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Impacts of Fermentation on the Phenolic Composition, Antioxidant Potential, and Volatile Compounds Profile of Commercially Roasted Coffee Beans

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Abstract: Fermented coffee beans are believed to have significantly different compositions of phenolic and volatile compounds and physicochemical properties compared to unfermented coffee beans. This study evaluated the effects of fermentation on coffee beans at a commercially roasted level by characterizing their phenolic compounds and semi-quantifying their volatile compounds using liquid chromatography–electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) and headspace/gas chromatography–mass spectrometry (HS-SPME-GC-MS). Coffee beans from two varieties of *Coffea arabica*, Geisha (G) and Bourbon (B), both fermented beans had higher contents of total phenolic compounds (G: 33.52 mg/g; B: 29.95 mg/g), total flavonoid (G: 0.42 mg/g; B: 0.35 mg/g), total tannins (G: 3.49 mg/g; B: 3.18 mg/g), and higher antioxidant potential in all assays. In total, 131 phenolic compounds were tentatively characterized via LC-ESI-QTOF-MS/MS, where 73 and 65 phenolic compounds were characterized from fermented Geisha and Bourbon, respectively. Regarding GC-MS, the fermented coffee beans had higher levels of phenols, pyrazines, furan, and furanic compounds. These findings substantiated that fermented coffee beans exhibit elevated levels of phenolic and volatile compounds and greater antioxidant activity, which could contribute to relatively higher nutritional values and organoleptic properties.

Keywords: *Coffea arabica*; fermentation; phenolic characterization; antioxidant activity; volatile aromatic compounds

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1. Introduction

In recent times, there has been a significant evolution in the coffee industry. The cost of coffee beans has surged to its peak in a decade, reaching approximately USD 2.0429/lb, and the consumption of 10,060 tons of coffee beans was achieved in 2022 [1]. Presently, coffee beans hold a status beyond that of a mere staple commodity. Consumers are in pursuit of coffee beans characterized by their premium quality, which promises to afford them enriched drinking experiences and health benefits. Hence, coffee that offers novel sensory perceptions and nutritional quality is greatly coveted by consumers. There are diverse elements such as coffee bean genetics, post-harvest processing, brewing, and serving techniques that can exert influence on the quality of coffee beans [2].

The variety of coffee beans could be linked to their chemical properties. Among the numerous Coffea species, *Coffea arabica* and *Coffea canephora* stand out as the most widely cultivated species globally. *Coffea arabica* is characterized by a smoother, sweeter taste with

flavor notes reminiscent of chocolate, making it more preferred in the market. On the other hand, *Coffea canephora* exhibits a stronger and harsher taste, often with bitter undertones, along with grainy or rubbery nuances [3]. Coffee beans contain a rich variety of bioactive compounds. Caffeine, the most well-known and extensively studied component, is responsible for the stimulating effects associated with coffee consumption [4]. Additionally, coffee beans contain other noteworthy bioactive compounds such as chlorogenic acid (CGA), cafestol, and kahweol, all of which could exert significant biological and pharmacological impacts on human health [5,6]. The presence of polyphenols, including feruloylquinic acid, dicaffeoylquinic acid, and CGA lactones, has been documented in substantial quantities within coffee bean extracts [7]. Notably, the occurrence of these bioactives and polyphenols in coffee beans could affect the overall taste of the brewed coffee [8].

The processing of coffee beans involves two primary methods: the dry method (DP), also known as the natural process, and the wet method. After harvesting, coffee cherries are meticulously chosen and sorted via either manual selection or immersion in water. Then, after selection, for the dry process (DP), which is also referred to as the natural process, the cherries are dried directly using sunlight or artificial technologies, followed by stripping the dried cherry to obtain the underlying green coffee beans (GCB) [2]. In the wet method, the cherries are initially depulped to remove their outer skin and incubated for a period to allow fermentation to happen; then, after fermentation, the beans are washed, dried, and depulped for the green coffee beans [9]. Fermentation serves a dual purpose, as it removes any residual mucilage adhering to the beans and concurrently contributes to enhancing the beverage flavor. This enhancement occurs via the generation of microbial metabolites, which can act as precursors to volatile compounds that develop during the roasting process [10].

Thus, the objective of this study was to assess the differences in phenolic content and phenolic profiles between fermented and unfermented Arabica coffee beans. The antioxidative potential of the phenolic profiles within the coffee beans was measured using various spectrophotometric methods. Furthermore, phenolic compound characterization was accomplished via liquid chromatography–electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS), followed by the semi-quantification of volatile compounds utilizing headspace/gas chromatography–mass spectrometry (HS-SPME-GC-MS). Additionally, an assessment of the physiochemical differences between the fermented and unfermented coffee beans was conducted. The findings from this study will contribute to the contrasting antioxidant potential and the profiles of the phenolic and volatile compounds exhibited in fermented and unfermented coffee beans.

2. Materials and Methods

2.1. Chemical and Reagents

Deionized Milli-Q water was used for all assays, obtained from the Millipore Milli-Q Gradient Water Purification System (Darmstadt, Germany). All standards used for the following antioxidant assays, namely gallic acid, quercetin, catechin, L-ascorbic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Chemicals used for testing polyphenols and antioxidant potential were purchased from Chem- Supply Pty Ltd. (Adelaide, SA, Australia), namely anhydrous sodium carbonate, sodium hydroxide pellets, and 30% hydrogen, and the 98% sulfuric acid from RCI Labscan Ltd. (Bangkok, Thailand). Other chemicals, including Folin–Ciocalteu reagent, hydrated sodium acetate, hexahydrate aluminum chloride, vanillin, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ferric chloride (Fe (III)$Cl_3 \cdot 6H_2O$), ferric (III) chloride anhydrous, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, iron (II) chloride, iron (II) sulfate heptahydrate, 3-hydroxybenzoic acid, ferrozine, and potassium ferricyanide, were all purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

2.2. Sample Preparation

To mitigate any potential confounding effects of roasting on the phenolic content influenced by fermentation in coffee beans, commercial dark-roasted coffee beans (225 °C, 10 min) were utilized in this whole research. Fermented and unfermented coffee beans were selected and purchased from ACOFFEE (Collingwood, VIC, Australia), Four Kilo Fish Coffee (Hawthorn, VIC, Australia), Proud Mary (Collingwood, VIC, Australia), ONA(Brunswick, VIC, Australia), Code Black Coffee (Brunswick, VIC, Australia), Manhattan Coffee Roaster (Rotterdam, BR, Netherlands), Tin Man Coffee Roasters (Coburg North, VIC, Australia), Bench Coffee (Melbourne, VIC, Australia), Vacation Coffee (Melbourne, VIC, Australia), respectively. Coffee beans were ground into a uniform-sized powder using a coffee grinder (Russell Hobbs Classic, model DZ-1613, Melbourne, VIC, Australia) and then stored in a dark area at a temperature of −20 °C until extraction.

2.3. Extraction of Free and Bound Phenolic Compounds

The extraction of free phenolic compounds from coffee samples was conducted according to Wu et al. [11] with some modifications. Each coffee powder was mixed sufficiently with 70% ethanol at 1:10 (*w*/*w*) and vortexed for 30 s at 2700 rpm using Vortex Agitator (VM1, RATEK, Boronia, VIC, Australia). This was followed by an agitated incubation at 4 °C with continuous shaking at 120 rpm in a shaking incubator for 18 h (ZWYR-240 incubator shaker, Labwit, Ashwood, VIC, Australia). Then, the mixture was centrifuged for 15 min at 8000 rpm under 4 °C using Hettich Refrigerated Centrifuge (ROTINA380R, Tuttlingen, BadenWürttemberg, Germany). The supernatant was collected after filtered using 0.45 µm syringe filter (Thermo Fisher Scientific Inc., Waltham, MA, USA) and was then ready for the estimation of free phenolic contents.

