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The Predicted Association between Microbial Community, Precursors, and Coffee Aroma from SIAF-Geothermal Coffee Processing

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ABSTRACT

The SIAF-Geothermal coffee processing is a novel method that applies the combination of self-induced anaerobic fermentation (SIAF) and coffee drying using geothermal heat. This study aimed to establish predictive associations between microbial community, precursors, and coffee aroma from the SIAF-Geothermal coffee processing as the basis for the selection of starter cultures. Shotgun metagenomics, liquid chromatography-tandem mass spectrometry (LC-MS/MS), solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GCMS), and the coffee taster's flavor wheel from the Specialty Coffee Association (SCA) were used to identify the microbial community, aroma precursors, aroma compounds, and aroma attributes of SIAF-Geothermal coffee, respectively. Furthermore, the predicted association between microorganisms, enzymes, non-volatile metabolites, aroma-active compounds, and aroma attributes from the SIAF-Geothermal coffee processing was constructed from various databases. The relative abundance of the microbial genera in the whole coffee cherries SIAF was 34% of *Tatumella*, 20% of *Leuconostoc*, and 17% of *Hanseniaspora*. In the pulped coffee cherries, *Tatumella* dominated the SIAF at 53%, followed by *Hanseniaspora* and *Leuconostoc* at 14% and 11%, respectively. The beta diversity, as measured by the Bray-Curtis index, was 0.26, which showed low diversity between samples. The predicted association between microbial community, precursors, and coffee aroma from SIAF-Geothermal coffee processing could serve as a knowledge base for choosing a starter culture. *Leuconostoc pseudomesenteroides* is preferred as a single-strain culture due to its possession of enzymatic potential to deliver the desirable notes of pineapple and grape and the Qualified Presumption of Safety (QPS) label. Other candidates for starter culture are projected based on the desired aroma attributes.

Keywords: aroma attributes, microbial community, SIAF-Geothermal coffee processing, starter culture

INTRODUCTION

Conventional coffee drying under direct sunlight brings several shortcomings, such as weather dependence, long duration process, and high workloads (Firdissa *et al*., 2022). The self-induced anaerobic fermentation (SIAF)-Geothermal coffee processing is a novel method to answer these limitations. This coffee processing applies the combination of SIAF and coffee drying using geothermal heat. SIAF produces higher secondary metabolites that contribute to sensory enhancement compared to aerobic metabolism (Batista da Mota *et al*., 2022). The excess heat from the geothermal installation dehydrates the coffee's moisture very fast, up to 40% in the first three hours, which results in a water activity reduction to 0.86 and reaches 0.62 on the final day (Gunawan *et al*., 2021; Pittia *et al*., 2007).

The microbes involved in the coffee SIAF are predicted to secrete microbial enzymes that enhance the abundance of precursors in green coffee beans before geothermal drying (Istighfarah *et al*., 2025). These precursors deliver aroma attributes in the brewed coffee after the roasting process (El Hosry *et al*., 2025; Poisson *et al*., 2017). Previous studies have predicted the association between microbial community from SIAF and produced volatiles which contribute to coffee aroma (Batista da Mota *et al*., 2022; da Silva Vale *et al*., 2023; Pereira *et al*., 2022). Yeast, especially *Saccharomyces,* was associated with ethanol, and lactic acid bacteria, especially *Lactobacillus,* was associated with alcohols and esters. Alcohols and esters impart the floral, fruity and buttery perceptions in the coffee beverage (Batista da Mota *et al*., 2022; da Silva Vale *et al*., 2023; Pereira *et al*., 2022). However, these studies applied SIAF

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and conventional coffee drying under direct sunlight. During this conventional drying, natural fermentation might still take place.

Throughout geothermal coffee drying, natural fermentation might not take place due to the very low water activity (Gunawan *et al*., 2021; Pittia *et al*., 2007). Therefore, the microbial community of SIAF-Geothermal coffee processing may deliver distinguished characteristics of brewed coffee. Since the SIAF-Geothermal coffee processing is unprecedented, the association between the microbial community, precursors, and coffee aroma from this method needs to be studied. The objective of this study was to predict the associations between microbial community, precursors, and aroma profile of SIAF-Geothermal coffee as the basis for the selection of starter cultures.

MATERIALS AND METHOD

SIAF-Geothermal coffee samples

Arabica coffee cherries were harvested from a coffee plantation in Kamojang, West Java, Indonesia. The SIAF-Geothermal coffee processing was referred to our previous study (Istighfarah *et al*., 2025). The coffee cherries were washed and pulped. The washed whole coffee cherries were fermented for 5 days applying SIAF without water addition and then dried using geothermal drying for 2–4 days. The pulped coffees were also processed by the same SIAF method, followed by 7–9 days of geothermal drying. The dry house for geothermal drying was set at 50 °C with RH 30%. The conventionally processed coffee samples, whole and pulped cherries that were directly dried under the sun, were used as controls.

