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# Volatile Compounds and Chemical Profiles in Unroasted Coffee: Impact of Lactic Acid Bacteria Species and Dose for On-farm Processing

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***Abstract*— While post-harvest fermentation is known to influence coffee quality, the specific impact of lactic acid bacteria (LAB) starter cultures on modulating the volatile aroma precursor diversity in unroasted green coffee beans remains underexplored. This knowledge gap limits the industry's ability to move beyond spontaneous fermentation to predictably target specific aroma profiles. This study investigated the species- and dose-dependent effects of locally cultured *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* (1.5–6% w/v inoculum) on the green bean's chemical profile. The unroasted beans were analyzed using HS-SPME-GC–MS for volatile aroma precursors alongside caffeine and proximate composition. The chemical profile diverged significantly following LAB inoculation, with volatile diversity increasing by 32–45% relative to the control. The two species demonstrated divergent impacts: *L. pseudomesenteroides* (3–4.5% inoculum) drove significant proteolysis (protein content decreased from a mean of 11.8% to 8.5%) and was associated with a ~47% increase in measured caffeine, enriching precursors for floral (2-phenethyl acetate) and buttery (2,3-pentanedione) notes. Conversely, *P. pentosaceus* produced nutty precursors (e.g., methylpyrazine) while preserving the bean's protein and fat matrix. These findings provide a clear biochemical roadmap for the coffee industry. They demonstrate that targeted LAB inoculation is a viable strategy to predictably engineer green bean chemistry, allowing producers to deterministically steer the final cup profile toward either complex floral notes or classic, matrix-preserved nutty characteristics.**

***Keywords*— Coffee Arabica, Aroma, Volatile, Fermentation, Lactic Acid Bacteria, GC-MS**

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## I. INTRODUCTION

Coffee is one of the most widely consumed beverages worldwide. Post-harvest processing is increasingly recognised as a decisive flavour-engineering step in coffee production. Coffee flavour is not created solely during roasting but is developed during this stage, where microorganisms transform the mucilage into an array of aroma precursors that persist within the green bean [1]. The vast majority of coffee aroma research has justifiably focused on the roasted bean, identifying hundreds of volatile compounds formed through Maillard and Strecker reactions that define the final cup aroma [2, 3]. Studies have extensively documented the influence of roasting degree on the formation of key furans, pyrazines, and phenols [4]. Pioneering work by De Bruyn et al. mapped the microbial diversity and general metabolite profiles during postharvest processing [1], yet this foundational research, along with the bulk of existing literature, focuses on the final roasted product or general metabolite shifts, not the specific formation of volatile aroma precursors in the green bean itself. By contrast, the chemistry of the unroasted green bean which provides the fundamental precursors for these pyrolytic reactions remains comparatively underexplored in the scientific literature.

This gap is widest for the increasingly popular anaerobic fermentations driven by lactic-acid bacteria (LAB). While previous studies have characterized LAB diversity, there is a critical lack of systematic, dose-response studies investigating how individual LAB starter cultures directly modulate the

volatile precursor landscape and macro-nutrient matrix of the unroasted green bean during farm-scale processing. Moreover, the field currently lacks the species- and dose-specific data needed for coffee producers to rationally select and calibrate LAB starter cultures toward a target aroma profile, shifting coffee fermentation from an unpredictable, spontaneous process to a precise, controllable strategy for flavor engineering. LAB are known to dominate many spontaneous fermentations and can amplify desirable floral esters, buttery diketones, and terpenoids [5-7]. Recent studies, such as those by Ribeiro et al., have successfully isolated and characterized LAB from wet processing, confirming their metabolic potential in pulped coffee mediums [8]. However, a critical gap persists: most reports either stop at microbial characterization, rely on mixed/undefined starter cultures, or evaluate volatile compounds only after roasting, where thermal degradation obscures the original microbial volatile signature [9], [10]. Consequently, the field lacks a clear understanding of how systematic, graded doses of individual, indigenous LAB species diverge the VOC landscape and macro-nutrient matrix of the green beans themselves.

The local microbial diversity in the growing region also influences the formation of coffee's chemical profile. Therefore, the present study addresses this gap by conducting on-farm anaerobic fermentations of Arabica parchment coffee from Kintamani Bali Indonesia, with stepwise doses (1.5, 3.0, 4.5, and 6%, w/v) of two locally isolated LAB: *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* originating from the same coffee-processing area. In the previous wet coffee fermentation study, selected mesophilic and lactic acid bacteria were inoculated onto pulped coffee at an initial level of $10^7$ CFU $g^{-1}$, with LAB populations ranging from 3 to 8 log CFU $g^{-1}$ during fermentation. Based on this benchmark of microbial density, LAB inoculum levels used in the present work in the range of 1.5–6% (w/v of active culture) were stated to be equivalent or predicted to be similar to the effective dose of Ribeiro et al. [8], because they are designed to achieve comparable LAB cell densities (3–8 log CFU $g^{-1}$ in the coffee mass) during fermentation, mirroring the successful population dynamics reported in that study and thereby directing the metabolic flux and the resulting profile of volatile precursors. This work represents the first systematic evaluation of inoculum-level effects of single LAB species under farm-scale washed processing in this origin. The objective of this research was to evaluate the alteration of volatile compound precursors and chemical properties of the unroasted green coffee beans following these different fermentation treatments.