The extraction method used for bound phenolics in samples was developed with some modifications based on Wu, Lu, Liu, Sharifi-Rad, and Suleria [11]. The sediments that remained after the collection of the supernatant were subjected to an alkaline hydrolysis process after complete drying in a fume hood for 4 days. The 2 M NaOH was added to the dry precipitates at a ratio of 1:2 (*w*/*w*), followed by adding the concentrated 4 M HCl to adjust the pH of the mixture to 7.0. Subsequently, 70% ethanol was added, and the mixture was incubated for 60 min (at 4 °C, 120 rpm) with sufficient agitation to allow the bound phenolic compounds to fully dissolve in the solvent. The supernatants were collected after centrifugation (20 min, 8000 rpm, 4 °C) and filtered with 0.45 µm syringe filter to obtain the bound phenolic extracts. Both the collected free and bound phenolic extracts were stored at −20 °C and were ready for further analysis.

2.4. Estimation of Phenolic Content and Antioxidant Potentials

The estimation of phenolic content (total phenolic content, total flavonoid content, and total condensed tannins) and antioxidant potentials (2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation-based assays, ferric reducing antioxidant power (FRAP) assay, hydroxyl radical ($^{\bullet}OH$) scavenging activity assay, Ferrous ion chelating activity (FICA), and Reducing Power Assay (RPA)) were conducted following the procedures developed by Jiang et al. [12]. These procedures were specifically designed for assays conducted in a 96-well plate (Corning Inc., Midland, NC, USA), and the absorbances were measured using the Multiskan® Go microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA). All samples were tested in triplicate, and the results were expressed as the equivalent of related standards.

2.4.1. Total Phenolic Content (TPC)

The Folin–Ciocalteu method used for determining the total phenolic content in coffee beans was based on Jiang, Subbiah, Wu, Bk, Sharifi-Rad, and Suleria [12] with some modifications. Briefly, 25 µL of sample extract was first added to the 96-well plate and mixed with 25 µL Milli-Q water-diluted Folin reagent and 200 µL of Milli-Q water. This mixture was then subjected to a 5 min dark incubation at ambient temperature. After

incubation, 25 µL of 10% (*w/w*) sodium carbonate solution was added, and the plate was further incubated for 1 h under the same dark conditions. Meanwhile, gallic acid in ethanol solution ranging from 0 to 200 mg/mL was prepared and used to generate calibration curve. To eliminate the sample's color interference on absorbance, a mixture of sample and water was used as blank. After incubation, the absorbance was measured at 765 nm using spectrophotometer (Multiskan® Go microplate photometer), and results were expressed as mg of gallic acid equivalents (GAE) per gram of coffee bean powder on a dry weight basis (mg GAE/g) ± standard deviation (SD).

2.4.2. Total Flavonoid Content (TFC)

The measurement of the total flavonoid content in fermented and unfermented coffee beans followed the methodology described by Suleria et al. [13] with slight modifications. In brief, 80 µL of sample extract was mixed with 80 µL of 2% aluminum chloride and 120 µL of sodium acetate solution (50 g/L) in a 96-well plate. Then, the plate was incubated in the dark condition at room temperature for 2.5 h. After the incubation, the absorbance was measured at 440 nm using a calibration curve of quercetin in methanol solution, with concentrations ranging from 0 to 50 µg/mL. The results were obtained by subtracting the blank and expressed as milligrams of quercetin equivalents (QE) per gram of coffee bean powder on a dry weight basis (mg QE/g), with the standard deviation (SD).

2.4.3. Total Condensed Tannins (TCT)

Similarly, the total content of condensed tannins in fermented and unfermented coffee bean samples was determined based on the method described by Wu, Lu, Liu, Sharifi-Rad, and Suleria [11] with some modifications. In this process, 25 µL of coffee bean extracts were added to a 96-well plate, followed by 150 µL of a diluted 4% vanillin in methanol solution and 25 µL 32% sulfuric acid. Meanwhile, a sample in water was used as the blank. The plate was then incubated in the dark at room temperature for 15 min, and the absorbance was measured at 500 nm. A standard curve was generated using catechin in methanol solution, with a concentration ranging from 0 to 1000 µg/mL. The total content of tannins was expressed as milligrams of catechin equivalent (CE) per gram of coffee beans on a dry weight basis (mg CE/g), with standard deviation (SD) reported.

2.4.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

A modified DPPH assay generated by Fan et al. [14] was applied to evaluate the free radical scavenging activity in the fermented and unfermented coffee beans. In total, 40 µL of coffee extract was mixed with 260 µL of diluted 0.1 mM DPPH in a methanol solution in the plate and incubated in the dark for 30 min at room temperature. Trolox in an ethanol solution, with concentrations ranging from 0 to 200 µg/mL, was used as the standard to produce the calibration curve. Additionally, 40 µL of the sample in the same reagent, along with an equal amount of water, was used as the blank. The reduction in absorbance was measured at 517 nm at the end of incubation, and the results of this assay were expressed as milligrams of Trolox equivalents (TE) per gram of coffee bean powder on a dry weight basis (mg TE/g) with the standard deviation (SD) reported.

2.4.5. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) Radical Cation-Based Assay

A modified $ABTS^{\bullet+}$ decolorization assay, as described by Hu et al. [15], was conducted with some modifications to assess the antioxidant capacity of the fermented and unfermented coffee bean extracts. In this assay, $ABTS^{\bullet+}$ solution was prepared by mixing 12.5 mL of 7 mM ABTS in water solution with 220 µL of 140 mM potassium persulfate, and it was incubated in the dark for 16 h at room temperature before use. The prepared $ABTS^{\bullet+}$ solution was then diluted with analytical-grade ethanol to achieve a final absorbance of 0.7 at 734 nm, indicating that it was ready to be used. Briefly, 10 µL of sample or standard was added and mixed with 290 µL of final prepared diluted $ABTS^{\bullet+}$ dye inside 96-well

plate. This mixture was then incubated at room temperature in the dark for 6 min. After incubation, the absorbance of sample was recorded. Trolox in ethanol solution, with concentrations ranging from 0 to 500 μg/mL, was used as the standard for the calibration curve, and sample in same reagent amount of water was used as the blank. Similarly, the results were expressed as mg TE/g ± SD.

2.4.6. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay used for fermented and unfermented coffee beans was conducted based on the methods of Wu, Lu, Liu, Sharifi-Rad, and Suleria [11] and Suleria, Barrow, and Dunshea [13]. The FRAP dye used for the assay was prepared by combining 300 mM sodium acetate solution, a 10 mM TPTZ solution, and a 20 mM Fe[III] solution in a ratio of 10:1:1 (*v/v/v*). In a 96-well plate, 20 μL of sample or standard was added to 280 μL of the prepared FRAP dye. The mixture was then incubated at 37 °C in the dark for 10 min, and the absorbance was measured as 593 nm. Trolox in ethanol solution, ranging from 0 to 200 μg/mL, was used as the standard to represent the FRAP radical scavenging activity. A sample with water was used as the blank. The results were expressed as milligrams of Trolox equivalents per gram of coffee powder on a dry weight basis (mg TE/g), along with the standard deviation (SD).