Shotgun metagenomic

The sample collection of the fermented coffee and the shotgun metagenomics analysis were described in our previous study (Istighfarah *et al*., 2025). Two composite samples, whole and pulped coffee cherries SIAF, from the top, middle, and bottom of three 200 L fermenters of each sample, were submerged in deoxyribonucleic acid (DNA) Shield (Zymo Research, US). In this study, the microbial genera and groups of enzymes were tentatively identified.

Shotgun metagenomics was conducted to identify all genome regions of the microbial community in the self-induced anaerobic fermented coffee, including the microbes and their functional annotations based on the National Center for Biotechnology Information (NCBI), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Carbohydrate-Active Enzyme (CAZy) databases. DNA extraction was conducted using the cetyltrimethylammonium bromide (CTAB) method. xGen DNA Library Prep EZ UNI Kit (IDT, USA) was used for library preparation. Sequencing was conducted using next-generation sequencer (Illumina NextSeq 2000, USA). The sequencing quality was set to Q40 above 90%, range of density between 450–650 K/mm2 and GC content 50%. Contigs shorter than 500 bp were filtered out. MetaGeneMark (v3.38) was used to predict the complete genome. The predicted open reading frames (ORFs) shorter than 100 bp were filtered out. Filtered ORFs from each sample were merged, and the redundant ORFs (percent identity= 100%) were filtered out, generating the unique gene catalogues. Other parameters were set to the application default. Taxonomy assignment and functional binning were performed based on the mentioned databases. Alpha diversity was measured by the Shannon-Weaver index, and beta diversity was measured by the Bray-Curtis index.

Precursors analysis

The sample collection and non-volatile metabolites analysis were referred to in our previous study (Istighfarah *et al*., 2025). Three composite samples, before SIAF, whole and pulped coffee cherries SIAF, were collected from the top, middle, and bottom of three 200 L fermenters of each sample. The samples were then manually peeled to obtain the green coffee beans and freeze-dried with liquid nitrogen. All samples were further distributed in a cool box with ice gel to our laboratory.

Non-volatile metabolites served as precursors of the coffee aroma in the final coffee beverages. The precursors available before and after SIAF were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to study metabolite alterations during SIAF (Yulianti *et al*., 2023). In this study, groups of non-volatile metabolites were tentatively identified.

The ground green beans were extracted based on the brewing method from the SCA procedures (Fernández-Alduenda and Giuliano, 2021). The filtrates were diluted with LC-MS grade methanol (1:1, v/v) and filtered through a 0.22 μm PTFE membrane (Merck, KGaA, Germany). A 5 μL volume of sample was injected onto an Accucore C18+ column (100×2:1 mm, 1.5 μm) (Thermo Fisher Scientific, Germany) at 40 °C. The mobile phases were LC-MS methanol (a) and 0.05% formic acid in LC-MS water (b). The flow rate was set at 0.2 mL/min, and the gradient elution was 0 min (5% A), 0–17 min (90% A), 17–20 min (90% A), 20–23 min (5% A), and 23–30 min (5% A). Ionization mode was used in positive and negative electrospray ionization (ESI) with Q Orbitrap mass analysis. Analysis was performed at m/z 70–900 accompanied by UV detection at wavelengths of 220, 265, 272, and 320 nm.

Compound identification was performed by analyzing raw data (chromatograms) using Compound

Discoverer 3.2 software (Thermo Fisher Scientific) and comparing the data to online libraries (FoodDB, NIST, PubChem, and MzCloud). The mass spectra of the identified compounds were analysed using ThermoXcalibur 4.2 (Thermo Fisher Scientific). Identified compounds were screened using annotation delta mass <5 ppm. Compound area data were normalized and displayed as heat maps.

Roasting, grinding, and brewing

Two kilograms of dried green coffee beans from four different processing methods (whole and pulped SIAF-Geothermal process, and whole and pulped conventional process) were randomly collected from several points. The roasting, grinding and brewing processes were carried out in accordance with the SCA procedures (Fernández-Alduenda and Giuliano, 2021). The Santoker Q10 Coffee Roaster (China) was used to fast roast all the dried green coffee bean samples. The roasting process was proceeded until the first crack was achieved (184–192 °C) and stopped at 194–196 °C. The roasting time of each sample was about 5–7 min. Medium-to-light-roasted coffee was intended to preserve its aroma and acidity. The roasted coffee was then ground and brewed for quality evaluation by Q-graders. Roasted beans were ground to medium-coarse, and 8.25 g of ground coffee were brewed with 150 mL of hot water (93 °C) and extracted for 4 min.