## II. Materials and Methods

### *A. Materials and Chemical Reagents*

*Coffea arabica L* (Arabica coffee) mature red cherry samples were hand-picked and collected from the Village of Belantih, Kintamani, Bangli, Bali, Indonesia, at an altitude of 1200-1300 meters above sea level.

Caffeine anhydrous standard and Tween 80 were purchased from Sigma-Aldrich (St. Louis, USA); magnesium oxide and peptone water were provided by Merck KGaA (Darmstadt, Germany). de Man-Rogosa-Sharpe (MRS) agar was from HIMEDIA LAB (Mumbai, India). Other reagents were analytical-grade $H_2SO_4$ (Merck KGaA, Darmstadt, Germany); D-glucose monohydrate (SIGMA-ALDRICH); petroleum ether (Merck KGaA, Darmstadt, Germany); $K_2SO_4$, $CuSO_4 \cdot 5H_2O$ (Merck KGaA, Darmstadt, Germany); $H_2SO_4$, NaOH, HCl (Merck KGaA, Darmstadt, Germany); and bromocresol green/methyl-red indicator (CARLO ERBA, Italy).

### *B. LAB Isolates*

The two LAB isolates used in this study, *Leuconostoc pseudomesenteroides* strain Himuka-SU6 and *Pediococcus pentosaceus* strain BPL5, were obtained from indigenous bacteria in a spontaneous coffee fermentation tank and coconut sap water, respectively. The *P pentosaceus* strain was found to be the dominant bacteria in the local coconut sap water which has been used by the local farmers in the growing area, for their fermented beverage. And it is hypothesized to be adaptable to the coffee mucilage which offers unique pathway for fermentation [11].

DNA was extracted from overnight MRS cultures of both isolates using a cetyl-trimethyl-ammonium bromide (CTAB) phenol/chloroform method [12]. The near-full-length 16S rRNA gene was amplified with universal primers 27F and 1492R. PCR products were purified and bidirectionally Sanger-sequenced. Consensus reads were queried against the NCBI nr/nt database using BLASTn. The sequences matched *Leuconostoc pseudomesenteroides* (99.9% identity; GenBank accession LC276830.1) and *Pediococcus pentosaceus* (99.7%; OR592509.1).

### *C. Fermentation Process*

The coffee was processed using a wet (washed) method. Cherries were first depulped to remove skins and mucilage using a depulper machine with flowing water. The cleaned beans were then subjected to anaerobic fermentation for 24 hours. The temperature of fermentation was 27 °C ± 3 °C.

For inoculated treatments, beans were mixed with LAB isolates at 4 levels of concentrations (1.5%, 3%, 4.5%, and 6% w/v) relative to the weight of the coffee fermentation tank. This resulted in 9 total treatment groups: one spontaneous control (0% inoculum) and four concentration levels for each of the two LAB isolates (*L. pseudomesenteroides* A1-A4 and *P. pentosaceus* N1-N4). Each sample group was replicated 5 times for analysis. The control samples underwent the traditional spontaneous wet fermentation for 24 hours without added isolates. Following fermentation, the beans were dried in a drying chamber to an ideal moisture content.

### *D. Bacterial Growth Analysis*

Fermenting coffee mass (10g) was aseptically collected at 0 h to 24 h, homogenised with 90 mL sterile 0.1% peptone water

containing 0.5 g $L^{-1}$ Tween 80, serially ten-fold diluted ($10^{-1}$–$10^{-6}$), and 0.1 mL of each dilution was spread in duplicate onto MRS agar. Plates were incubated at 30°C for 48-72 h, after which colonies in the 30-300 range were counted and reported as colony-forming units per gram (CFU $g^{-1}$).

*E. Volatile Compounds Analysis using HS-SPME-GCMS*

The method to extract volatile compounds from the beans follows Aurum et al. [13] with slight modification. Headspace volatiles were extracted from 1 g coffee powder placed in 20 mL crimp-top vials, pre-equilibrated at 60°C for 10 min and then sampled for 30 min with a 50/30 µm DVB/CAR/PDMS SPME fibre (Supelco, Bellefonte, USA). The fibre was conditioned at 250°C for 30 min before first use and 10 min between runs [14]. The fibre was desorbed for 5 min in the inlet of an Agilent GC system (Agilent Technologies, Santa Clara, USA) in splitless mode at 250°C. Helium was the carrier gas at a constant 1 mL $min^{-1}$. Compounds were separated on a DB-WAX capillary column (30 m × 0.25 mm i.d., 0.25 µm; J&W Scientific, USA). The oven programme was: 40°C (1 min), 5°C $min^{-1}$ to 180°C, then 10°C $min^{-1}$ to 250°C (36 min total run time). The mass detector operated in electron-ionisation mode (70 eV) scanning m/z 30-550.

All measurements were performed in five replicates. Peak areas were normalised to an internal standard (10 µL $L^{-1}$) of 4-methyl-2-pentanol (CAS: 108-11-2; Sigma-Aldrich, St. Louis, MO, USA).

*F. Data Preprocessing of GCMS Spectra*

Raw chromatogram files from the GCMS were converted to the ABF (Analysis Base File) format using the Reifycs ABF Converter. Data processing was performed in MS-DIAL (v5.1.230807) [15]. For peak detection, parameters were set as follows: mass accuracy (m/z 0.5 Da), minimum peak height (1,000 amplitude), and mass slice width (0.1 Da). Spectral deconvolution was performed to separate co-eluting compounds with a sigma window value of 0.5, an EI similarity cutoff of 70%, and a minimum peak purity of 70%.