2.4.7. Hydroxyl Radical (•OH) Scavenging Activity Assay

The hydroxyl radical scavenging activity of both fermented and unfermented coffee beans was assessed using a modified Fenton-type reaction method, as described by Lee et al. [16]. In this procedure, 50 μL of sample extract or standard was added into the 96-well plate and mixed with 50 μL of 6 mM ferrous sulfate heptahydrate and 50 μL of 6 mM hydrogen peroxide. The plate was then incubated for 10 min under 25 °C in the dark. After 10 min, 50 μL of 6 mM 3-hydroxybenzoic acid was added to the plate cell and it was further incubated under the same condition for another 10 min. The absorbance of the plate was measured at 510 nm after the final incubation. A calibration curve was established using Trolox in ethanol solution as standard (ranging from 0 to 400 μg/mL), and a sample in water served as the blank. The results for hydroxyl radical scavenging activity were reported as milligrams of Trolox equivalents per gram (mg TE/g) with the standard deviation (SD).

2.4.8. Ferrous Ion Chelating Activity Assay

The FICA assay used for fermented and unfermented coffee beans was modified and produced by Zhu et al. [17]. To conduct this assay, 15 μL of sample or standard solution was added, along with 85 μL of water, 50 μL of 2 mM ferrous chloride, and 50 μL of 5 mM ferrozine, in the 96-well plate. The plate was then incubated for 10 min in the dark at room temperature, and absorbance was measured after incubation at 562 nm. EDTA solution ranging from 0 to 50 μg/mL was used as the standard to create the calibration curve, and a sample in water was used as the blank. The results were reported as mg EDTA equivalents per dry weight of coffee powder (mg EE/g) ± SD.

2.4.9. Reducing Power Assay (RPA)

The estimation of reducing power in fermented and unfermented coffee was conducted using the modified method developed by Wang et al. [18], which is indicated by a color change from yellow to green. In this assay, 10 μL of sample or standard was added to the plate, followed by the addition of 25 μL of a 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (III) solution. The plate was then incubated at room temperature in the dark for 20 min. After incubation, 25 μL of 10% trichloroacetic acid, 85 μL of water, and 8.5 μL of 0.1% ferric chloride solution were added to the plate. The plate was incubated under the same condition for an additional 15 min. Trolox in ethanol solution ranging from 0 to 500 μg/mL was used as the standard, and the sample in water was used as the blank. The results were measured after the last 15 min incubation and expressed as milligrams

of Trolox equivalents per gram of coffee powder on a dry weight basis (mg TE/g) with standard deviation (SD).

2.5. Liquid Chromatography–Electrospray Ionization Quadrupole Time-of-Flight Mass Spectrometry (LC-ESI-QTOF-MS/MS)

The characterization of phenolic compounds was performed using the LC-ESI-QTOF-MS/MS method, which was generated and modified by Peng et al. [19] and Wu, Lu, Liu, Sharifi-Rad, and Suleria [11]. The analysis involved the use of an Agilent 1200 series HPLC coupled with an Agilent 6520 Accurate-Mass Q-TOF LC/MS system equipped with an electrospray ionization source (ESI) for initial identification and characterization. The sample separation was carried out by a Synergi Hydro-RP 80A LC column (250 mm × 4.6 nm, 4 µm) (Phenomenex, Lane Cove, NSW, Australia) at 25 °C. The mobile phase consisted of 98% acetic acid in water (mobile phase A) and a mixture of acetonitrile, water, and acetic acid in a ratio of 100:1:99 (*v*/*v*/*v*) (mobile phase B). Mass spectra were obtained in the m/z range from 50 to 1300, and peaks were identified in both positive and negative ionization modes. The acquisition and analysis of data were conducted using Mass Hunter Data Acquisition Software Version B.03.01 (Agilent Technologies, Santa Clara, CA, USA) and Personal Compounds Database and Library (PCDL). For compounds that exhibited a mass error lower than 5 ppm and a PCDL score above 80, further MS/MS identification and m/z characterization were carried out.

2.6. Headspace Solid-Phase Microextraction Coupled to Gas Chromatography–Mass Spectrometry (HS-SPME-GC-MS)

The identification and semi-quantification of volatile compounds in ground coffee samples were analyzed via HS-SPME-GC-MS following the methods by Rocchetti et al. [20] and modified by Wu, Lu, Liu, Sharifi-Rad, and Suleria [11]. The analysis involved utilizing a gas chromatograph (6850 series II Network GC System, Agilent Technologies, Santa Clara, CA, USA) coupled with an HS-SPME system (PAL RSI I20, CTC Analytics AG, Zwingen, Switzerland) and a mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies, Santa Clara, CA, USA). A 30 m DB-Wax capillary column (Agilent Technologies, Santa Clara, CA, USA) with an internal diameter of 0.25 mm and a film thickness of 0.25 µm was selected, along with a 65 µm PDMS/DVB fiber (Fused Silica, Sigma Aldrich, St. Louis, MI, USA). Helium was used as the carrier gas with a column head pressure of 60 kPa, and samples were incubated at 60 °C for 15 min, followed by a 15 min extraction and a 6 min desorption period. The GC oven program was set as follows: it started with an initial temperature of 40 °C for 5 min, followed by a gradual increase to reach 190 °C at a rate of 5 °C/min and was kept at that temperature for 8 min. Later, the temperature was further raised to 240 °C at a rate of 10 °C/min and maintained for 10 min. The acquisition was performed in SCAN mode within the mass range of 35 to 350 m/z, with a solvent delay time of 2 min. Samples were prepared as follows: A concentration of 100 mg/L 4-Octanol was prepared and used as the internal standard for semi-quantification. It was mixed with 1g of ground coffee sample inside the GC vials and followed the set temperature gradient program. The linear retention index (LRI) was calculated following the equation below using an alkane standard (C_7–C_{20}) by comparing the retention time of a target compound (RTx) with the retention times of n-alkanes (RTn and RTn+1) that eluted before and after the target compound.

$$\text{LRI (target compound)} = 100 \times ((\text{RTx} - \text{RTn}/(\text{RTn+1} - \text{RTn}) + \text{n})$$

The LRI and mass spectrum of the volatile compounds detected in coffee samples were then compared with data from the NIST Chemistry WebBook spectrum library (NIST2017) and the NIST mass spectra database, respectively. Semi-quantification was conducted by comparing the response area of the target compound with a closely eluted compound of known concentration after confirming the LRI and compound's MS.

2.7. Statistical Analysis

The results were presented after subtracting blank or control values as the mean ± standard deviations (SD) from triplicated independent analyses (n = 3). Statistical analysis was performed using Minitab 18.0 Statistical (Minitab®, Minitab Inc., State College, PA, USA). To assess significant differences among the samples, one-way analysis of variance (ANOVA) and Tukey's honestly significant differences (HSD) test were utilized, and a significance level of $p < 0.05$ was achieved.

3. Results and Discussion

3.1. Phenolic Content Estimation Assays (TPC, TFC, TCT)

The phenolic contents in the coffee samples were estimated based on the results of three phenolic assays, which are TPC, TFC, and TTC, as shown in Table 1. Overall, the amount of free phenolic compounds was higher than the amount of bound phenolic compounds for all samples, and fermented coffee samples showed a significantly higher phenolic content than unfermented coffee samples ($p < 0.05$).

Table 1. The total phenolic compounds and antioxidant activities from different coffee samples.

