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Yeast Strain Inoculation in Coffee Wet Fermentation: Impact on Volatile Compound Formation

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Abstract

Starter cultures enhance coffee sensory quality by producing yeast-derived volatile organic compounds (VOCs). This study evaluated five native yeast strains—*Hanseniaspora guilliermondii*, *Pichia membranifaciens*, *Schizosaccharomyces pombe*, *Torulaspora globosa*, and *Rhodotorula aurantiaca*—for their VOC contributions in mucilage, green beans, and roasted coffee. A 2×2+1 factorial randomized complete block design assessed two consortia (c1, c2), two inoculation growth phases (lag, t1; exponential, t2), and a spontaneous fermentation control. VOCs were analyzed via HS-SPME-GC-MS, and sensory quality was evaluated per SCA protocols. Multivariate and univariate statistical analyses were conducted across matrices, demonstrating a shift from fermentation-derived alcohols and esters in mucilage and green beans to thermally generated pyrazines, furans, and aldehydes in roasted coffee. Treatment c2t2 exhibited higher metabolic activity in mucilage, with increased peak areas for acids, alcohols, and aldehydes, while c1t1 showed a greater chromatographic response for acids and ketones in green coffee. In roasted coffee, peak areas of compounds associated with enhanced aroma and body, such as 1-(2-furanyl)ethanone, 1-(2-furanyl)-1-propanone, and pyrazines, were significantly higher for c2, whereas 1-hydroxy-2-propanone, 1-hydroxy-2-butanone, and 1-acetyloxy-2-propanone were higher for t1. Although non-parametric sensory tests did not reveal statistically significant differences among treatments, c1t1 achieved the highest numerical overall cupping score (82.63 SCA points) due to improved flavor, acidity, and balance. Yeast inoculation obtained SCA total scores 2.6 to 3.5 points higher than that of spontaneous fermentation (79.13 points) without developing defective off-flavors. The evaluated yeasts show potential in modulating specific volatile profiles and sensory attributes in coffee effectively.

Keywords: mucilage fermentation; yeast consortium; volatile organic compounds; coffee quality

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1. Introduction

The primary role of coffee fermentation in the development of volatile organic compounds has been well established in different investigations, as has the possibility of modifying coffee's composition by varying the fermentation process [1–4]. Fermentation is a

promising candidate for developing new methodologies that can improve overall quality, with the possibility of starter cultures as a potential advantage. Yeasts emerge as classic microorganisms for enhancing aroma in many products, such as wine, beer, and chocolate. Currently, it is understood that the role of yeast in coffee fermentation is much more than just pectinase production, as it is likely to be essential in the production of organic volatile compounds, which are very beneficial to coffee quality [5]. By producing esters and alcohols that contribute to flavor complexity, yeasts have strong potential in enhancing coffee attributes and meeting market demand for differentiated coffee.

It has been shown that these desirable volatile compounds are significantly reduced when yeast growth is suppressed by natamycin, resulting in coffee with lower scores for flavor and aroma [6]. In conjunction with yeast-assisted fermentation processes, increased concentrations of specific organic acids, primary and secondary alcohols, esters, and ketones, among other chemical functional groups, are observed in the coffee beans, thereby positively impacting quality, reducing variability, yielding more stable products, and promoting their safety and preservation [4,7]. Even though in the roasting process of coffee beans, a considerable number of compounds that determine coffee's quality are produced, the relation between green beans' composition and the final product is unmistakable. VOCs from different fermentation methods reveal metabolic processes that benefit the coffee bean's composition in subsequent stages, such as roasting [1,8]. This has been well documented given the influence of the chosen process on the chemical composition of the more abundant groups [9]. For instance, the wet process yields more polysaccharide and protein extractions than the dry method, suggesting higher free amino acid content associated with balanced, valued sensory attributes [10]. It has also been demonstrated that some volatile organic compounds (VOCs), such as pyrroles and furans, might help identify the fermentation method [5].

Saccharomyces cerevisiae, *Torulaspora delbrueckii*, and *Pichia fermentans* have been the most studied yeasts in coffee fermentation, mainly in Brazil, and they promote the formation of different compounds depending on the processing method, with notable effects on sensory attributes [1,11,12].

Colombian coffee is processed chiefly using the wet method, where endophytic microbiota play a significant role. In this highly biodiverse environment, non-Saccharomyces yeasts belonging to the genera *Candida*, *Pichia*, *Hanseniaspora*, *Torulaspora*, *Meyerozyma*, *Kazachstania*, and *Wickerhamomyces* are the leading representatives of the yeast community, whose presence is influenced by the producing region, variety, processing method, and other factors [13–17]. Using native yeasts could improve the sensory profile of high-quality coffees. A methodology for the selection process is required to select appropriate strains that ultimately correspond to the desired profiles.

This study presents the selection and inoculation of yeasts isolated from the native microbiota across various fermentation processes [18]. Previous work demonstrated behavioral differences in these microbial communities [19] that were directly related to the sensory characteristics of coffee. This research reveals changes in the volatile composition of the mucilage, the primary substrate; green coffee beans, as the intermediate stage and determining factor in chemical composition; and roasted coffee beans, as the final product, as well as the sensory quality of the coffee beverage. Furthermore, this study highlights the vast potential of these native yeast strains for coffee fermentation and for future study and assessment.

2. Materials and Methods

2.1. Yeast Strain Selection

Yeast strains utilized in this study were obtained from the SaBio Research Group's microorganism collection at the Science Faculty, Universidad Nacional de Colombia.

Identification was performed using culture-dependent methods and metabolic profiling with the BIOLOG™ system (Biolog Inc., Hayward, CA, USA), which assesses 95 substrates. The strains originated from fermentation processes associated with high-quality coffee production [18]. The selection criteria focused on the ability to grow on coffee mucilage as the primary substrate. The VOC profiles of the isolates were subsequently analyzed as described below. Seven strains that demonstrated growth in a medium containing 5% mucilage were selected: *Hanseniaspora guilliermondii*, *Rhodotorula aurantiaca*, *Schizosaccharomyces pombe*, *Wickerhamomyces domercquiae*, *Candida apicola*, *Torulaspora globosa*, and *Pichia membranifaciens*. These yeast strains were then evaluated for their capacity to produce VOCs on the mucilage medium, as detailed in the Statistical Analysis Section.

Following strain selection based on volatile compound profiles, growth curves were generated to determine the durations of the lag and exponential phases. Each yeast strain was inoculated into a liquid medium containing 5 $g \cdot L^{-1}$ yeast extract and 10 $g \cdot L^{-1}$ glucose. Subsequently, 100 µL of each culture was transferred to microplates and monitored photometrically at 600 nm at hourly intervals for 24 h at room temperature (approximately 20 °C) using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific Inc., Vantaa, Finland). In addition, we assessed the yeast growth through plate count in Petri dishes using YGC Agar (YGC; Merck®, Darmstadt, Germany) as culture medium to determine the average CFU/mL in each phase.

2.2. Experimental Design

The effect of yeast inoculation on fermentation and its capacity for VOC production was evaluated using a randomized complete block design. Five treatments were arranged in a 2×2+1 factorial structure, with the quality of raw material serving as the blocking variable. Experimental factors included two yeast consortia designated C1 and C2, two growth phases denominated t1 and t2, and an absolute control representing non-inoculated or spontaneous fermentation. The experiments were distributed across four blocks, resulting in a total of 20 fermentations.