Peak alignment across all 45 samples was performed with a retention time tolerance of 0.2 min and a mass tolerance of 0.5 Da. Compound identification was performed by matching deconvoluted mass spectra against the MS-DIAL EI-MS open source public database (https://systemsomicslab.github.io and GNPS public database) with a match score cutoff of ≥80%. Identifications were further verified by comparing linear retention indices (LRI), calculated using a $C_8$-$C_{20}$ n-alkane series (Sigma-Aldrich, St. Louis, MO, USA) run under the same GC conditions. The final data matrix, consisting of aligned peak areas normalized to the internal standard, was exported for chemometric analysis.

*G. Macro Chemical Properties Analysis*

All analyses in this section were performed in quintuplicate (n=5).

*Caffeine.* Caffeine was assayed by reversed-phase HPLC-UV based on Naveen et al. [16]. Sample (0.500 g) was Soxhlet-extracted in 70% ethanol. The extract was dried, re-dissolved in 70% methanol, and filtered. Analysis was performed on a Shimadzu Prominence LC-20AC system with a Kinetex C18 column, using an isocratic elution of water:methanol (50:50, v/v) at 1.0 mL $min^{-1}$ and detection at 272 nm.

*Carbohydrates.* This analysis was performed according to AOAC 925.35 [17]. Freeze-dried coffee (1 g) was hydrolysed in hot water to release reducing sugars. The extract was boiled with Fehling's solution. The resulting cuprous-oxide ($Cu_2O$) precipitate was filtered, dried, and weighed.

*Total Protein.* Nitrogen content was measured by the macro-Kjeldahl procedure (AOAC 2001.11) [18]. Ground coffee (0.700 g) was digested with $H_2SO_4$ and a catalyst. Released $NH_3$ was steam-distilled into 4% boric acid and titrated with 0.1 N HCl. Crude protein was calculated as N × 6.25.

*Total Fat.* Lipids were quantified by continuous Soxhlet extraction (AOAC 963.15) [19]. Ground sample (2.0 g) was extracted for 6 h with petroleum ether. The solvent was evaporated, and the residue was dried to constant mass.

*Ash Content.* Sample (2.0 g) was incinerated in porcelain crucibles at 550°C for 6 h (AOAC 923.03) [20].

*Moisture Content.* Sample (5 g) was dried at 105°C to constant weight (AOAC 925.10) [21].

*H. Chemometrics Data Analysis*

Chemometric analysis was performed using the mixOmics package (v6.22.0) in R (v4.3.2) [22, 23]. The data matrix (45 samples × 68 compounds) was first log10 transformed to reduce heteroscedasticity and then autoscaled (mean-centered and scaled to unit variance) prior to analysis.

Hierarchical cluster analysis (HCA) was performed on the autoscaled data. We employed the Ward's linkage method ("ward.D2") with Euclidean distance as the dissimilarity measure to visualize sample clustering and compound relationships.

Partial least-squares discriminant analysis (PLS-DA) was performed to maximize the separation between the 9 treatment groups. The optimal number of components for the PLS-DA model was determined using 10-fold cross-validation (CV) repeated 100 times, selecting the component number that achieved the lowest balanced error rate. Model performance was assessed using the $Q^2$ (goodness of prediction) and $R^2Y$ (goodness of fit) values. A permutation test (1000 permutations) was conducted to validate the model and ensure the separation was statistically significant ($p < 0.01$). Discriminative biomarkers were identified using the Variable Importance in Projection (VIP) scores, selecting compounds with a VIP score > 1.0 from the final validated model [24].

*I. Statistical Analysis*

Univariate statistics were executed in R 4.3.2 [23]. Data normality and homoscedasticity were verified. One-way analysis of variance (ANOVA) or Kruskal-Wallis test was applied. Significant ANOVA results ($\alpha$=0.05) were followed by Tukey's honest significant difference (HSD) for pair-wise comparisons.

## III. RESULTS AND DISCUSSION

The inoculation of indigenous LAB as starter cultures was a successful metabolic intervention that fundamentally altered the green bean chemical composition. The results demonstrate a clear, controllable, and species-specific modulation of the aroma precursor landscape, underpinned by distinct shifts in the bean's macro-chemical composition.

### *A. The GC-MS-based Volatile Compounds and Microbial Dynamics*

A broad survey of the volatile compounds (**Figure 1**) reveals the diverse chemical classes produced during fermentation. The details of each compound are illustrated in **Figure 2** as an HCA heatmap. Across all treatments, including spontaneous control, the green bean precursor profile was dominated by pyrazines, alcohols, and aldehydes. This is consistent with the known chemistry of green coffee, where these compounds serve as the fundamental building blocks for the Maillard and Strecker reactions during roasting [25]. While the diversity (count) of compounds across classes was relatively stable, with *P. pentosaceus* (N3) showing a slight increase in pyrazine diversity, this high-level view obscures the more dramatic, species-specific changes in compound intensity and identity. It further indicates that pyrazines were the dominant chemical class in every treatment, accounting for ~18-24 % of the distinct volatiles per group (8–11 individual pyrazines of the 42-51 compounds identified). Alcohols (7-9 species) and aldehydes (4-8 species) formed the next two most diverse classes, followed closely by furan derivatives. Increasing the inoculation rate of *Leuconostoc pseudomesenteroides* (A-group) produced a modest enrichment in total volatiles, peaking at 51 compounds in A4, and was characterised by a rise in acid (from 2 to 4 species) and ester counts (up to three). In contrast, the *Pediococcus pentosaceus* treatments (N-group) maintained a stable acid profile but exhibited a concentration-dependent increase in pyrazine diversity, with N3 registering the highest