	Fermented Coffee Bean		Unfermented Coffee Bean	
	Geisha	**Bourbon**	**Geisha**	**Bourbon**
		Free Phenolic		
TPC (mg GAE/g)	33.52 ± 2.31 ^{a}	29.95 ± 1.75 ^{b}	29.19 ± 0.74 ^{abc}	23.82 ± 0.81 ^{c}
TFC (mg QE/g)	0.42 ± 0.07 ^{a}	0.35 ± 0.06 ^{ab}	0.35 ± 0.08 ^{ab}	0.25 ± 0.08 ^{b}
TCT (mg CE/g)	3.49 ± 0.38 ^{a}	3.18 ± 0.23 ^{a}	4.91 ± 0.74 ^{b}	4.45 ± 0.81 ^{b}
DPPH (mg TE/g)	116.79 ± 0.55 ^{a}	91.34 ± 0.23 ^{b}	32.81 ± 2.97 ^{c}	32.68 ± 3.20 ^{c}
FRAP (mg TE/g)	110.06 ± 0.55 ^{a}	86.83 ± 1.97 ^{b}	62.39 ± 4.95 ^{c}	30.99 ± 0.16 ^{d}
ABTS (mg TE/g)	285.45 ± 1.77 ^{a}	266.61 ± 3.84 ^{a}	144.41 ± 1.94 ^{b}	256.27 ± 1.06 ^{ab}
OH-RSA (mg TE/g)	255.08 ± 3.91 ^{a}	279.66 ± 1.18 ^{a}	230.51 ± 3.35 ^{ab}	196.61 ± 4.82 ^{b}
FICA (mg EE/g)	0.79 ± 0.13 ^{a}	0.77 ± 0.42 ^{a}	0.68 ± 0.10 ^{ab}	0.63 ± 0.29 ^{b}
RPA (mg TE/g)	45.37 ± 0.61 ^{ab}	52.95 ± 3.90 ^{a}	29.56 ± 1.47 ^{c}	34.16 ± 0.15 ^{b}
TAC (GAE mg/g)	6.48 ± 0.28 ^{a}	5.83 ± 0.66 ^{b}	5.89 ± 0.27 ^{ab}	5.20 ± 0.36 ^{b}
		Bound Phenolic		
TPC (mg GAE/g)	7.59 ± 0.12 ^{a}	6.97 ± 0.08 ^{a}	3.14 ± 0.12 ^{b}	2.56 ± 0.15 ^{b}
TFC (mg QE/g)	0.07 ± 0.01 ^{a}	0.04 ± 0.16 ^{a}	-	-
TCT (mg CE/g)	-	-	-	-
DPPH (mg TE/g)	13.30 ± 2.30 ^{a}	9.79 ± 0.70 ^{ab}	0.60 ± 0.86 ^{b}	0.53 ± 0.15 ^{b}
FRAP (mg TE/g)	12.89 ± 1.23 ^{ab}	20.00 ± 0.78 ^{a}	5.56 ± 0.87 ^{b}	8.08 ± 0.12 ^{b}
ABTS (mg TE/g)	75.63 ± 1.03 ^{a}	73.62 ± 0.38 ^{a}	41.81 ± 1.13 ^{b}	8.89 ± 3.11 ^{c}
OH-RSA (mg TE/g)	53.25 ± 1.53 ^{a}	49.15 ± 1.38 ^{ab}	48.31 ± 1.38 ^{ab}	28.25 ± 1.44 ^{b}
FICA (mg EE/g)	0.79 ± 0.16 ^{ab}	0.97 ± 0.15 ^{a}	0.78 ± 0.06 ^{ab}	0.72 ± 0.20 ^{b}
RPA (mg TE/g)	8.01 ± 0.83 ^{a}	9.31 ± 0.05 ^{a}	3.59 ± 0.06 ^{b}	2.63 ± 0.54 ^{b}
TAC (GAE mg/g)	0.99 ± 0.13 ^{a}	0.94 ± 0.02 ^{a}	0.75 ± 0.08 ^{b}	0.81 ± 0.18 ^{ab}

Values are mean ± standard deviation per gram powder weight; n = 3 samples per sample. Mean values within the same column with different superscript letters ($^{a–d}$) are significantly different from each other ($p < 0.05$). GAE (gallic acid equivalents); QE (quercetin equivalents); CE (catechin equivalents); TE (Trolox equivalents).

The free phenolic content in fermented Geisha and Bourbon coffee beans was found to be 33.52 mg and 29.95 mg GAE/g, respectively, which was higher than that in the unfermented beans (29.19 mg and 23.82 mg GAE/g, respectively). This increase in total phenolic content aligns with Kwak et al. [21], where all groups of fermented green coffee beans exhibited higher phenolic content compared to the unfermented control group. The increased phenolic content after fermentation can be attributed to the liberation of bound phenolic compounds from the coffee beans [22]. Coffee beans contain various groups of phenolic compounds, with chlorogenic acids (CGAs) being the major phenolic components in coffee brews. These CGAs are commonly found in the conjugated form

of free hydroxycinnamic acids and quinic acids, primarily including caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), p-coumaroylquinic acids (pCoQAs), dicaffeoylquinic acids (diCQAs), as well as caffeoylquinic acid lactones (CQLs) and feruloylquinic acid lactones (FQLs) [23]. Some previous studies have documented the biotransformation process of conjugated hydroxycinnamic acids via the fermentation of lactic acid bacteria or yeast decarboxylases/reductases in various plant sources [22,24,25]. According to Lee et al. [26], following fermentation, the decrease in phenolic acid concentration could be attributed to an increase in volatile phenol levels. This is the result of phenolic acid catabolism, in which the breakdown of l-phenylalanine via the Ehrlich pathway is mediated via yeast, contributing to a 1.9-fold increase in 2-phenylethanol levels. Simultaneously, the reductions in the levels of alkanes, acids, and aldehydes can be attributed to the unique hydrophobic substrate metabolizing pathways.

Since all the sample beans used in this study were commercially roasted coffee beans, it is important to note that during the roasting process of coffee beans, the Maillard reaction leads to the accumulation of melanoidins. These melanoidins can react with the Folin–Ciocalteu reagent used in total phenolic content (TPC) analysis, thereby potentially increasing the measured results. This could lead to confounding effects in the context of fermentation [27].

In the results of total flavonoid content (TFC), the fermented coffee beans showed slightly higher levels of both free and bound phenolic compounds compared to the unfermented coffee samples. Additionally, in the results for total condensed tannin (TCT), the fermented coffee samples exhibited lower TCT levels compared to the unfermented coffee beans. However, it should be noted that both TFC and TCT assays were unable to accurately determine the bound phenolic content, as the levels of flavonoids and condensed tannins were too low to produce a discernible effect on the absorbance of the reagent used in the assays. These two results align with the findings of a study conducted by Kwak, Jeong, and Kim [21], where all groups of fermented coffee beans exhibited higher TFC values compared to the unfermented control group. The increase in flavonoid content can be attributed to the accumulation of acidic compounds during the fermentation process. This accumulation may liberate bound flavonoids from the food matrix, leading to the transformation of flavanols into their aglycone forms, a process that occurs during coffee fermentation of lactic acid bacteria (LAB) and yeast [28]. Also, the fermentation effect on increasing flavonoid content in coffee beans has correlated to the TFC results from several previous studies on different plant sources [29].

For TCT, condensed tannins from the condensation of flavanols could be metabolized by several species of LAB, for example, *Lactobacillus plantarum* and *Lactobacillus pentosus*, fungi and yeasts that possess tannic acid hydrolase into the free gallic and ellagic acid monomers [30,31]. Therefore, the fermented coffee beans exhibit lower results in TCT compared to unfermented coffee beans due to the microbiologic metabolism. But as intensive commercial roasting could also break the condensed tannin structure into the flavonoids in a lower molecular mass, like anthocyanin, this breakdown could potentially increase the free TFC values for both fermented and unfermented coffee beans [32,33].