A liquid culture medium was prepared with 5% coffee mucilage, supplemented with 5 g/L glucose and essential elements including nitrogen, magnesium, potassium, sodium, calcium, phosphorus, and sulfur. The medium was sterilized at 116 °C for 10 min to prevent sugar caramelization. Each pure yeast strain was independently inoculated into the liquid medium by transferring a colony from a solid YGC agar (YGC; Merck®, Darmstadt, Germany) culture using a sterile swab and suspending it in sterile distilled water. The cell density was adjusted to 47% of transmittance, consistent with the procedure used for identification with the Biolog™ system (Biolog Inc., Hayward, CA, USA). A 1 mL aliquot of the cell suspension was inoculated into glass tubes containing 9 mL of the culture medium. Incubation was performed at room temperature for the time specified for each growth phase. For each strain, one tube was used to verify the concentration (CFU/mL) on YGC agar (YGC; Merck®, Darmstadt, Germany). Consortia were formed by combining two tubes of each strain, which resulted in an application rate of 6 mL per kilogram of depulped coffee. Yeast populations were not monitored during the fermentations.

The two consortia were established based on two factors. The first factor was each yeast strain's volatile organic compound (VOC) profile and its coffee-processing origin. The second factor involved evaluating the adaptive capacity of the strains during coffee fermentation, specifically assessing the lag phase (time 1, t1) and exponential phase (time 2,

t2) as determined by their respective growth curves. Then, t1 and t2 represent a combined effect of culture age and cell density.

2.3. Coffee Fermentation Processes

For each experimental block, 100 kg of *Coffea arabica* L. cv. Castillo fruits were used, cultivated at altitudes between 1260 and 1400 m in the central Colombian coffee zone (4.59 N, 75.35 W). The coffee fruits were handpicked to achieve over 85% ripeness, and lower-quality fruits were eliminated by flotation into water. The exocarp was removed using a horizontal cylinder pulper (JM Estrada Plus #5, Medellín, Colombia). The resulting pulped coffee was divided into five equal portions of 10 kg each, and randomly assigned to different treatments. Fermentation was performed for 24 h in open tanks at approximately 20 °C, without water addition.

Following fermentation, a sample of the residual mucilage was stored at −20 °C for subsequent chemical analysis. The coffee was washed with fresh water and then dried under forced air at 38 °C for approximately 48 h, until the moisture content reached 10–12% on a wet basis (w.b.). One 1 kg sample of dry parchment coffee (dpc) was collected for chemical and sensory analyses. This procedure was conducted independently for each treatment to ensure representative samples.

2.4. Volatile Organic Compound Determination

Volatile organic compounds (VOCs) were determined for each yeast strain on mucilage media, for each fermentation mucilage sample, and for both green and roasted coffee beans using Head Space Solid Phase Microextraction (HS-SPME) coupled with Gas Chromatography–Mass Spectrometry (GC-MS). Each sample type was transferred to SPME vials and processed according to the following protocol.

The yeast strains were analyzed by suspending each one in sterile distilled water at 1.5×10^8 CFU·mL^{-1}. One milliliter of this suspension was inoculated into tubes containing 9 mL of sterile medium supplemented with 5% mucilage as a carbon source and with essential elements for microbial growth, such as magnesium, nitrogen, phosphorus, and sulfur. After two hours, 3 mL of the fermenting medium was transferred to SPME vials and sealed, leaving a 2 mL head space for analysis. The remaining 6 mL from each fermentation was stored in sealed vials as counter-samples. Following 48 h of fermentation at room temperature, all vials were stored at 4 °C to slow yeast activity and subsequently kept at −20 °C until analysis. Each fermentation was performed in triplicate. Four samples were analyzed for coffee mucilage, green coffee beans, and roasted coffee.

Coffee mucilage: Samples of 3.0 g were taken from the treatments' end products. It was stored at −20 °C in sealed SPME vials for subsequent analysis.

Green coffee beans: The parchment of the dpc was manually removed from 50 g coffee samples to avoid temperature excess caused by the threshold. Then, the green coffee bean samples were cryogenically ground and stored at −20 °C in SPME vials containing 0.5 g of ground coffee for subsequent analysis.

Roasted coffee: Samples of 100 g from the sensory analysis were milled to a medium grind, and ground roasted coffee samples of 1.0 g were immediately placed into the SPME vials.

The same procedures for extraction, separation, and compound identification were used for all sample types. To define the methodological parameters, preliminary tests were conducted prior to finalizing the experimental conditions. These tests estimated desorption and adsorption times, sample quantities, and temperatures to confirm the extraction technique's stability and robustness.

For compound extraction, the vials were allowed to rest at room temperature for 60 min before analysis. Afterward, they were taken into 35 °C ovens for 30 min until they reached

equilibrium for head space, as mentioned. The compounds were trapped on a 75 µm SPME fiber of Carboxen/Polydimethylsiloxane (CAR/PDMS, Supelco Ref. 57319, Milwaukee, WI, USA) at the equilibrium temperature, with an exposure duration of 45 min. The desorption of the compounds was performed in the injection port of the gas chromatograph for 3 min at 280 °C in split-less mode.

An Agilent DBWAX 50 m × 320 µm × 1 µm film thickness column (Agilent Technologies, Santa Clara, CA, USA) in a gas chromatograph, Agilent HP 6890 GC (Agilent Technologies, Santa Clara, CA, USA), was used to separate the compounds, coupled with a mass spectrometer, Agilent 5973 MS, for detection. The carrier gas used was helium at a flow rate of 1.2 mL·min^{-1}. The column had a controlled temperature of 40 °C for 3 min, followed by a 7 °C·min^{-1} rate until a temperature equal to 110 °C was reached and kept for 1 min. Afterwards, it was elevated to 280 °C at a 10 °C·min^{-1} rate. The mass was scanned from 40 to 550 AMU at a scanning rate of 2.89 scans·s^{-1}. The ionization method used was electron impact at 70 eV. To monitor background contamination and system stability, blank runs (empty vials and fiber) and Quality Control (QC) samples were analyzed every 10 injections and verified for each sample due to manual injection. All measurements were performed in biological replicates ($n = 4$). Data in tables are presented as mean and standard deviation (SD). Volatile compounds below the detection limit (non-detects) were treated as zero for statistical processing.

Each sample's volatile compounds in the head space (HS) were identified by comparing their mass spectrum with the database (Wiley 275 Mass Spectral Data by NIST 98 Mass Spectral Library; John Wiley & Sons. Inc., Hoboken, NJ, USA). A minimum spectral match factor of 80% was applied for tentative identification. Due to the lack of retention index benchmarking (alkane series), all reported volatiles represent MSI Level 2 (Tentatively Annotated Compounds) according to Metabolomics Standards Initiative guidelines.

The area under the curve (Arbitrary Units) of the chromatographic peaks obtained determined the chromatographic response of identified compounds for each chemical family and sample type (matrix).