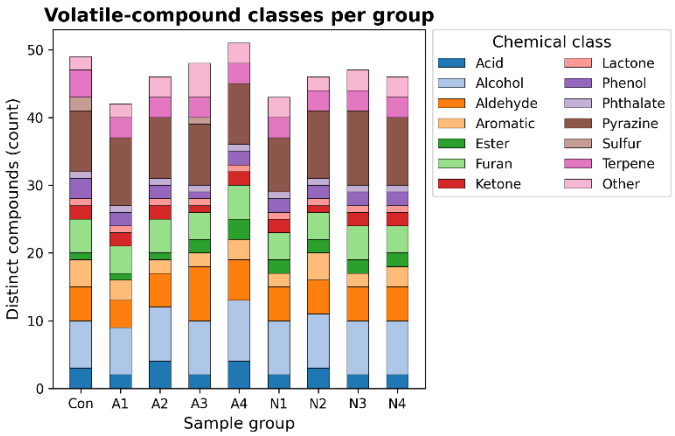


Fig 2 Diversity of volatile compound classes identified in each treatment. Con = Spontaneous Control. A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.5%, 4 = 6%.

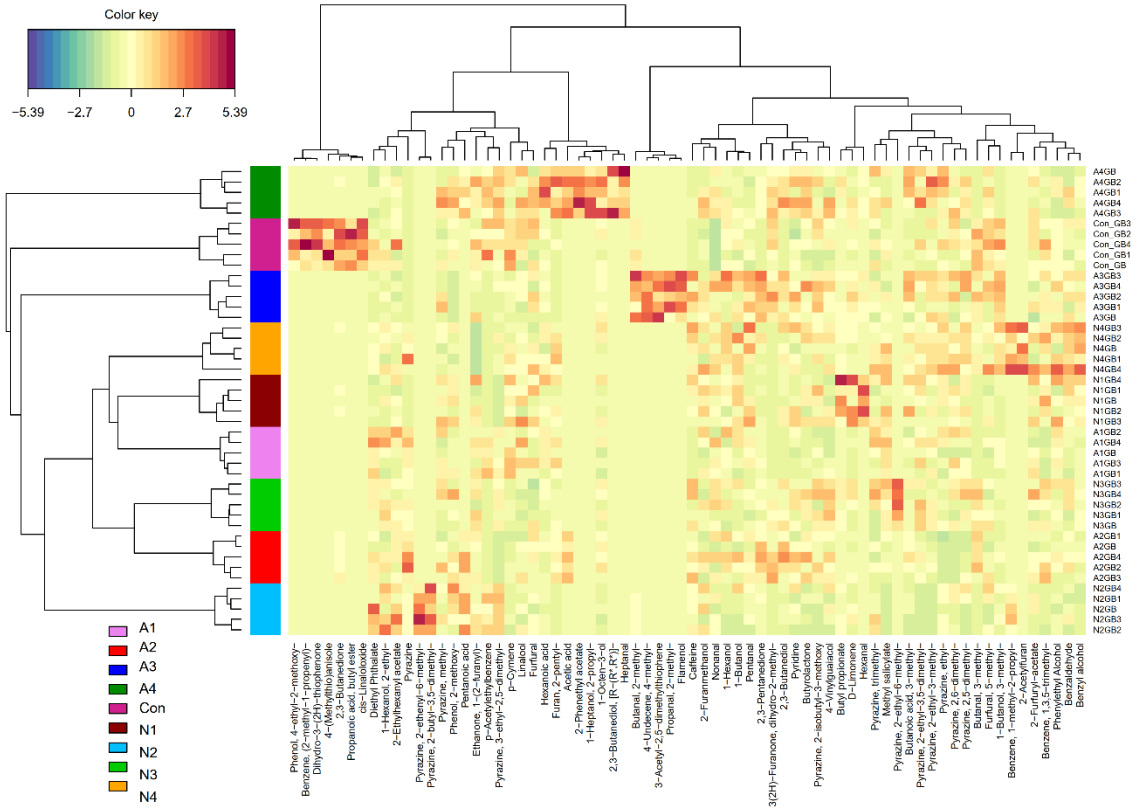


Fig 1. Hierarchical clustering analysis (HCA) heatmap of volatile compound intensities. Rows represent sample replicates (Con = Spontaneous Control; A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.5%, 4 = 6%)

share (11 distinct pyrazines). This relatively flat diversity profile suggests that the metabolic impact is not in creating entirely new chemical classes, but in selectively promoting or suppressing specific compounds within these classes.

identify the specific chemical signatures driving this separation. The control group was defined by a block of compounds showing high intensity for 'Con' samples but low intensity or absence in all inoculated treatments. In particular, this group