3.2. Antioxidant Activities of Coffee Bean Estimation

Due to the complexity of bioactive compounds profiles in plants, mixed analytical procedures are generally used to estimate the antioxidant capacity of samples [34]. In this study, the antioxidant capacity of coffee beans was estimated using the result analyzed from the six antioxidant activity assays, as shown in Table 1. Overall, all assay results were correlated with the phenolic content assays and were consistent with previous studies, as fermented coffee beans exhibit higher antioxidant activity than unfermented coffee beans [35].

DPPH and ABTS assays are commonly used to determine the antioxidant capacity in samples based on their free radical scavenging capacity by transferring the hydrogen atom [36]. Both the fermented and unfermented coffee beans show a higher result for these

two assays in the free phenolic compounds extracts than the bound phenolic compounds. Also, fermented coffee beans are shown to have a higher value in both assays compared with unfermented coffee beans. The increased DPPH and ABTS results after fermentation indicate that fermentation in coffee beans has the potential to generate some metabolites that improve the radical scavenging activity, i.e., the antioxidant capacity of the beans, which then suggests a health-promoting processing [37]. The highest value in the DPPH assay was observed in the fermented Geisha coffee beans, measuring 116.79 mg TE/g, which was 25.45 mg TE/g higher than the fermented Bourbon, which had a value of 91.34 mg TE/g. On the other hand, the unfermented Geisha, with a value of 32.81 mg TE/g, exhibited a significantly lower antioxidant activity compared to the fermented Geisha. A similar trend can be observed in both the Geisha and Bourbon coffee beans in the ABTS assay, with the highest value achieved in the fermented Geisha sample at 285.45 mg TE/g compared to 144.41 mg TE/g in the unfermented Geisha.

RPA and FRAP assays were both based on the reducing powder of antioxidants by transferring one electron to turn Fe^{3+} into an Fe^{2+} ion [38]. The reducing capacity of fermented Bourbon coffee beans is the highest among the other groups of coffee beans, measuring 52.95 mg TE/g, and it is significantly higher than the value of the unfermented Bourbon group, which has a value of 34.16 mg TE/g. For FRAP, the highest value is also shown in the fermented Geisha coffee bean as 110.06 mg TE/g, which has a significant difference compared to the unfermented Geisha at 62.39 mg TE/g. Furthermore, the reducing capacity of fermented Bourbon coffee beans is also significantly higher than that of the unfermented Bourbon coffee beans, with values of 86.83 mg TE/g and 30.99 mg TE/g, respectively.

The $^{\cdot}$OH-RSA assay has a different mechanism compared to the previous four assays, which is based on the Fenton and Haber–Weiss reaction, producing Fe^{3+} and hydroxyl radicals from Fe^{2+} by reacting with hydrogen peroxide [39]. Similarly, both groups of fermented coffee beans have a higher value than the unfermented coffee beans (Fermented Geisha: 255.08 mg TE/g; Fermented Bourbon: 279.66 mg TE/g; Unfermented Geisha: 230.51 mg TE/g; Unfermented Bourbon: 196.61 mg TE/g).

In FICA assays, the mechanism of testing the antioxidant potential of compounds is achieved by chelating ferrous ions to form the ferrous and ferrozine complexes [13]. The results showed a slight increase in both groups of fermented coffee beans compared with unfermented coffee beans as 0.79 mg EE/g, 0.68 mg EE/g, 0.77 mg EE/g, and 0.63 mg EE/g (fermented Geisha, unfermented Geisha, fermented Bourbon, and unfermented Bourbon, respectively).

Generally, the results of these six antioxidant assays align with previous studies, suggesting that the antioxidant activity of coffee beans increases after the fermentation process, primarily due to the release of bound phenolics from the beans [28,35]. As all samples underwent commercial dark roasting, a significant accumulation of melanoidins occurred during the roasting, which potentially introduced complexity when assessing the fermentation's impact on phenolic content and antioxidant activity. Since the comparison is drawn against commercially dark roasted coffee beans, the potential confounding effect of melanoidins could be controlled. Also, as lower molecular mass polyphenols were generated from the degradation of high molecular mass polyphenols during fermentation, the total antioxidant capacity of the coffee bean is increased. The metal chelation activity that came from these high molecular mass polyphenols will be reduced and reflected in FICA assays [40].

3.3. Correlation between Phenolic Compounds and Antioxidant Potential

To investigate the correlation between the content of phenolic compounds (TPC, TFC, and TCT) and the related antioxidant activities in the sample (DPPH, FRAP, $ABTS^{\bullet+}$, $^{\bullet}$OH-RSA, FICA, TAC), the pairwise Pearson's correlation test was implemented, and the results were shown in Table 2. An absolute value of a correlation coefficient closer to 1 indicates that a stronger correlation exists. TPC and TFC were positively correlated to most

antioxidant assays except FICA, indicating that the phenolic and flavonoid compounds in the coffee beans were primarily responsible for the antioxidant capacity that the sample presented [41].

Table 2. Pearson's correlation coefficient (r) for the pairwise correlation between phenolic content assays (TPC, TFC, and TCT) and antioxidant potential assays (DPPH, FRAP, ABTS, RPA, •OH-RSA, and FICA).

	TPC	TFC	TCT	DPPH	FRAP	ABTS·+	·OH	FICA	RPA	TAC
TPC	1									
TFC	0.997 *	1								
TCT	0.918 *	0.900 *	1							
DPPH	0.859 *	0.873 *	0.605	1						
FRAP	0.918 *	0.935 *	0.702	0.966 *	1					
ABTS·+	0.917 *	0.900 *	0.808	0.876 *	0.837 *	1				
OH	0.986 *	0.979 *	0.904 *	0.856 *	0.905 *	0.919 *	1			
FICA	−0.357	−0.348	−0.601	−0.061	−0.092	−0.290	−0.382	1		
RPA	0.953 *	0.942 *	0.820	0.908 *	0.909 *	0.960 *	0.975 *	−0.279	1	
TAC	0.992 *	0.985 *	0.949 *	0.824	0.884 *	0.910 *	0.985 *	−0.451	0.943 *	1

* Significant correlation with $p \leq 0.05$.

TCT assay was negatively correlated with DPPH and FRAP, while it could result from the complex processing methods used in coffee bean commercial processing. The condensed tannins could be degraded into flavonoids and other simple phenolics during the fermentation and roasting processing, resulting in improved TFC and TPC values.

3.4. LS-ESI-QTOF-MS/MS Characterization of Phenolic Compounds in Roasted Fermented and Unfermented Coffee Beans

The phenolic compounds present in both fermented and unfermented coffee beans, including phenolic acids, lignans, stilbenes, flavonoids, isoflavonoids, and other polyphenols, were analyzed in an untargeted manner and were tentatively identified and characterized based on their m/z values and MS spectra, using both negative and positive modes of ionization. The compounds selected for further analysis and verification underwent MS/MS analysis and were chosen based on criteria such as a mass error of less than 5ppm and a score higher than 80 in the PCDL library.