2.5. Sensory Analysis Determination

The samples were then evaluated by three coffee tasters with Q-Grader qualifications regarding the sensory characteristics per the roasting protocols, preparation, and analysis outlined by SCA [20], allowing comparisons between the different samples. Green coffee samples from five experimental blocks were roasted to a light-medium degree (Agtron Gourmet score 63 ± 1) within an 8–12 min duration, rapidly air-cooled, and rested for 24 h prior to cupping. Roasted samples were ground coarsely (70–75% passing through a US Mesh #20/850 µm sieve) immediately before evaluation. Cupping was performed using a ratio of 8.25 g of coffee per 150 mL of water at 93 °C. A total of 5 cups per sample replicate were evaluated across the sessions. This analysis entailed the assessment of coffee attributes, including fragrance/aroma, flavor, aftertaste, acidity, body, balance, clean cup, sweetness, defects (negative taste sensations), and the taster's overall rating. The scale allows for each attribute to be rated from good (6.00 to 6.75), very good (7.00 to 7.75), excellent (8.00 to 8.75), and exceptional (9.00 to 9.75). The sum of these ratings accounts for the final or total score of the sample, which likewise allows for the coffee to be categorized according to the following scale: inferior below specialty quality (<80.0), good quality (80 to 84.99), excellent (85.00 to 89.99), and exceptional (90 to 100).

The procedure established by SCA was meticulously followed to obtain roasted coffee beans in pursuit of maintaining the same roasting grade, as per the protocol recommendations [20].

2.6. Statistical Analysis

Multidimensional Scaling (MDS) was used to identify the similarity of the volatile profiles produced by yeasts on the mucilage medium.

Multivariate and univariate statistical analyses were performed separately for each sample type (matrix): mucilage, green coffee, and roasted coffee. Before analyzing the data, volatile organic compound (VOC) peak areas were standardized using Z-score auto-scaling to account for differences in scale between major and trace volatile metabolites. Unsupervised Principal Component Analysis (PCA) was performed to identify groupings and patterns in the samples based on the first two principal components (PC1 and PC2), and 95% confidence ellipses were calculated for each treatment. Permutational Multivariate Analysis of Variance (PERMANOVA) based on Euclidean distance matrices was executed with 9999 permutations. Pairwise PERMANOVA comparisons between individual factor treatments and the control were subsequently performed.

The chromatographic response of individual volatile compounds was analyzed using Analysis of Variance (ANOVA) for a randomized complete block design. The statistical model assessed the block effect, the main experimental factor effects, their interaction, and included a pre-planned orthogonal contrast to compare the means of the factorial-arrangement treatments against the control treatment. For compounds exhibiting significant main effects without interaction, differences between level means were examined. When a significant interaction was observed, the simple effects of the factors were evaluated. All statistical analyses were conducted at a 5% significance level ($\alpha = 0.05$).

The average, standard deviation, median, minimum, and maximum of the sensory scores were obtained for the attributes (fragrance/aroma, flavor, aftertaste, acidity, body, balance, taster's overall rating), and a total score was produced for each treatment. Sensory total scores from the 2×2+1 factorial structure were evaluated using non-parametric statistics suitable for randomized block designs ($N = 4$ blocks). Overall treatment differences were assessed using the Friedman test. Post hoc pairwise comparisons of each treatment against the control treatment were performed using two-tailed Wilcoxon signed-rank tests with Holm–Bonferroni *p-value* adjustments. Statistical significance was declared at $p < 0.05$.

Statistical computations were executed using R software (version 4.3.2; R Core Team, Vienna, Austria) with the stats package and Python (version 3.10 and 3.11.5). Statistical significance was set at $\alpha = 0.05$.

3. Results and Discussion

The HS-SMPE/GC-MS identification technique and the CAR/PDMS fiber used allowed accurate identification of the volatile profile, particularly the affinity for small molecules such as acids, esters, and alcohols in all three of the evaluated matrices, as shown for both green and roasted coffee beans [3,12,21–24].

3.1. Yeast Strains Selected

The seven selected yeast strains produced 14 compounds in the supplied medium, which contained mucilage and essential nutrients for microbial growth (see Table S1). *S. pombe*, *T. globosa*, and *W. domercquiae* generated the highest chromatographic area of ethanol, acetaldehyde, and other alcohols, including 1-propanol, 2-methyl-1-propanol, and 3-methyl-1-butanol. Previous studies have reported high ethanol production from glucose by *T. globosa* [25] and *S. pombe* [26]. In contrast, *R. aurantiaca* and *C. apicola* produced the lowest ethanol peak area, suggesting limited fermentative capacity. Additionally, species within the *Candida* genus are frequently observed at higher relative abundance in wine following fermentation [27].

Ethanol represents the primary volatile product of yeast metabolism, with diols, higher alcohols, and esters produced in lesser amounts. During alcoholic fermentation, ethanol is generated through the reduction of acetaldehyde by alcohol dehydrogenase (ADH) enzymes and also serves as an intermediate in pyruvate decarboxylation; regulation of acetaldehyde levels is essential for maintaining cellular homeostasis and supporting glycolysis [28]. At low concentrations, acetaldehyde imparts a pleasant, fruity aroma. In contrast, elevated fermentation temperatures can increase acetaldehyde accumulation, resulting in an undesirable, irritating odor [29].

Esters were the most prominent chemical family, followed by alcohols. Ethyl acetate was abundant, particularly in *H. guilliermondii* and *P. membranifaciens*. Ethanol, the main alcohol present, reacts with acetyl-CoA to form ethyl acetate, a process mediated by acyltransferases [1]. All apiculate yeasts produce high levels of ethyl acetate [30], accounting for its greater accumulation in *H. guilliermondii*. Ethyl esters such as ethyl acetate and ethyl propanoate contribute desirable floral and fruity aromas. *P. membranifaciens* produced high levels of these esters and was the only yeast to produce phenylethyl acetate, placing it among yeasts that can increase ester content in wine [29,31].

Ketones, including 2,3-butanedione (diacetyl) and 3-hydroxy-2-butanone (acetoin), are key contributors to coffee aroma, imparting buttery, fruity, and caramel characteristics. Furthermore, yeasts can reduce diacetyl to acetoin [32]. In the present study, *non-Saccharomyces* genera, particularly *Schizosaccharomyces*, *Rhodotorula*, and *Hanseniaspora*, produced higher amounts of acetoin.

Similarity analysis with the MDS method of volatile profiles identified three distinct yeast groups (Figure 1), consistent with previous classifications. The group containing *C. apicola* and *R. aurantiaca* produced low chromatographic areas of ethanol and related esters, such as ethyl acetate and ethyl propanoate. In contrast, *W. domercquiae*, *T. globosa*, and *S. pombe* generated higher quantities of ethanol and other alcohols, including 1-propanol and 2-methyl-1-propanol, as well as intermediate levels of acetaldehyde and acetic acid. *P. membranifaciens* and *H. guilliermondii* exhibited the highest levels of esters linked to fruity and floral aromas, including ethyl acetate, ethyl propanoate, and phenylethyl acetate [33]. Excessive production of 3-methyl-1-butanol and acetic acid can cause undesirable aromas; these compounds were produced at higher levels by *W. domercquiae* and *C. apicola,* respectively. Based on this analysis, the selected yeasts for consortium formation in coffee fermentations were *Hanseniaspora guilliermondii* (code 1), *Pichia membranifaciens* (code 7), *Schizosaccharomyces pombe* (code 3), *Torulaspora globosa* (code 6), and *Rhodotorula aurantiaca* (code 2).