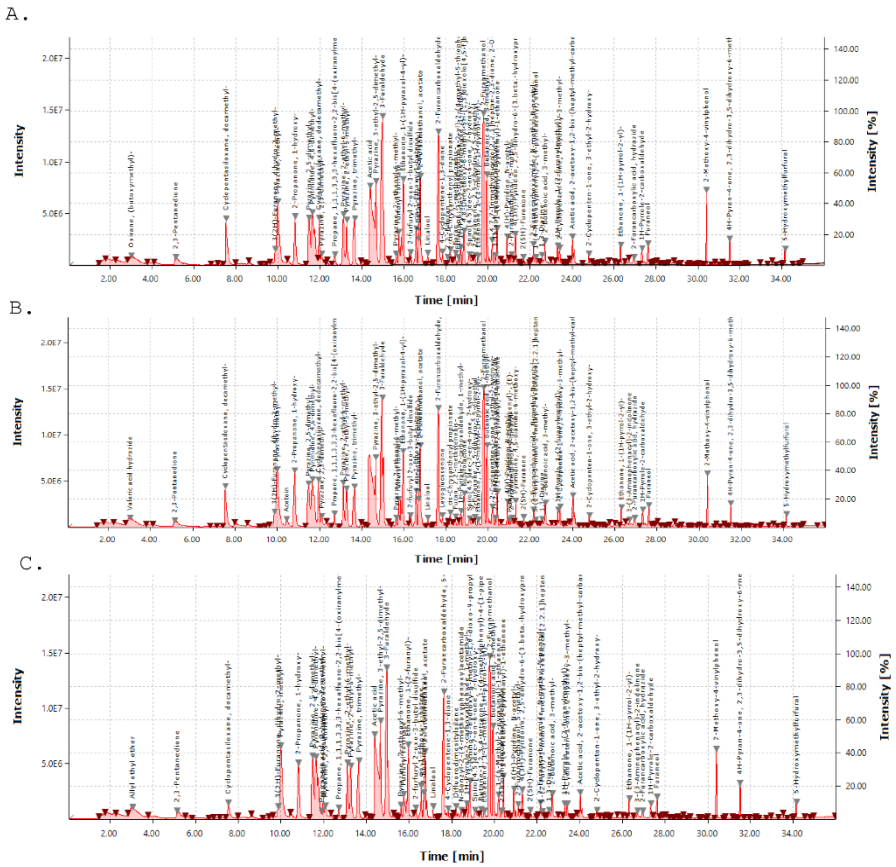


Fig 3. Representation of chromatogram from GC-MS analysis. (A) Chromatogram of Control Sample ; (B) Chromatogram of A group and (C) Chromatogram of N group

This intense, species-driven metabolic activity left a distinct chemical fingerprint, clearly visible in the HCA (**Figure 2**). This heatmap provides the first major piece of evidence supporting our central finding that inoculum identity is a primary driver of the final chemical profile. The spontaneous control (Con) samples formed their own cluster, but more importantly, the two LAB genera diverged into their own distinct chemotypes. The *Leuconostoc pseudomesenteroides* (A-series) and *Pediococcus pentosaceus* (N-series) treatments are clearly separated. This demonstrates that while the Lactobacillaceae family is often discussed as a monolith, different genera possess unique enzymatic toolkits that may yield different chemical products [8], [26].

All treatments formed a tight, distinct cluster, indicating that the inoculant were separated into different groups. This demonstrates that inoculation, regardless of species, might be single most influential factor in altering the bean's volatile chemical profile. By examining the compound clusters, we can

was uniquely rich in phenols (e.g., Phenol, 4-ethyl-2-methoxy), benzene derivatives (e.g., Benzene, (2-methyl-1-propenyl)-), and sulfur compounds like dihydro-3-(2H)-thiophenone and 4-(Methylthio)anisole. This confirms the spontaneous pathway is chemically unique.

The *L. pseudomesenteroides* (A-group) signature was defined by its own distinct block of compounds, which intensified with dosage, culminating in the A4 samples. This signature is

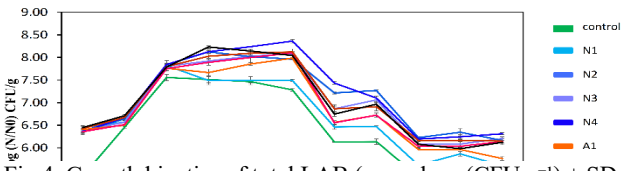


Fig 4 .Growth kinetics of total LAB (mean $\log_{10}$(CFU $g^{-1}$) ± SD, n=5) during fermentation. (Con = Spontaneous Control; A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.5%, 4 = 6%.).

characterized by high levels of acetic acid which is a key marker for heterofermentative metabolism and a notable presence of the aldehyde-alcohol pair, heptanal and 1-heptanol, 2-propyl. This profile is chemically distinct from both the control and the N group. *P. pentosaceus* (N-group) signature was defined by at least two major compound blocks. First, it is strongly defined by a large group of pyrazines (e.g., pyrazine, methyl-, pyrazine, 2-ethenyl-6-methyl-), confirming the 'Pyrazine' pathway from **Figure 1**. Second, this group is also uniquely enriched in other benzene derivatives, particularly at the N4 dose, including p-cymene and p-acetylethylbenzene.

Therefore, **Figure 2** visually illustrates and confirms the underlying data. This finding provides direct context to the work of Aurum et al. [24], who identified pyrazines and terpenes as critical differentiators for nutty, roasty-profiled Indonesian origins. It proves that the (Con), (A), and (N) groups are chemically divergent and identifies the specific volatile compounds that define these engineered chemical signatures.

The representative GC–MS chromatograms **(Figure 3**) revealed clear differences in the overall volatile complexity among the three groups. The control chromatogram (**Figure 3A**) showed fewer mid- to late-eluting peaks, reflecting a simpler aromatic profile. In contrast, the A4 (**Figure 3B**)fermentation produced a noticeably denser cluster of peaks between 12–20 min, consistent with enhanced formation of esters, alcohols, and pyrazines characteristic of LAB-driven carbohydrate and amino-acid metabolism [27]. Meanwhile, N4 (**Figure 3C**) displayed intensified peaks in the later retention window (20–30 min), suggesting a greater contribution of Strecker- and Maillard-related volatiles, aligning with its stronger preservation of the bean's macromolecular matrix.