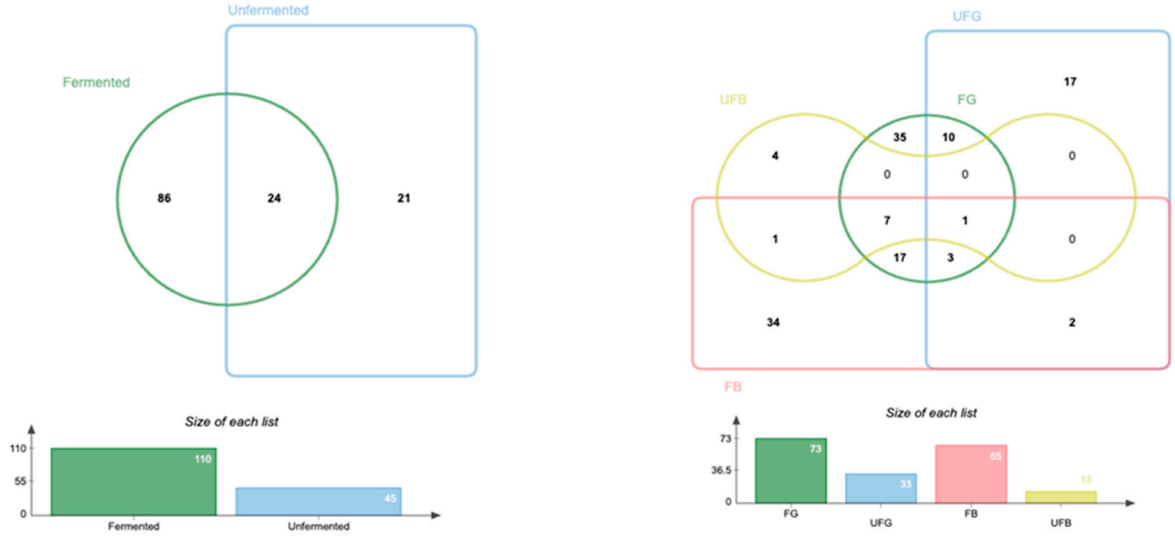
The two Edward's Venn diagrams in Figure 1 were generated based on the phenolic compound profiles that were identified in the fermented and unfermented coffee beans. A total of 131 phenolic compounds were identified based on the LC-ESI-QTOF-MS/MS method in this study, and there were 24 phenolic compounds identified in both fermented and unfermented coffee beans. As shown in Figure 1, the fermented coffee beans contain more unique (n = 86) and total phenolic compounds (n = 110) than the unfermented coffee beans (n = 21). Fermented Geisha has the largest phenolic profiles (n = 73) identified among the other group of coffee beans, followed by fermented Bourbon coffee beans (n = 65), sharing 18 compounds in total and 17 compounds identified only in the fermented coffee beans. One phenolic compound (Dihydromyricetin 3-O-rhamnoside) was identified in all coffee samples. These Edward's Venn graphs also demonstrate that fermentation has the potential to enhance the production of a variety of phenolic compounds via the degradation of free phenolic compounds and the release of bound phenolic compounds, which are consistent with the TPC results [42].

As observed in Table 3, 18 phenolic compounds were identified and characterized in the fermented and unfermented coffee samples, in which 3 were Hydroxycinnamic acids, 4 were lignans, 1 stilbene, 3 flavonols, 1 flavone, 1 anthocyanin, 2 hydroxycoumarins, and 1 furanocoumarin.

Table 3. LC-ESI-QTOF-MS/MS characterization of phenolics from coffee samples.

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Error (ppm)	MS^2 Product Ions	Coffee Samples
Phenolic acid										
Hydroxycinnamic acids										
1	*p*-Coumaroyl tartaric acid	$C_{13}H_{12}O_8$	9.185	$[M-H]^-$	296.0541	295.0468	295.0470	0.7	115	FG
2	3-Caffeoylquinic acid	$C_{16}H_{18}O_9$	25.788	** $[M-H]^-$	354.0942	353.0869	353.0871	0.6	253, 190, 144	* FB, UFG
3	5-5′-Dehydrodiferulic acid	$C_{20}H_{18}O_8$	27.889	$[M+H]^+$	386.0999	387.1072	387.1073	0.3	369	* FB, FG
Lignans										
4	Enterolactone	$C_{18}H_{18}O_4$	16.535	$[M+H]^+$	298.1192	299.1265	299.1263	−0.7	281, 187, 165	FG
5	7-Oxomatairesinol	$C_{20}H_{20}O_7$	45.824	$[M+H]^+$	372.122	373.1293	373.1297	1.1	358, 343, 328, 325	FB
6	Schisandrol B	$C_{23}H_{28}O_7$	89.329	$[M+H]^+$	416.1825	417.1898	417.1892	−1.4	224, 193, 165	FB
7	Schisantherin A	$C_{30}H_{32}O_9$	86.857	$[M+H]^+$	536.2054	537.2127	537.2130	0.6	519, 415, 385, 371	* FG, FB
Stilbenes										
8	4-Hydroxy-3,5,4′-trimethoxystilbene	$C_{17}H_{18}O_4$	39.862	$[M+H]^+$	286.1191	287.1264	287.1264	0.0	271, 241, 225	* FB, FG
Flavonoids										
Flavonols										
9	Quercetin 3-*O*-(6″-malonyl-glucoside)	$C_{24}H_{22}O_{15}$	24.64	$[M+H]^{l+}$	550.0956	551.1029	551.1028	−0.2	303	* FB, FG
10	Prodelphinidin dimer B3	$C_{30}H_{26}O_{14}$	34.76	$[M+H]^+$	610.1332	611.1405	611.1394	−1.8	469, 311, 291	FB
11	Quercetin 3-*O*-xylosyl-rutinoside	$C_{32}H_{38}O_{20}$	40.43	$[M+H]^+$	742.1962	743.2035	743.2009	−3.5	479, 317	FG
Flavones										
12	Gardenin B	$C_{19}H_{18}O_7$	88.235	$[M+H]^+$	358.1066	359.1139	359.1148	2.5	344, 329, 311	FB
Anthocyanins										
13	Pelargonidin 3-*O*-rutinoside	$C_{27}H_{31}O_{14}$	21.545	$[M+H]^+$	579.1701	580.1774	580.1775	0.2	271, 433	* FB, FG
Isoflavonoids										
14	6′′-*O*-Malonylgenistin	$C_{24}H_{22}O_{13}$	4.081	$[M+H]^+$	518.107	519.1143	519.1118	−4.8	271	UFG
15	6′′-*O*-Acetylglycitin	$C_{24}H_{24}O_{11}$	10.044	$[M+H]^+$	488.1327	489.14	489.1397	−0.6	285, 270	UFG
Other polyphenols										
Hydroxycoumarins										
16	Esculin	$C_{15}H_{16}O_9$	26.85	$[M+H]^+$	340.0794	341.0867	341.0855	−3.5	179, 151	FG
17	Coumarin	$C_9H_6O_2$	24.106	$[M+H]^+$	146.0369	147.0442	147.0442	0.0	103, 91	* UFG, FG
Furanocoumarins										
18	Isopimpinellin	$C_{13}H_{10}O_5$	80.679	$[M+H]^+$	246.0539	247.0612	247.0611	−0.4	232, 217, 205, 203	* FG, FB

The ** mark in the ionization mode represented that the compound was detected in both positive and negative modes, while only one mode's data were presented in the table; and * mark used for when compounds were found in more than one sample, and only results for samples with * were shown in the table; coffee samples mentioned in abbreviations were Fermented Geisha "FG", Unfermented Geisha "UFG", and Fermented Bourbon "FB", Unfermented Bourbon "UFB"; and abbreviation "RT" used for retention time.



(**a**) (**b**)

Figure 1. (**a**) Edward's Venn diagram of phenolic compounds in fermented and unfermented coffee beans; (**b**) Edward's Venn diagram of phenolic compounds in fermented/unfermented Geisha and Bourbon; the number in the figure represents how many phenolic compounds that have been identified in the samples using LC-ESI-QTOF-MS/MS.