Hanseniaspora, *Schizosaccharomyces*, *Torulaspora*, *Pichia*, and *Rhodotorula* are among the 32 yeast genera commonly associated with wine production, with *Saccharomyces* being the most prevalent [27]. Additionally, species of the *Torulaspora*, *Hanseniaspora,* and *Pichia* genera have been investigated as starter cultures for coffee fermentation due to their impact on coffee quality through the production of esters, fatty acids, and alcohols [7,12].A single yeast strain from each group, selected for similar volatile organic compound (VOC) profiles, was used to establish the first consortium (c1). These strains were isolated from the same original fermentative process [18] to maximize compatibility within the medium. The strains, which experienced identical environmental conditions during fermentation, included *H. guilliermondii* (code 1), *S. pombe* (code 3), and *R. aurantiaca* (code 2). In contrast, *S. pombe* and *T. globosa* share the same VOC profile group but originate from different fermentative processes, while *R. aurantiaca* was present in all coffee processes involving yeast-strain origin. Therefore, consortium 2 (c2) comprised strains isolated from different fermentative processes: *P. membranifaciens* (code 7), *T. globosa* (code 6), and *S. pombe* (code 3).

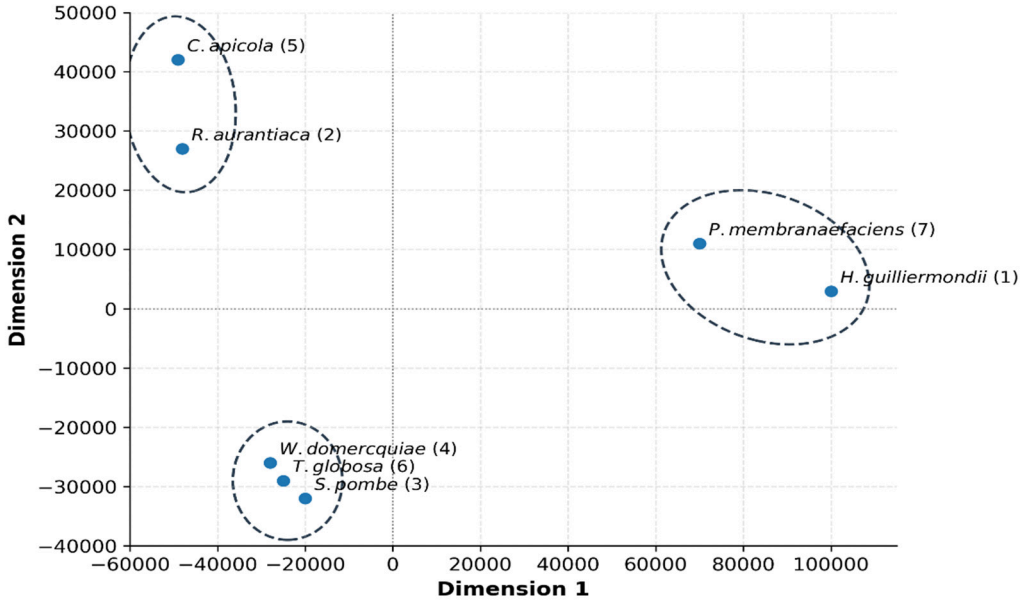


Figure 1. Similarity map of the volatile profile produced by seven native yeast strains on coffee mucilage. *Hanseniaspora guilliermondii* (1), *Rhodotorula aurantiaca* (2), *Schizosaccharomyces pombe* (3), *Wickerhamomyces domercquiae* (4), *Candida apicola* (5), *T. globosa* (6), and *Pichia membranifaciens* (7).

Four of the five selected yeast strains exhibited two distinct growth phases (Figure S1): an initial lag phase during the first eight hours, followed by a logarithmic phase beginning at nine hours. During the lag phase, cell concentrations ranged from 10^4 to 10^5 CFU·mL^{-1}. In the logarithmic phase, concentrations increased to 10^7 to 10^8 CFU·mL^{-1}. Since equal culture volumes were used for inoculation without adjusting the final cell doses, the second experimental factor, defined as time 1 (t1, 0–4 h, lag phase) and time 2 (t2, 10–12 h, logarithmic phase), reflects both the culture's physiological age and the initial inoculum density, not just the physiological state. *Rhodotorula aurantiaca* showed minimal growth in the initial screening medium because it is unable to use glucose as a main carbon source [19]. Instead, it metabolizes other carbohydrates found in coffee mucilage. Species in this genus are known as oxidative yeasts with low fermentation activity [34]. Because the growth curve of this strain was not determined before the experiment, its inoculation times (t1: 0–4 h; t2: 10–12 h) were aligned with those of the other consortium members for consistency. Consequently, the precise cell counts and physiological state of *R. aurantiaca* at inoculation remain unknown, representing an operational assumption and a limitation in characterizing this oxidative yeast strain.

The two consortia and the two yeast growth phases were combined to inoculate pulped coffee during fermentation. Volatile organic compounds were subsequently monitored after fermentations in coffee mucilage, green coffee beans, and roasted coffee samples.

3.2. Organic Volatile Compounds

A total of 81 compounds were identified in the three sample types. The number of compounds detected varied among the samples, with 34 in mucilage, 28 in green coffee, and 51 in roasted coffee (see Tables S2–S4). Pure mucilage produced a greater number of compounds, including alcohols and esters, compared to yeasts grown on media supplemented with 5% mucilage (see Table S1). This difference is attributed to the higher availability of carbon sources in pure mucilage, which facilitates fermentation. Alcohols and esters, which are associated with fermentation, were the predominant compounds in mucilage; however, their relative abundance decreased during bean transformation, consistent with the findings of Elhalis et al. [6]. In contrast, the number of aldehydes and ketones increased, becoming

the most representative groups in roasted coffee. The area under the chromatographic peaks also varied by sample type and increased for aldehydes and ketones in roasted coffee (Figure 2). The formation of aldehydes and ketones in green coffee beans is likely due to thermal reactions during drying or to the oxidation of amino acids and polyphenols by polyphenol oxidase [9]. Sugars and amino acids are well established as the main precursors of organic volatile compounds generated by thermal reactions [9,24,35]. During roasting, furans, pyrazines, pyridines, and pyrroles are formed, with the Maillard reaction playing a particularly significant role [6,9,10,24].

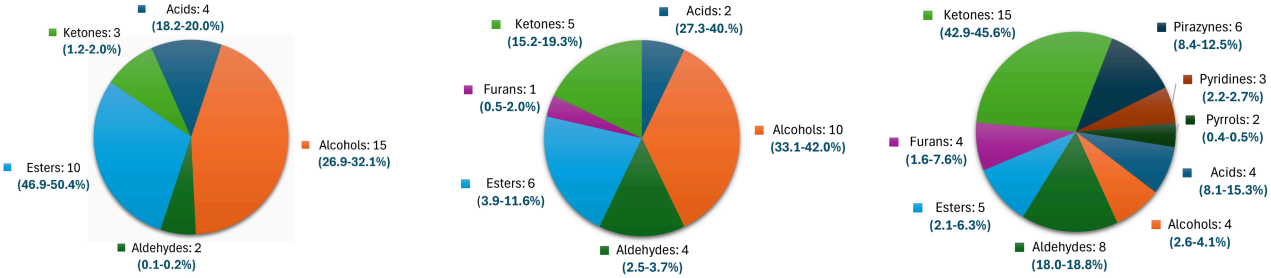


Figure 2. Number of volatile organic compounds (VOCs) and minimum and maximum percentages of total chromatographic peak area by chemical family (dark blue numbers) for each sample type: (**a**) mucilage, (**b**) green coffee beans, and (**c**) roasted coffee.