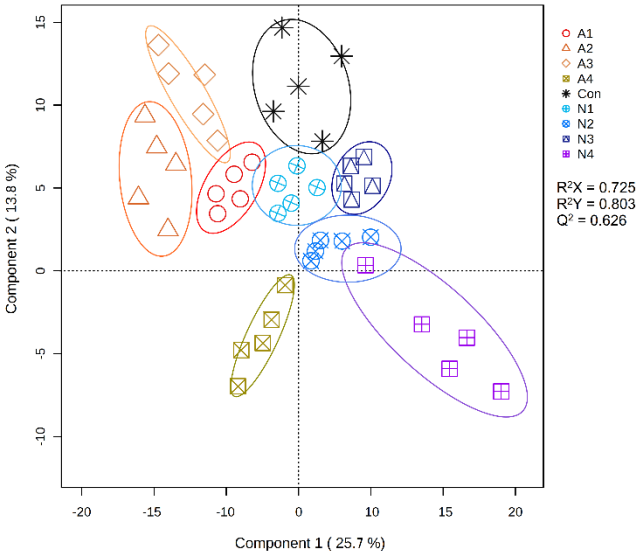


Fig 5. PLS-DA scores plot of volatile profiles. (Con = Spontaneous Control; A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.5%, 4 = 6%.)

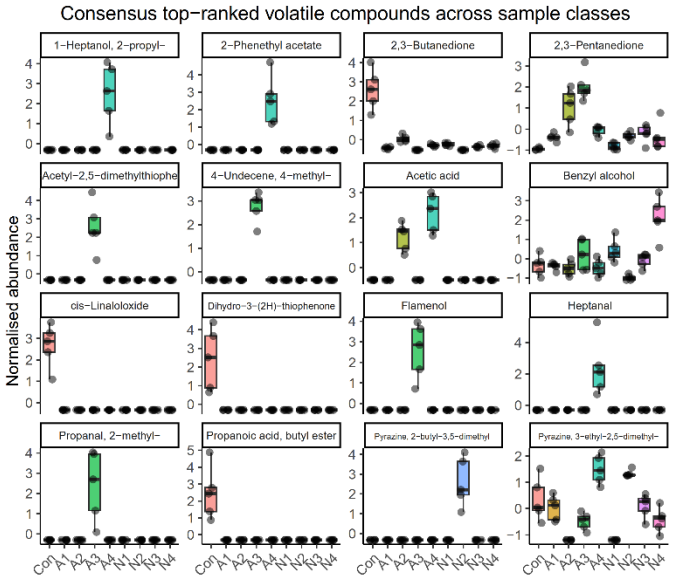


Fig 6. Boxplots of top-ranked volatile markers (VIP > 1) from the PLS-DA model. (Con = Spontaneous Control; A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.5%, 4 = 6%.)

The possible cause of this diversity was microbial growth. As shown in **Figure 4**, the fermentation was defined by a rapid and intense burst of microbial activity. All inoculated LAB populations surged from their starting points to peak densities of 8.0–8.4 log CFU $g^{-1}$ within a 12-hour window. This establishes the critical timeframe during which the vast majority of metabolic transformation occurred. The chemical changes observed were not the result of a slow, prolonged process, but of this high-density microbial activity. This aligns with modern studies of microbial succession in fermentation, which identify this peak growth phase as the period of maximum metabolic output and enzymatic activity [28], [26, 29].

*B. Multivariate Validation and Divergent Metabolic Signatures*

The PLS-DA model (**Figure 5**) statistically confirmed this visual separation of the 9 different groups or treatments. The model was robust ($R^2Y$=0.803, $Q^2$=0.626) and showed clear, non-overlapping 95% confidence ellipses for the high-dose (A4, N4) and control groups. This model not only showed clustering; it was a robust classification and potential predictive separation. Component 1, which captured the largest variance (25.7%), cleanly separated the two genera, demonstrating that the metabolic signature of *L. pseudomesenteroides* is fundamentally different from that of *P. pentosaceus* .

The VIP-score analysis (**Figure 6**) allowed us to identify the specific volatile compounds, or "chemical architects," responsible for this separation. The results revealed two divergent metabolic strategies, i.e., the Leuconostoc Signature (heterofermentative) which illustrated of the A-series separation which was driven by compounds such as 2-phenethyl acetate

(rose, honey), 2,3-pentanedione (buttery, creamy), and acetic acid. This is a classic signature of Leuconostoc's heterofermentative metabolism. This genus readily converts citrate, which is abundant in coffee mucilage, into acetoin and diacetyl (the precursor to 2,3-pentanedione), as well as acetic acid [7], [28], [30]. Furthermore, its known possession of alcohol acetyltransferase enzymes allows it to esterify alcohols diketone factory," while *P. pentosaceus* acts as a "pyrazine and alcohol promoter."