3.4.1. Phenolic Acids

Three hydroxycinnamic acids (*p*-coumaroyl tartaric acid, 3-caffeoylquinic acid, and 5-5′-dehydrodiferulic acid) were characterized in the fermented and unfermented coffee samples. *p*-coumaroyl tartaric acid and 5-5′-dehydrodiferulic acid can only be traced in fermented coffee samples, and 3-caffeoylquinic acid can be traced in both the unfermented and fermented coffee samples. The fermentation of coffee could lead to the degradation of hydroxycinnamoyl esters, for example, the caffeoyl-*O*-methylquinic acid, and release quinic acid and the corresponding hydroxycinnamic acid [43].

Hydroxycinnamic Acids

Compound **1** (RT = 9.185 min) with $[M\text{-}H]^-$ ion m/z at 295.0470 was detected only in the fermented Geisha coffee bean extract and characterized as *p*-coumaroyl tartaric acid. The compound 3,5-5′-dehydrodiferulic acid, with a retention time of 27.889 min, can be detected in the positive mode with $[M+H]^+$ ion at m/z 387.1073 and was present in both fermented Geisha and Bourbon phenolic extracts. Compound **2** (RT = 25.788 min) characterized as 3-caffeoylquinic acid (3-CQA) was detected in both unfermented coffee bean and fermented coffee bean, performed product ions in both positive and negative mode at m/z 353.0871. The identification of 3-CQA has confirmed fragments at m/z 253, m/z 190, and m/z 144, which indicated the loss of HCOOH-3H_2O (100 Da), $C_6H_5O_2$-3H_2O (163 Da), and $C_7H_{11}O_6$-H_2O (209 Da) from the parent ion [11,13]. 3-CQA is the most abundant isomer of the caffeoylquinic acid that belongs to one of the major subclasses of chlorogenic acids in coffee beans. It is naturally found in green coffee beans and has essential biological antioxidant activity among several chronic diseases like cardiovascular disease and obesity [44]. 3-CQA was also detected in several cultivars of coffee beans in both roasted and green conditions by Ho et al. [45] and Moon et al. [46].

3.4.2. Lignans and Stilbenes

Four lignans were all detected in fermented coffee samples, enterolactone (compound **4**), 7-oxomatairesinol (compound **5**), schisandrol B (compound **6**), and schisantherin A (compound **7**). Lignans are classified as phytoestrogens due to their bioactive benefits for humans, potentially influencing estrogenic activities by acting as agonists/antagonists of estrogen receptors. Additionally, their antioxidant properties may help reduce the risk

of chronic diseases [47]. Compound **4** was detected in this study with $[M+H]^+$ ion m/z at 299.1263 and was confirmed as enterolactone via its fragments at m/z 281, m/z 187, and m/z 165. According to Heinonen et al. [48] and Landete [49], enterolactone (ENL) is a mammalian lignans that has been converted from plant lignans using several bacteria via chemical transformation. Compound **6** was identified as schisandrol B, detected in the positive mode with $[M+H]^+$ ion at m/z 417.1892, and exhibiting fragmented ions at m/z 224, m/z 193, and m/z 165. This identification was confirmed via comparison with a previous study by Zou et al. [50]. Compound **7**, schisantherin A, is a dibenzo-cyclooctadiene lignan that was identified in this study in a positive ionization mode at m/z 537.2130 and had product ions at m/z 519, m/z 415, m/z 385, and m/z 371 due to the loss of H_2O, C_6H_5COOH, C_6H_5COOH-CH_2O, and C_6H_5COOH-C_2H_4O, as confirmed in a previous study [51]. The anti-inflammatory potential of schisantherin A was also reported, which could downregulate NF-κB and MAPK signaling by reducing the synthesis of inflammatory cytokines in lipopolysaccharide-induced inflammation [52].

4-hydroxy-3,5,4′-trimethoxystilbene (compound **8**) was the only stilbene detected in the fermented coffee sample in the positive mode, with $[M+H]^+$ ion m/z at 287.1264. It was previously detected in both cherry and blackberries [15,38]. It was shown to have some anticancer effects according to Androutsopoulos et al. [53], which could inhibit the protein kinase C and D, induce apoptosis in cells, and decrease the activity of transcription factors (NF-κB and AP-1).

3.4.3. Flavonoids and Isoflavonoids

There were three flavonols (compounds **9**, **10**, **11**), one flavone (compound **12**), one anthocyanin (compound **13**), and two isoflavonoids (compounds **14**, **15**) identified in this study. All flavonols, flavones, anthocyanins, and isoflavonoids were detected only in the fermented coffee beans, while the two isoflavonoids were only detected in unfermented Geisha coffee beans. Compound **9** was detected only in fermented coffee beans in the positive mode with $[M+H]^+$ ion m/z at 551.1028. Its identification was confirmed via the loss of malonyl conjugates (malonyl + glycosyl) 248 Da at m/z 303 from the precursor ions [54]. Quercetin 3-O-(6″-malonylglucoside) was not previously reported to be detected in fermented coffee samples but was mostly found in lettuce, aquatic *Ranunculus* species, *Gerbera* flowers, and *Clitoria ternatea* flowers [55]. Compound 10, prodelphindin dimer B3 (prodelphinidin B3), was detected in fermented coffee beans in the positive mode with $[M+H]^+$ ion m/z at 611.1394. Prodelphinidin B3 is a flavan-3-ol that was previously identified in colored barley grains and pomegranate peels and has antioxidant properties that are health promoting to humans [56,57]. Quercetin 3-*O*-xylosyl-rutinoside was identified based on the precursor ion detected at positive mode $[M+H]^+$ ion with m/z at 743.2009. This compound was previously detected in the hops (*Humulus lupulus* L.) [58].

Compounds **12** and **13** were detected in fermented coffee beans giving $[M+H]^+$ at m/z 359.1148 and 580.1775. They were characterized as flavones gardenin B and anthocyanins pelargonidin 3-*O*-rutinoside, respectively. 6″-*O*-Malonylgenistin and 6″-*O*-Acetylglycitin were the two isoflavonoids detected only in unfermented Geisha coffee beans, with precursor $[M+H]^+$ ions at m/z 519.1118 and 489.1397, respectively. During the coffee bean fermentation, isoflavones could be transformed into a more bioavailable aglycones form via lactic acid bacteria (LAB) and, therefore, remain undetected in fermented coffee beans [59].

3.4.4. Other Polyphenols

Three other polyphenol compounds were detected in coffee samples, except compound **17**, coumarin, which was detected in both fermented and unfermented coffee beans. The other two compounds (**16** and **18**) were only detected in fermented coffee beans. Compounds **16** and **17** were hydroxycoumarins, detected with the precursor ions $[M+H]^+$ at m/z 341.0855 and 147.0442, respectively. As the product ions of compound **17** were located at m/z 103 and m/z 91 by losing CO_2 (44 Da) and CO (28 Da), it was then characterized as coumarin [50].

3.5. Volatile Compounds in Fermented and Unfermented Coffee Beans

As shown in Table 4, a total of 18 compounds (3 phenols, 5 pyrazines, 4 acids and esters, 3 furan and furanic compounds, 2 ketones, and 1 anethole) were identified and semi-quantified in the coffee samples, and some showed significant differences between the fermented and unfermented coffee samples.