Particularly, isobutyraldehyde (2-methylpropanal) was produced by the yeasts, as it was not detected in the initial mucilage samples. Notably, it was produced in higher chromatographic areas by *S. pombe* and *R. aurantiaca* (see Table S1). In roasted coffee, this compound is the result of the Strecker reaction, as an intermediate step of the Maillard reaction involving carbohydrates and amino acids such as alanine, valine, and leucine [33,36]. Consequently, the presence of this compound was quite evident in green and roasted coffee, which could have been formed by the effect of high temperature in each process. This compound has also been noted as a primary compound for the characteristic coffee fragrance, especially in Colombian Arabica coffee [37]. Additionally, it is reported as a key component of the aroma of "outstanding" coffee samples for providing floral and fruity notes [33].

3.3. Volatile Organic Compounds in Coffee Mucilage

Most compounds detected in the mucilage matrix are products of fermentation processes (see Table S2). Furthermore, mucilage exhibited the largest cumulative chromatographic peak area. Esters were the most abundant chemical family based on chromatographic peak area, with acetate esters, particularly ethyl acetate, being the most prevalent. These acetate esters result from the esterification of alcohols with acetic acid generated during fermentation [1]. Therefore, the elevated acetate ester content is attributable to the high abundance of acetic acid and alcohols in the samples.

Of the volatile compounds analyzed, 67.6%—mainly alcohols, esters, and acids—showed significant differences between experimental blocks. This result indicates that the blocking factor, based on each block's coffee characteristics, strongly affected the chromatographic response of these compounds. Fruit maturity plays a key role because it provides substrates for microorganisms. Yeasts are especially well suited to the mucilage medium, which starts the process of creating the desired volatile profile [8,9]. As a result, the initial quality of coffee depends mostly on factors like species, variety, and maturity.

Six of the 34 compounds identified in the mucilage exhibited significant effects attributable to the experimental factors (Table S2). Only the growth phase (t1 and t2) sig-

nificantly influenced the chromatographic area of 2-methyl-1-propanol ($p = 0.0427$). In contrast, the inoculated consortia (c1 and c2) significantly affected ethyl acetate ($p = 0.0304$) and 3-methyl-2-butenol acetate ($p = 0.0325$). For the control treatment, chromatographic areas of 3-methylbutanoic acid ($p = 0.0189$), 3-hydroxy-2-butanone ($p = 0.0174$), and ethyl propanoate ($p = 0.0272$) were significantly higher than in the other treatments. No interaction effects were observed for any compound in the coffee mucilage matrix ($p > 0.05$). This indicates that the significant changes in the compounds' chromatographic areas are attributable to the independent effect of the consortia and yeast growth phases.

The numerical variations detected may be attributed to the similar profiles of the microbial consortia employed. Specifically, *H. guilliermondii* and *P. membranifaciens* in each consortium demonstrated the highest capacity to produce ethyl acetate, consistent with the results obtained from the individual strains (see Table S1). Additionally, accumulation of this compound was significantly greater in fermentations involving *P. membranifaciens* (Table S2).

It has been reported that discontinuous fermentations enable the adaptation of inoculated yeasts to external changes, which are reflected in metabolic changes associated with growth phases and in enzymatic activity across different metabolic pathways [38]. This phenomenon may explain the differences in the compound's accumulation, depending on the specific growth phase in which the yeasts were inoculated. The c2t2 treatment showed the highest abundance of volatile organic compounds (VOCs), as evidenced by a larger chromatographic total area than in c1t2, which contained fewer VOCs (Table S2). This observation indicates a reduced fermentative capacity in c1t2. Within the c1 consortium, *R. aurantiaca* and *H. guilliermondii* are the primary contributors; *Rhodotorula* generally dominates the initial stages of fermentation [34], while *Hanseniaspora* is known for its capacity to regulate ethanol levels and acid composition [30]. The elevated VOC production in c2t2 is likely attributable to the presence of both *S. pombe* and *T. globosa*, as reflected by increased ethanol and ethyl acetate peak areas in coffee mucilage (see Table S2), consistent with the elevated ethanol production (see Table S1).

3.4. Volatile Organic Compounds in Green Coffee Beans

The aroma of green coffee beans is subtle yet distinctive and has primarily been investigated to assess product quality. The significance of volatile organic compounds (VOCs) is evident in the development of roasted coffee beans [9,10,35] and in differentiating coffee species [39]. For example, *Coffea arabica* exhibits a significantly greater area of VOCs compared to *C. canephora* var. robusta [10,40], and the emission of those compounds is lower in Robusta than in Arabica [33]. Additionally, evaluating the potential to detect defects prior to roasting is a key objective [9]. Although green coffee beans are not suitable for consumption, they contain significant quantities of VOCs. Most of these compounds increase during roasting, while others decrease due to thermal degradation or evaporation [33,35].

Of the 28 compounds identified in the green coffee matrix, only three were significantly affected by the experimental block, indicating that the volatile profile of green coffee in this study was independent of coffee fruit quality. Three esters demonstrated significant effects attributable to the experimental design (Table S3). Methyl acetate exhibited a significant interaction effect ($p = 0.0232$), with its chromatographic response influenced by the yeast strains in the consortia and their growth phase. The chromatographic area of ethyl acetate was significantly higher with consortium 1 ($p = 0.0384$), whereas ethyl isovalerate was significantly higher in the control ($p = 0.0417$). Elhalis et al. [6] found that ethyl acetate was present in higher quantities in green coffee beans fermented with yeasts (25 times) than in beans fermented without yeast activity. *H. guilliermondii* produced this compound in greater quantities (peak area) as part of the c1 consortium (Table S1).

A key distinction arises from the environmental conditions present in coffee bean production areas. Plantation altitude influences the relative composition of volatile organic compounds (VOCs), as agro-climatic variables such as temperature, UV radiation, and precipitation exhibit a linear, positive, and significant correlation with altitude during the growth stage [10,33,35]. Consequently, compounds such as butanol, 3-methylbutanol, acetic acid, 2,3-butanedione, and 3-hydroxy-2-butanone are present at higher chromatographic areas in coffee cultivated at higher altitudes [41]. The coffee beans used for fermentation with a starter culture were grown at altitudes of 1260–1400 m, which are lower than those associated with the fermentations from which the yeasts in this study were isolated (1850 m). These differences are attributable to agro-climatic factors, resulting in a slightly reduced accumulation of these compounds. In contrast, hexanal and pentanoic acid, which are characteristic of green coffee beans, did not show significant variation with respect to altitude [33,41].

The reduced chromatographic area of acetate esters is likely due to lower levels of alcohols and acetic acid in the samples. The drying temperature likely contributed to the early degradation of these compounds. The presence of 2-methylpropanal and 3-methylbutanal is explained by the auto-oxidation of 2-methylpropanol and 3-methylbutanol [9], both of which were present in the analyzed samples. In the green coffee samples, the inoculum growth phase (t1 and t2) did not significantly affect the chromatographic response of any analyzed compounds ($p > 0.05$).

