food may reduce the flavonoid contents depending on the techniques used [66].

The yeast-fermented coffee extracts had a significant difference ($p < 0.05$) compared to non-fermented coffee with regard to TTC in the light and dark roasting conditions (Figure 8). The TTC of non-fermented coffee was 0.53, 0.33, and 0.21 mg tannic acid/mL of coffee extract in light, medium and dark roasting conditions, respectively. In the fermented coffee, the TTC was 0.73, 0.28, and 0.15 mg tannic acid/mL coffee extract in the light, medium, and dark roasting conditions, respectively. In the light roasting condition, the tannin content was higher in fermented coffee than the control and it was lower than the control in the medium and dark roasting conditions (Figure 8). It has been reported that fermentation significantly decreased the tannin content of fermented sorghum cultivars [67], and in fermentation of *Xuan mugua* fruit with lactic acid bacteria, where it caused a significant decrease in the tannin content [68]. We have also found a supporting result in our previous experiment that indicates the fermentation of green coffee beans with *S. cerevisiae* and *S. fibuligera* strains reduced the TTC compared to non-fermented coffee [22]. Regardless of the fermentation, the TTC was reduced as the roasting time increased. The average TTC in each roasting condition was 0.63, 0.31 and 0.19 mg tannic acid mg/mL coffee extract in the light, medium, and dark roasting condition (Figure 8). Likewise, the tannin content was decreased in soya bean flour (0.01–0.30 g/100 g dry weight) [69], and 22% in maize [70]; because of soaking in water for 48 h and roasting. Tannin content has an astringent characteristic that contributes to the bitterness of coffee, so the reduction in tannins can be viewed as a positive aspect.

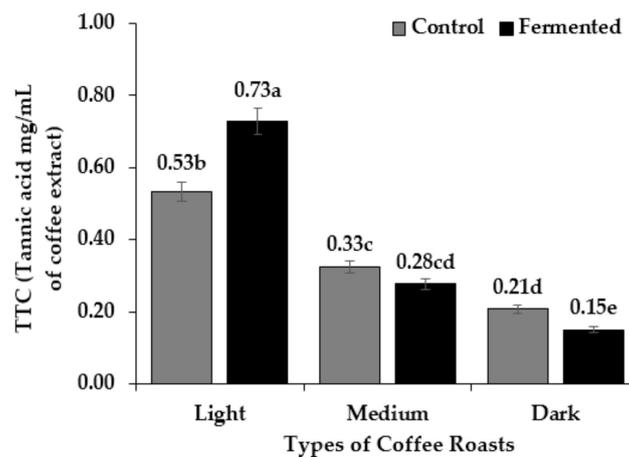


Figure 8. Total tannin contents (tannic acid mg/mL of coffee extract) of fermented coffee. Bars with different letters indicate a significant difference ($p < 0.5$) among treatments.

3.5. Sensory Evaluation

Based on the results of consumer acceptance ratings, the non-fermented coffee received a higher score than the fermented coffee in terms of its color, sourness, mouthfeel, acidity, and overall quality. However, the fermented coffee received the highest rating with respect to aroma, bitterness, and astringency. The average score for the overall quality of the coffee was 5.22 and 4.51 for non-fermented and fermented coffee, respectively (Figure 9). The average score for acidity was 3.75 for the fermented coffee and 4.15 for the non-fermented coffee. Kwak et al. [21] have evaluated the consumer acceptability of fermented green coffee beans with different yeasts, noting that one of their fermented coffee treatment acceptability ratings was lower but insignificant when compared to the control treatment. In addition, they mentioned that all the fermented coffee received lower ratings when compared to the control. Fermentation has both positive and negative influences on the flavor and aroma characteristics of coffee. Based on the overall quality rating, we have categorized the consumer responses into two groups: people who preferred the non-fermented coffee over the fermented coffee (55.48%) and people who preferred the fermented coffee over the non-fermented coffee (44.52%). A supporting result was published by Kwak et al. [21], who found that 39.4% of consumers preferred fermented coffee while 60.6% preferred non-fermented coffee by disliking the control (non-fermented) treatments based on the overall quality ratings. However, consumer acceptance evaluations are very subjective to the individual.

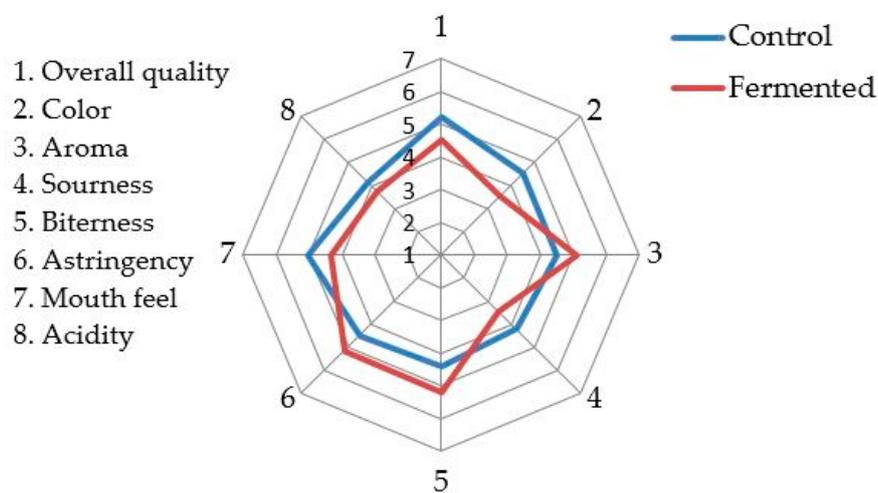


Figure 9. The consumer acceptability ratings of fermented and non-fermented coffee.

4. Conclusions

This study showed the responses of the coffee beans fermented with *W. anomalus* strain KNU18Y3. The fermentation of green coffee beans with *W. anomalus* for 24 h increased the DPPH, FRAP, TPC, and TFC when compared to non-fermented coffee, whereas the SOD-like and ORAC did not significantly differ between the fermented and non-fermented coffee beans. We have also found that the fermentation of coffee beans for 24 h is enough to modify the functionality of coffee beans. The extent of roasting had both negative and positive effects on the overall antioxidants, TPC, TFC, and TTC. The degradation of the TPC, TFC, and TTC was observed as the roasting time increased from 11.5 to 16 min, regardless of the fermentation. The fermented coffee received better ratings in terms of its aroma, bitterness, and astringency parameters. Moreover, 44.52% of participants chose the fermented coffee over the non-fermented one based on the overall quality ratings. This result shows that fermented coffee is also preferred by consumers. Since the consumer evaluation experiment was conducted blind (the participants were not informed of the coffee types), the increased antioxidants and phenolic and flavonoid compounds will probably attract more consumers when they are informed of these positive aspects of fermented coffee. While the *W. anomalus* yeast has the potential to be used for green coffee bean fermentation, further selection and evaluation of microorganisms should be continued to maximize the functionality of the coffee bean and its health benefits.

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