Both acetic acid and propanoic acid were organic acids that were detected relatively higher in fermented coffee beans, which is correlated to the study conducted by Avallone et al. [60]. The presence of organic acids in coffee beans has confirmed the microbial action that is involved in the fermentation process, mainly due to the degradation of the mucilage layer by lactic acid bacteria (LAB) and acetic acid bacteria (AAB) [59,61]. As shown in Table 4, acetic acid was found to be the predominant organic acid in fermented coffee beans, which has an average amount of 9.058 μg/mL. However, there was a negative influence of acid presence on the quality or acceptance of the coffee beverages according to Louzada Pereira et al. [62]. Propanoic acid was detected in all fermented coffee samples, with an average amount of 0.298 μg/mL. The bacteria of the genus *Bacillus*, specifically *B. megaterium*, could be responsible for this acid production during coffee bean fermentation [63].

Although all five pyrazines identified in this study could be found in both fermented and unfermented coffee samples, the content of all five pyrazines detected in the fermented coffee samples was relatively higher than in the unfermented coffee samples. The compound 1, pyrazine, was the highest at 1.286 μg/mL compared to the other four pyrazines. Pyrazine compounds were generally related to nutty, roasty, and earthy aromas [64]. It arises as the same Maillard reaction occurred in both fermented and unfermented coffee samples due to the same roasting degree achieved. The formation of Amadori-type conjugates differs in the Amadori rearrangement between the dipeptide and sugar adduct, resulting this pyrazine clusters [65]. All five pyrazines were identified before in a previous study conducted by Elhalis et al. [9] and showed the same outcome, as fermented coffee beans presented a higher level of pyrazines than unfermented coffee beans.

There were three furan and furanic compounds detected in the coffee samples. The content of 2-furanmethanol and furfural was relatively higher than the 5-methyl-2-Furancarboxaldehyde. As furans are commonly developed from the reaction between sugars and amino acids, during fermentation, the amount of sugar in the coffee bean will be reduced and result in less furan production than the unfermented coffee bean [66]. Thus, microbial activities could contribute to the formation of these furan and furanic compounds via their impacts on the volatile precursor during fermentation [67,68].

2,3-Butanediol has the relatively highest content of the three phenolic compounds identified in the fermented coffee samples, while the concentrations of the other two compounds were not significantly different between the fermented and unfermented coffee samples. 2,3-Butanediol can be produced during yeast fermentation, involving the reduction in diacetyl formed from the valine biosynthetic pathway, and contributes to a fruity, creamy, buttery flavor. It is commonly detected in commercially purchased coffee beans due to uncontrolled spontaneous fermentation [69,70].

A controlled and suitable fermentation process could be used and included in coffee bean processing for the desired quality of coffee bean products. However, further sensory analysis needs to be implied to test the relative sensory attributes generated in the coffee fermentation process.

Table 4. The content of volatile compounds identified in fermented and unfermented coffee samples via HS-SPME-GC-MS.

No.	Compound Name	Molecular Formula	RT (mins)	Aroma	Content (mg/g)			
					Fermented		Unfermented	
					Geisha	Bourbon	Geisha	Bourbon
Phenols								
1	Phenol	C_6H_6O	31.02	Phenolic/Rubbery	0.07 ± 0.01	0.10 ± 0.03	0.05 ± 0.03	0.09 ± 0.01
2	1,6-Octadien-3-ol, 3,7-dimethyl- (linalool)	$C_{10}H_{18}O_2$	20.97	Citrus/Floral/Woody/Green	0.09 ± 0.03	0.05 ± 0.03	0.07 ± 0.03	0.03 ± 0.01
3	2,3-Butanediol	$C_4H_{10}O_2$	21.43	Fruity/Creamy/Buttery	0.41 ± 0.09	0.23 ± 0.12	0.20 ± 0.02	0.12 ± 0.19
Pyrazines								
4	Pyrazine	$C_4H_4N_2$	11.36	Nutty/Roasted	1.25 ± 0.17	1.32 ± 0.44	1.21 ± 0.06	1.11 ± 0.33
5	Pyrazine, 2,5-dimethyl-	$C_6H_8N_2$	14.76	Nutty/Peanut/Musty/Earthy	0.10 ± 0.27	0.93 ± 0.20	0.99 ± 0.36	0.64 ± 0.01
6	Pyrazine, 2,3-dimethyl-	$C_6H_8N_2$	15.44	Butter/Coffee/Caramellic/Roasted	0.33 ± 0.04	0.27 ± 0.03	0.25 ± 0.01	0.16 ± 0.06
7	Pyrazine, 2-ethyl-5-methyl-	$C_7H_{10}N_2$	16.75	Coffee/Roasted/Nutty	0.42 ± 0.09	0.41 ± 0.10	0.38 ± 0.15	0.28 ± 0.02
8	Pyrazine, 2,6-diethyl-	$C_8H_{12}ON_2$	17.93	Nutty/Hazelnut	0.06 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Acid and esters								
9	Acetic acid	$C_2H_4O_2$	18.24	Sour/Overripe fruit	9.80 ± 2.61	8.31 ± 0.93	9.13 ± 0.55	7.73 ± 1.00
10	Butanoic acid, 3-methyl-	$C_5H_{10}O_2$	23.83	Mentholic/Fruity	1.18 ± 0.17	1.32 ± 0.44	1.21 ± 0.06	1.34 ± 0.33
11	Propanoic acid	$C_3H_6O_2$	20.61	Acidic/Cheesy/Vinegar/Oniony	0.26 ± 0.01	0.34 ± 0.03	0.35 ± 0.01	-
12	2-Butenoic acid, 3-methyl-	$C_5H_8O_2$	26.67	Green/Phenolic/Dairy	0.28 ± 0.06	0.24 ± 0.06	0.28 ± 0.02	0.32 ± 0.11
Furan and Furanic compounds								
13	Furfural	$C_5H_4O_2$	18.56	Bready/Woody/Baked	4.57 ± 0.13	5.34 ± 0.67	4.11 ± 0.92	5.25 ± 1.35
14	2-Furanmethanol	$C_5H_6O_2$	23.62	Sweet/Caramellic/Bready/Coffee	5.02 ± 0.37	4.97 ± 0.52	4.52 ± 0.40	4.86 ± 0.92
15	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	21.37	Caramellic/Spice/Maple/Bready	2.70 ± 0.41	3.28 ± 0.56	2.25 ± 0.47	3.14 ± 0.93
Ketones								
16	Butyrolactone	$C_4H_6O_2$	22.65	Creamy/Oily/Caramellic	0.58 ± 0.13	0.55 ± 0.04	0.50 ± 0.01	0.44 ± 0.11
17	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	$C_7H_{10}O_2$	28.86	Caramellic/Maple/Brown/Toasted	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Other compounds								
18	Anethole	$C_{10}H_{12}O$	27.41	Licorice/Anise/Spice/Sweet	0.95 ± 0.13	1.05 ± 0.16	0.80 ± 0.10	0.99 ± 0.21

4. Conclusions

This study has found that commercially fermented coffee beans contain relatively higher total phenolic compounds and exhibit greater antioxidant potential compared to unfermented ones. A total of 18 phenolic compounds were detected and characterized in fermented and unfermented coffee beans via LC-ESI-QTOF-MS/MS. Additionally, 18 volatile compounds were identified and semi-quantified in both fermented and unfermented coffee beans using HS-SPME-GC-MS. In general, fermented coffee beans were found to have more characterized phenolic compounds and higher concentrations of volatile compounds than their unfermented counterparts. Moreover, the analysis revealed that different coffee bean varieties possess distinct profiles and quantities of phenolic compounds after analysis, leading to unique physicochemical characteristics that can potentially influence their sensory properties and taste as perceived by humans. For instance, Geisha exhibits more complex phenolic profiles than Bourbon both before and after fermentation, suggesting a potentially more intricate and rich sensory profile following processing. Further sensory evaluation is needed to provide a more specific understanding of the sensory differences between fermented and unfermented coffee beans.

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