3.5. Volatile Organic Compounds in Roasted Coffee Beans

The roasting process is a crucial phase in coffee production, as it determines the final product's flavor and aroma. Numerous studies have examined the chemical reactions and compounds produced during roasting. Research findings indicate that the conditions and outcomes of preceding processing stages substantially influence the overall quality of the coffee beverage [9,10,33,35,42,43].

The most representative compounds of roasted coffee, based on their chromatographic areas, were acetic acid, 2,3-butanedione, 1-hydroxy-2-propanone, 2-furan carboxaldehyde, and 2-methylpyrazine. Acetic acid was the most abundant acid, attributed to sugar fragmentation during roasting and the subsequent formation of formic acid [44].

Chromatographic responses of major ketone compounds varied significantly across experimental blocks. Among these, 2,3-pentanedione is identified as one of the most abundant odorants in roasted coffee [9] and is typically present at higher concentrations in Arabica than in Robusta coffees [35]. These compounds are generally formed through the thermal interaction of glucose and cysteine during roasting and are associated with the ripeness of the coffee fruit. Notably, 2,3-butanedione was detected at a higher chromatographic area and is known for its characteristic buttery aroma and spicy notes, which contribute positively to the beverage's sensory profile [1,9,33,35]. The fermentations evaluated in this study produced significant effects in 19 of the 51 compounds analyzed in roasted coffee (see Table S4). The interaction among experimental factors influenced the peak area of 2-methylpyrazine ($p = 0.0288$). Additionally, compounds such as acetic acid, 1-hydroxy-2-propanone, 1-hydroxy-2-butanone, 1-acetyloxy-2-propanone, 2-furanmethanol, 2-furancarboxaldehyde, and 2,3-dihydrofuranone exhibited significantly larger chromatographic areas ($p < 0.05$) during the lag phase (t1) of inoculum growth. In contrast, 2,5-dimethylfuran displayed a larger peak area during the exponential phase (t2). Most pyrazines, pyridines, and pyrroles demonstrated a significant consortia effect, with chromatographic areas being predominantly larger ($p < 0.05$) for c2 level. Both consortia and growth phases influenced the peak area of 2-furancarboxaldehyde and 1H-pyrrole.

Pyridines and pyrroles were among the chemical families present in smaller proportions, but more affected by the treatments. Pyrazines, which are associated with burnt and roasted flavors, were identified in six compounds in this study and play an important role in fragrance and flavor. Pyrazines accounted for less than 10% of the total chromatographic area, with 2-methylpyrazine being the most prominent. This compound's chromatographic response depends significantly on the interaction between the two consortia used (c1, c2) and the two growth phases (t1, t2). It also has a nutty, cocoa, and roasted aroma profile, is linked to coffee during fermentation, and contributes to the fruity flavor [24]. Additionally, 2,5-dimethylpyrazine, the second compound in this chemical family, demonstrated a significantly higher peak area when the c2 consortium was utilized. This compound is associated with chocolate and nutty aromas, as well as fermentation influences, consistent with findings reported by Wu et al. [24].

Esters and alcohols also exhibited low representation. Esters primarily contribute to the floral and fruity aroma of coffee [35]. The ester family was numerically higher in the c1t2 treatment, with a notably greater presence than in spontaneous fermentation (control treatment), which showed the lowest chromatographic area for this chemical family.

Alcohols showed a marked decrease in both number and in chromatographic area. However, higher values were recorded in treatments with lag-phase inoculation (t1). Acids demonstrated a comparable pattern.

Principal Component Analysis (PCA) indicated that the cumulative variance for the first two components (PC1 and PC2) was 57.26% for mucilage, 44.97% for green coffee, and 69.10% for roasted coffee (Figure 3). The green coffee matrix, comprising 28 compounds, demonstrated the highest multidimensionality and independence, with PC1 accounting for 25.89% of the variance and PC2 for 19.08% (Figure 3b). In mucilage, which contained 34 compounds, the variability was distributed almost equally between PC1 (29.98%) and PC2 (27.28%) (Figure 3a). In contrast, the roasted coffee matrix, including 51 compounds, exhibited the greatest concentration of information within the two-dimensional plane, with PC1 explaining 42.17% and PC2 26.93% (Figure 3c). These results indicate that the thermal processes involved in roasting generate correlation patterns among the volatile compounds.

For mucilage, sample distribution along PC1 was primarily influenced by block variability, with treatment replications forming tight, overlapping clusters within each block. Within each of the four blocks, treatment points were closely grouped. These results show that the treatments did not significantly affect the overall aromatic profile of the mucilage. The differences between blocks were much larger than the treatment effects, which supports the ANOVA results for each compound.

In the case of green coffee, most data points, representing factorial treatment replicates, cluster near the origin. The treatments applied during fermentation did not significantly influence the volatile composition of green coffee. Additionally, the experimental variability associated with blocking factors is minimal and does not cover the treatment profiles.

Unlike the mucilage and green coffee matrices, the roasted coffee plot displays clear dispersion and separation of samples across the two-dimensional plane. The blocks are arranged in a crosswise pattern throughout the plot, without introducing any systematic or clustered bias.

When evaluating the complete aromatic profile while controlling for the block effect (Block-Controlled PERMANOVA), the results reveal clearly distinct behaviors depending on the sample type. For the mucilage, no significant differences were observed between the treatments and the control ($Pseudo\text{-}F = 0.427$, $p = 0.5771$); variability was dominated by the block effect, as in green coffee beans ($Pseudo\text{-}F = 0.838$, $p = 0.6695$) and roasted coffee ($Pseudo\text{-}F = 1.425$, $p = 0.121$).