*C. Linking Precursor Formation to the Bean's Chemical Matrix*

The data from **Figure 7**, which details the macro-chemical composition of the green bean itself, might provide the underlying mechanistic explanation for these divergent volatile

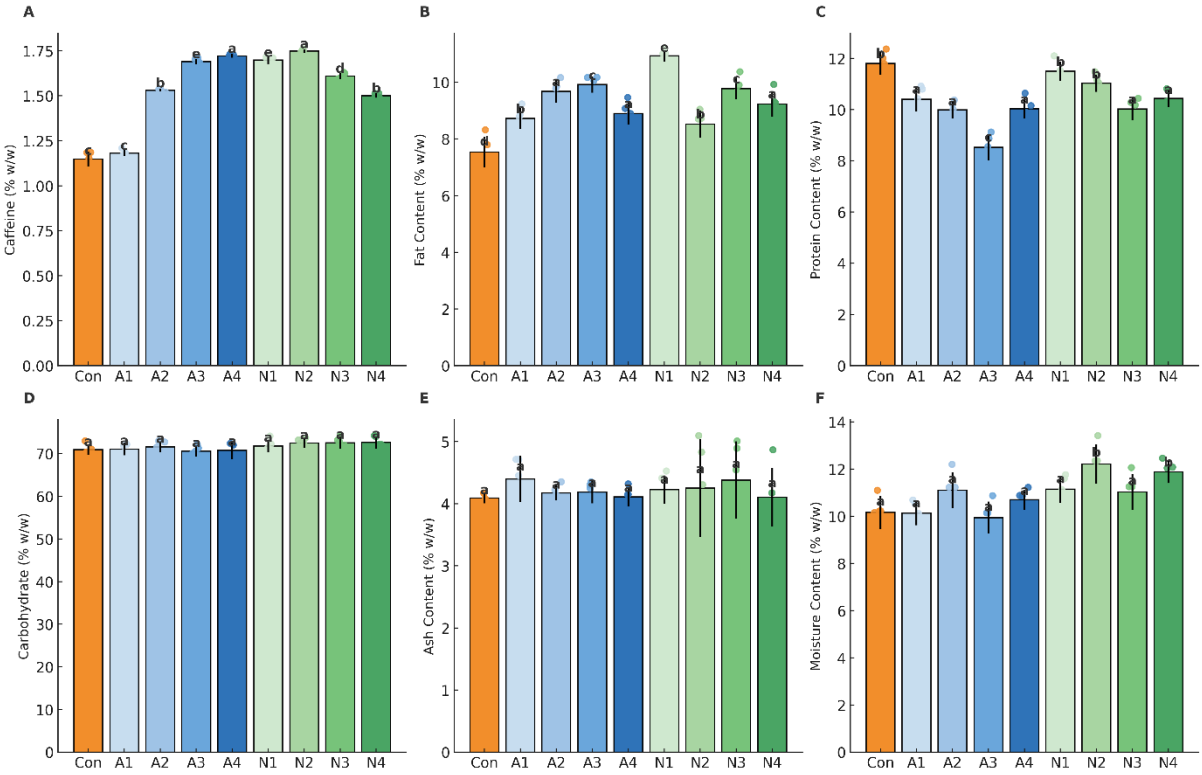


*Figure 7. Proximate composition and caffeine content of green beans (mean ± SD, n=5). Different letters denote significant differences ($p < 0.05$). (Con = Spontaneous Control; A = L. pseudomesenteroides. N = P. pentosaceus. Inoculum levels: 1 = 1.5%, 2 = 3%, 3 = 4.)*

(like phenethyl alcohol) into their corresponding acetates [31]. This aligns strongly with recent metabolomic studies that have linked Leuconostoc dominance to desirable acetate esters and high sensory quality in wet-processed coffees [31], [32].

On the other hand, the Pediococcus signature (homofermentative) which is the inoculum of the N-series, the profile was defined by different compounds, primarily benzyl alcohol (almond, floral) and a suite of alkyl-pyrazines. *P. pentosaceus* is a facultative homofermentative organism, rapidly converting hexose sugars primarily to lactic acid, which creates a different chemical environment and redox potential [28][33]. This, combined with a different enzymatic toolkit, clearly favours the formation or preservation of these specific alcohol and pyrazine precursors. This finding is directly supported by recent (2025) literature where *P. pentosaceus* (in a sequential fermentation) was also shown to enhance almond-floral alcohols [34]. This side-by-side comparison elucidates a key finding: *L. pseudomesenteroides* acts as an "ester and profiles. The most significant finding was the opposing effect of the two LAB on the bean's protein content. *P. pentosaceus* (N-series) preserved the protein content (~11%), showing no significant difference from the control (11.8%). Conversely, *L. pseudomesenteroides* (A-series) inoculation led to a steep decrease in total protein, dropping it to 8.5% (in A3). This finding might provides the basis for a proposed mechanism explaining the divergent volatile profiles. *L. pseudomesenteroides* is known for its strong proteolytic and peptidolytic enzyme systems [35, 36], [37]. It may actively break down the bean's native protein (the loss in **Figure 6**) which logical consequence of this proteolytic action would be the liberation of free amino acids (FAAs) from the bean's protein matrix [29]. And then they might be catabolised into fusel alcohols (e.g., phenethyl alcohol, 3-methylbutanol), which are the direct precursors to the esters seen in **Figure 5** [38], [39]. As a result, protein loss in **Figure 7** is mechanistically and directly linked to the 2-phenethyl acetate gain in **Figure 6**. *L.*

*pseudomesenteroides* uses protein to generate desirable floral aroma precursors.