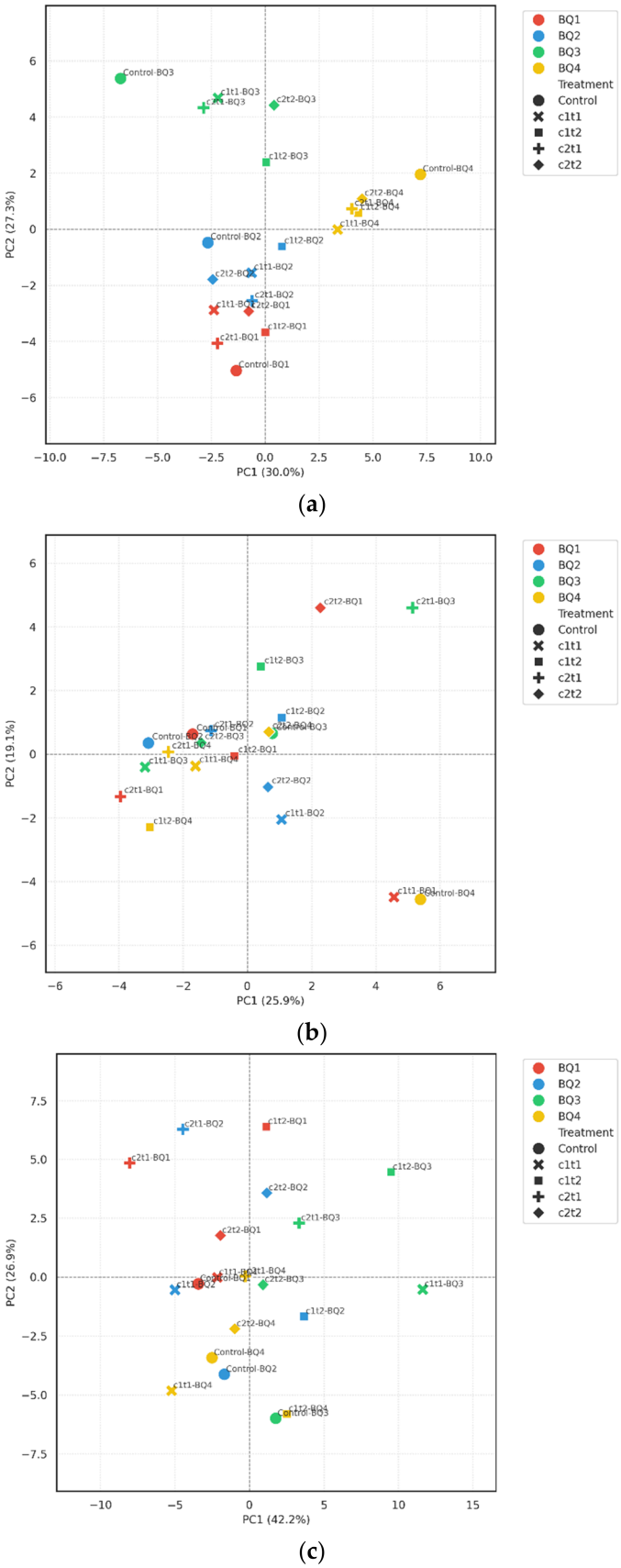


Figure 3. PCA score plot of the volatile profiles produced by the consortia used for fermenting coffee mucilage, for each sample type: (**a**) mucilage, (**b**) green coffee beans, and (**c**) roasted coffee.

Univariate analysis using two-way ANOVA (Treatment and Block) identified 8volatile compounds in roasted coffee that changed significantly ($p < 0.05$) compared with the control (Table 1). The c2t1 treatment produced the most pronounced effect and the greatest deviation from the control, significantly increasing all pyrazines as well as key furans and pyrroles. In contrast, c1t2 significantly increased furans and aldehydes, including 2-methylfuran, 2,5-dimethylfuran, acetaldehyde, and methyl formate, relative to the control. The c2t2 treatment displayed an intermediate profile, significantly increasing key pyrazines (2,5-dimethylpyrazine and 2-methylpyrazine) and 2,5-dimethylfuran compared to the control. The c1t1 treatment was most similar to the control, with no statistically significant differences ($p > 0.05$) observed for any volatile compound.

Table 1. Volatile organic compounds in roasted coffee with higher significant differences ($p < 0.05$) vs. the control treatment (VOC expressed as peak area × 10^5).

Chemical Family	Compound	Control	c1t1	c1t2	c2t1	c2t2	*p-Value*	Treatments Significantly Different from the Control ($p < 0.05$)
Pyrazines	2,5-Dimethylpyrazine	82.9	80.73	88.57	111.88	100.96	0.0049	c2t1, c2t2
	2-Methylpyrazine	547.91	548.1	588.26	692.17	624.57	0.0055	c2t1, c2t2
	2,3-Dimethylpyrazine	14.71	13.84	14.89	18.45	16.45	0.0127	c2t1
	2-Ethyl-6-methylpyrazine	16.00	14.76	16.27	20.13	18.22	0.0317	c2t1
	2-Ethyl-3-methylpyrazine	6.31	5.97	6.5	7.93	7.23	0.0273	c2t1
	Trimethylpyrazine	13.56	12.43	13.65	17.26	15.37	0.0404	c2t1
Furans, Esters and Aldehydes	2-Furancarboxaldehyde	750.49	774.78	697.73	884.39	789.02	0.0063	c2t1
	5-methyl-2-furancarboxaldehyde	132.98	137.3	123.96	172.21	145.98	0.0341	c2t1
	2-Methylfuran	73.3	215.97	525.46	261.00	291.5	0.0415	c1t2
	2,5-Dimethylfuran	11.08	14.22	26.22	19.09	21.61	0.0224	c1t2, c2t2
	Acetaldehyde	94.29	138.52	379.04	247.01	229.88	0.0440	c1t2
	Methyl formate	25.41	57.56	190.75	110.46	93.32	0.0337	c1t2
	Methyl acetate	25.40	186.20	378.80	211.20	197.54	0.0433	c1t2
Ketones and Pyrroles	1-(2-Furanyl) ethanone	104.75	107.51	99.84	129.8	114.04	0.0286	c2t1
	5-Methyl-3-hexanone	18.58	20.19	21.83	24.05	22.84	0.0439	c2t1, c2t2
	1H-Pyrrole	12.94	13.58	12.55	16.36	13.14	0.0201	c2t1
	1-Methyl-1H-pyrrole	20.16	23.08	31.73	30.00	29.73	0.0388	c1t2, c2t1, c2t2
	2-Butanone	54.40	125.10	257.50	143.80	154.80	0.0342	c1t2

3.6. Sensory Analysis

Samples from fermentations inoculated with the consortia showed no sensory defects. Consequently, clean cup, sweetness, and uniformity received the maximum score (10 points), a rating achieved when all five cups of the sample are entirely free of defects, the coffee's characteristic sweetness is maintained, and the flavor is uniform. For this reason, Table 2 does not include values for these attributes. Conversely, one evaluated cup of a control treatment exhibited a defect related to a *phenolic off-flavor*, affecting the total score of the sample (73 SCA points).

Sensory scores across the five experimental treatments were evaluated using a non-parametric Friedman test for randomized block designs. Descriptively, treatments had interquartile ranges (IQR): 2.12 for c1t1, 2.25 for c1t2, 1.44 for c2t1, 1.38 for c2t2, and 3.62 for the control (Table 2). Contrary to the volatile description of roasted coffee, the overall Friedman test indicated no statistically significant differences among treatments across blocks (χ^2 (4) = 3.69, $p = 0.449$), suggesting that the coffee sensory analyses did not detect the differences identified by the volatile profile. Pairwise Wilcoxon signed-rank tests comparing each treatment against the control similarly revealed no statistically

significant differences after Holm–Bonferroni correction ($p > 0.05$). Fermentations with added yeast had SCA mean scores 2.6 to 3.5 points numerically higher than the control, which used spontaneous fermentation. Although these differences were not statistically significant because of high variability, particularly in the control group, yeast-inoculated coffee fermentation showed a distinct biochemical transformation process compared with the control treatment. This difference was ultimately reflected in the coffee attributes (Table 2). Inoculation with the yeast consortia used in this research resulted in numerically higher scores than the control, especially in body, flavor, and acidity, thereby distinguishing the coffee beverage with a specialty classification [20].

Table 2. Descriptive statistics for coffee quality (SCA points) obtained via the fermentation of coffee inoculated with different yeast consortia and at different growth times, and without an inoculation control.

Statistics	Treatment	Attributes							
		Fragrance /Aroma	Flavor	Aftertaste	Acidity	Body	Balance	Taster's Overall Rating	Total Score
Mean	c1t1	7.56	7.56	7.44	7.56	7.56	7.44	7.50	82.63
	c1t2	7.50	7.50	7.31	7.44	7.50	7.44	7.56	82.25
	c2t1	7.50	7.44	7.19	7.31	7.56	7.31	7.38	81.69
	c2t2	7.69	7.38	7.31	7.38	7.63	7.31	7.44	82.13
	control	7.56	7.19	7.06	7.25	7.19	7.19	7.19	79.13
Standard deviation	c1t1	0.13	0.24	0.38	0.43	0.13	0.31	0.35	1.75
	c1t2	0.20	0.20	0.38	0.43	0.20	0.43	0.31	2.02
	c2t1	0.20	0.24	0.24	0.24	0.13	0.24	0.32	1.46
	c2t2	0.38	0.14	0.24	0.25	0.25	0.38	0.31	1.65
	control	0.13	0.24	0.43	0.35	0.24	0.24	0.24	4.33
Median	c1t1	7.50	7.63	7.50	7.63	7.50	7.50	7.63	82.75
	c1t2	7.50	7.50	7.25	7.38	7.50	7.38	7.50	81.75
	c2t1	7.50	7.38	7.13	7.38	7.50	7.38	7.38	81.63
	c2t2	7.75	7.38	7.38	7.50	7.50	7.25	7.50	82.25
	control	7.50	7.13	7.13	7.13	7.13	7.13	7.13	80.25
Minimum	c1t1	7.50	7.25	7.00	7.00	7.50	7.00	7.00	80.50
	c1t2	7.25	7.25	7.00	7.00	7.25	7.00	7.25	80.50
	c2t1	7.25	7.25	7.00	7.00	7.50	7.00	7.00	80.00
	c2t2	7.25	7.25	7.00	7.00	7.50	7.00	7.00	80.00
	control	7.50	7.00	6.50	7.00	7.00	7.00	7.00	73.00
Maximum	c1t1	7.75	7.75	7.75	8.00	7.75	7.75	7.75	84.50
	c1t2	7.75	7.75	7.75	8.00	7.75	8.00	8.00	85.00
	c2t1	7.75	7.75	7.50	7.50	7.75	7.50	7.75	83.50
	c2t2	8.00	7.50	7.50	7.50	8.00	7.75	7.75	84.00
	control	7.75	7.50	7.50	7.75	7.50	7.50	7.50	83.00

The SCA protocol establishes a standardized framework for characterizing primary sensory coffee descriptors during sensory analysis. Most samples were characterized as sweet, with honey and chocolate being the predominant descriptors, particularly in the treatment inoculated with consortium 1 (c1). In contrast, samples inoculated with consortium 2 (c2) were more frequently described as fruity and citric, which may explain the higher fragrance score observed in this treatment. Yeast used during fermentation produces compounds that influence fragrance, especially [6,9]. However, the low chromatographic response of the alcohol and ester families in roasted coffee may explain the low reports of fruity aromatic notes. A notable observation was the absence of significant differences between the

compounds of c1t1 and the control (Table 1), potentially reflecting their comparable average fragrance scores (Table 2). The control treatment showed honey, chocolate, and almond notes but also had a cereal, straw, herbal, or dry aftertaste, associated with altered flavor and an overall decline in quality. In contrast, the c1t1 treatment showed honey, chocolate, and peanut notes, with no negative descriptors. In the c2t1 treatment, 2,5-dimethylpyrazine was a prominent compound, corresponding to nut, peanut butter, leather, cacao, wood, straw, and medicine profiles [33,37]. These findings may explain the lowest aftertaste and balance scores for this treatment, leading to a lower overall rating. The lower aftertaste scores in treatments c1t2, c2t1, and c2t2 may be related to the greater chromatographic area of aldehydes (acetaldehyde and propionaldehyde) and furans (2-methylfuran and 2,5-dimethylfuran) (see Table S4), compounds associated with undesirable aromas including ethereal, pungent, earthy, burnt, bitter, roasted grain, and tobacco notes. Additionally, aldehydes are associated with defective beans and excessive fermentation [9,33]. In contrast, c1t1 demonstrated the numerically highest acidity score, coinciding with the higher peak area for the acid chemical family in roasted coffee.

4. Conclusions

This study evaluated the impact of introducing native non-*Saccharomyces* yeast consortia during coffee wet fermentation on the volatile organic compounds (VOCs) present in mucilage, green coffee beans, and roasted coffee.

Two compatible consortia of native yeast strains (c1 and c2) were evaluated based on their volatile profiles. Alcohols and esters predominated in mucilage and green beans, whereas roasting shifted the dominant volatiles to pyrazines, furans, aldehydes, and pyrroles. These findings indicate that yeast metabolism during fermentation could modify precursor compounds, subsequently influencing chemical reactions during roasting. Further research using the individual yeast strains may improve understanding and optimize chemical transformations in mucilage and coffee for each yeast.

Consortium 1, comprising *H. guilliermondii*, *S. pombe*, and *R. aurantiaca*, was added during the lag phase (c1t1) and could help to retain organic acids and flavor precursors. This approach resulted in the sensory quality, with an average score of 82.63 SCA points, characterized by bright acidity and a clean flavor profile.

Consortium 2, consisting of *P. membranifaciens*, *T. globosa*, and *S. pombe*, was introduced during the exponential phase (c2t2). This treatment elevated alcohol and ester peak areas in the mucilage and increased key pyrazine levels, specifically 2,5-dimethylpyrazine and 2-methylpyrazine, in roasted coffee.

The incorporation of the evaluated yeast strains during coffee fermentation resulted in a final coffee score of 2.6 to 3.5 SCA points, numerically higher than spontaneous fermentation without sensory defects, such as phenolic off-flavors, detected in the control treatment. Although variations in coffee fruit quality influence the volatile organic compound profile of both mucilage and roasted coffee, the use of native yeast starter cultures could provide a reliable method to standardize wet fermentation, reduce risk, and develop specific flavor profiles in specialty coffee. Further research on these yeast strains is necessary to more comprehensively assess their limitations and potential, thereby generating more robust data on coffee quality. Additionally, investigations into their effects on other chemical families, such as sugars, organic acids, and amino acids, are required to clarify their broader relationship with coffee composition.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation12110504/s1, Table S1. Chromatographic response of VOCs (Mean ± SD) expressed as peak area × 10^5 produced by yeasts from coffee mucilage; Table S2. Volatile organic compounds—VOCs (Mean ± SD) identified in coffee mucilage from fermentations

with and without yeast consortia inoculation. (VOCs expressed as peak area × 10^5); Table S3. Volatile organic compounds—VOCs (Mean ± SD) identified in green coffee beans from fermentations with and without yeast consortia inoculation. (VOCs expressed as peak area × 10^5); Table S4. Volatile organic compounds—VOCs (Mean ± SD) identified in roasted coffee from fermentations with and without yeast consortia inoculation. (VOCs expressed as peak area ± 10^5); Figure S1. Growth curves of the yeasts used for the formation of the consortia, (a) *Hanseniaspora guilliermondii*, (b) *Schizosaccharomyces pombe*, c) *Pichia membranifaciens*, and (d) *Torulaspora globosa*.

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Abbreviations

The following abbreviations are used in this manuscript:

VOCs	Volatile Organic Compounds
SCA	Specialty Coffee Association
MDS	Multidimensional Scaling
HS-SPME	Head Space Solid Phase Microextraction (HS-SPME)
GC-MS	Gas Chromatography–Mass Spectrometry

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