This hypothesis may also explains the *P. pentosaceus* results. Known for weaker extracellular protease activity [11], it spares the bean's protein. While it cannot produce the same high levels of floral esters, its metabolic output is directed elsewhere, possibly, toward pyrazine formation an aroma of vegetal green, which relies on different precursors that may be more available in its specific metabolic niche [25], [40].

*D. Impact on the Green Bean's Macro-Chemical Matrix*

Beyond the volatile aroma precursors, the fermentation process also induced significant, species-specific changes to the green bean's non-volatile and macromolecular composition (**Figure 6**). The Pediococcus N1 treatment significantly increased the fat content (**Figure 7B**) (10.9% vs. 7.5% in the control). This protein-sparing, fat-enhancing profile suggests a pathway toward a fuller-bodied coffee. This finding aligns with studies such as Darwin et al. who also noted significant changes in protein and fat contents in Indonesian Arabica coffee when fermented with mixed cultures from civet faeces [41]. Our study, however, isolates this effect, suggesting *P. pentosaceus* specifically promotes a preservation-type strategy, providing the roaster with a bean matrix rich in raw materials for the Maillard and Strecker reactions [42].

The fact that total carbohydrates (**Figure 7D**) remained statistically unchanged ($p=0.16$) is a critical finding. It indicates that the LAB were metabolizing the simple sugars in the mucilage, and not degrading the bean's structural carbohydrates (e.g., hemicellulose) [32]. This means they added significant aromatic value without compromising the bean's physical integrity.

Regarding the caffeine content, while the data on carbohydrate, moisture content and ash content did not suggest a statistically significant trend across all treatments, some inoculated groups (e.g., A4, A3, N2, and N1) showed a higher apparent mean caffeine concentration compared to the control. This observed variation was unlikely to be due to new caffeine biosynthesis, as LAB are not known to produce this alkaloid.

A more plausible and hypothetical explanation was related to the broader enzymatic activity during fermentation. It is possible that the breakdown of complex plant cell wall structures by various microbial enzymes could altered the bean matrix [43]. This structural modification might, in turn, improve the extractability of compounds like caffeine during the analytical process. Alternatively, such enzymatic action could potentially liberate caffeine that was previously complexed with other compounds, such as chlorogenic acids or other phenolics [43], [44]. The green bean is a rich reservoir of such phenolic compounds, whose extractability is highly dependent on the surrounding matrix [45]. Caffeine in coffee also forms molecular complexes with chlorogenic acids and other polar compounds [46]. Phenolics and caffeine may also create covalent bonds with proteins, therefore, arguably the protein decomposition in this study could indirectly improve caffeine extractability. However, further analyses are required to provide clearer evidence. For example, quantifying the total phenolic content and specific chlorogenic acid compounds would enrich the scientific rigor of future studies.

Further analysis showed that the fermentation treatments did not significantly alter the green beans' ash or moisture content (**Figure 7D, 7F**). The ash content, reflecting the inorganic mineral composition, remained stable across all groups, as expected. Moreover, the final moisture levels for all samples were maintained within the 9.1–11.5% range. This consistency demonstrates that the inoculation protocols do not negatively impact these fundamental quality parameters and that the beans were processed to an acceptable and stable final moisture level.

*E. Practical Implications for the Coffee Industry*

This sequential analysis provides a clear metabolic roadmap for coffee producers and roasters. The findings present distinct, practical strategies for coffee producers to engineer green bean chemistry based on desired sensory targets. For a modern profile characterised by floral and bright complexity, inoculation with *L. pseudomesenteroides* at a 3–4.5% dose (A2-A3) proved to be an effective choice. This specific treatment optimizes the formation of floral (2-phenethyl acetate) and creamy (2,3-pentanedione) precursors, likely via enhanced proteolysis, while also advantageously reducing bean moisture. Conversely, for producers targeting a classic nutty and full-bodied profile, inoculation with *P. pentosaceus* is the recommended intervention. This strain enriches the bean with nutty pyrazine precursors and almond-like benzyl alcohol. Importantly, it also preserves the protein and fat matrix, ensuring the roaster has ample raw material for the Maillard-driven reactions that build body and complexity. This study moves beyond the general consensus that "fermentation matters" and provides a species- and dose-specific guide for producers to purposefully engineer green bean precursor chemistry.

## IV. CONCLUSION

In conclusion, this research confirms that the targeted inoculation of coffee fermentation with specific lactic acid bacteria is an effective, predictable strategy for engineering the green bean's aroma precursor chemistry for on-farm processing. The metabolic outcomes were confirmed to be both species- and dose-dependent, revealing divergent pathways. A clear practical recommendation emerged, an inoculation of 3–4.5% *L. pseudomesenteroides* offered the optimal balance, significantly enriching desirable floral (2-phenethyl acetate) and buttery (2,3-pentanedione) precursors. Conversely, *P. pentosaceus* inoculation preserved the bean's macromolecular matrix, favouring the development of nutty precursors. This work provides a clear biochemical roadmap for these interventions; however, future studies are needed to validate these precursor-driven findings. This should involve systematic sensory analysis to connect this engineered green bean chemistry to final cup quality, as well as the direct quantification of key substrates,

such as free amino acids, to further elucidate the precise metabolic pathways. As well as, total phenolic content and chlorogenic acids compounds.

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## CONFLICT OF INTEREST

The authors do not have any conflict of interest.

## DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

## DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors utilized Google Gemini AI for the purpose of English grammar checking and language editing to improve clarity and avoid ambiguity. The authors reviewed and revised the content thoroughly, and assumed full responsibility for the accuracy, integrity, and originality of the published article.

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