Volatiles analysis

The volatiles analysis was based on a previous study (Laukaleja and Koppel, 2021) with adjustments. A 5 mL volume of each brewed coffee sample and 0.4μl ethyl decanoate 0.01% (as internal standard) were put into a 22 mL gas chromatography (GC) vial. The vial was heated at 70 °C for 9 min for extraction using solid-phase microextraction (SPME) fiber divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm. GC Agilent 7890A (USA) and mass spectrophotometry (MS) Agilent 5975C (USA) with Triple Axis Detector were used. Samples were then desorbed with a split-less injector at 250 °C and helium as the carrier gas at a 0.8 mL/min rate. The heating parameter was initially started at 60 °C, and then increased at a 7 °C/min rate to 120 °C, followed by an 11 °C/min rate to 250 °C. The separation was conducted in an Agilent HP-5MS column (30 m×250 µm×0.25 µm) (USA) with an interface at 250 °C, mass scan range 29–550 m/z, MS source 230 °C, and MS Quad 150 °C. Library NIST23 (MS) was used, and a similarity index above 80% was chosen. Double replication was applied. Further, the linear retention index (LRI) of the tentatively detected volatiles was calculated based on the retention times of alkane C8–C23. The calculated LRI was then compared to National Institute of Standards and Technology (NIST) Chemistry WebBook, SRD 69, with a 20–50 difference allowed. The average relative abundance of the volatiles from duplicates was delivered as a heat map after data normalization.

Sensory analysis

Five Q-graders, three females and two males aged 25–35, from the Indonesia Specialty Coffee Campus (ISCC), Jakarta, described the perceived aroma attributes of SIAF-Geothermal and control coffees based on the coffee taster's flavor wheel from SCA (Fernández-Alduenda and Giuliano, 2021). Every Q-grader evaluated the aroma attributes of all coffee samples once. The four samples were coded A, B, C, and D for the pulped control coffee, pulped SIAF-Geothermal coffee, whole control coffee, and whole SIAF-Geothermal coffee, respectively. Tasting room was quiet and decorated in neutral colors, while the isolation booths were kept dark, quiet, and nondescript. This study was approved by the Ethics Committee of IPB University (1772/IT3.KEPMSM-IPB/SK/2025). The prediction of aroma active compounds was based on the former studies.

Prediction of the association between microbial community, precursors, and coffee aroma from SIAF-geothermal coffee processing

The tentatively identified microorganisms, enzymes, non-volatile metabolites, aroma-active compounds, and aroma attributes from the previous analyses were associated by mapping these data to various databases, such as NCBI, KEGG, and Cazy for microbial communities, enzymes, and metabolites, as well as FoodDB, PubChem, ChemSpider, NIST, and MzCloud for non-volatile and volatile chemical compounds, and Coffee Taster's Flavor Wheel from SCA for the aroma attributes. Microsoft Excel 365 was used to map this association as a network diagram.

RESULTS AND DISCUSSION

Microbial community and metabolites of SIAF

Good metagenomics data quality is often characterized by a high N50 value and a high percentage of assembly coverage. One study reported that the minimum N50 value for short-read analysis was 20 kilo base pairs (kbp) (Eisenhofer *et al*., 2024). However, the minimum contig length analyzed in the study by Eisenhofer *et al.* (2024) was 1,500 bp, which resulted in a lower assembly coverage of 93–97%. In microbial diversity studies, contigs can be directly mapped to databases, allowing metagenomics data with low N50 values to be used to predict microbial communities and their functional annotations. Several studies also used low N50 values (750–1,000 bp) but managed to identify microbial diversity (Kumar *et al*., 2023; Perez-Mon *et*

al., 2021; Wu *et al*., 2022). In these studies, the minimum contig length analyzed was only 500 bp. However, assembly coverage of these three studies was 98.6–99.7%, higher than in the previous one (Eisenhofer *et al*., 2024).

In our study, the minimum contig length was also 500 bp, resulting in low N50 values at 1,303 bp for the whole coffee cherries self-induced anaerobic fermentation (SIAF) sample and 1,175 bp for the pulped coffee cherries SIAF sample. However, the assembly coverage of both samples was high, 99.3% and 99.63%, respectively. The justification of our current study is that short contigs were not discarded and were included in further analyses, resulting in high assembly coverage.

The detected microbial genera in SIAF of Arabica coffee at room temperature (20±1.5 °C) without added water for 5 days are available in Figure 1. In the fermented whole coffee cherries, *Tatumella* led with 34%, followed by *Leuconostoc* 20% and *Hanseniaspora* 17%. While in the fermented pulped coffee cherries, *Tatumella* dominated with 53%, followed by *Hanseniaspora* and *Leuconostoc,* 14% and 11%, respectively. These microbial communities were dominated by *Enterobacterales,* yeast, and lactic acid bacteria (LAB).

The putative species-level assignments and specific enzymes were obtained in our previous study (Istighfarah *et al*., 2025). These microbes presumably secreted specific microbial enzymes. In this study, microbial enzymes are categorized into three major groups: amylolytic, proteolytic, and lipolytic (Table 1). The microbial enzymes were uncovered from their open reading frames (ORF), which were mapped to the databases. Since the enzymes were not identified directly from the enzyme assays or the gene expressions, the results of this study are predictive. Therefore, further studies utilizing complementary approaches such as enzyme assays and transcriptomics are recommended.

Our previous study predicted that the microbial community of coffee SIAF, with its enzymes, formed a synergistic symbiosis to establish an environment that supported the production of metabolites or aroma precursors of SIAF-Geothermal coffee (Istighfarah *et al*., 2025). Each microbial species played a distinct role in this symbiotic relationship. *Tatumella* sp. broke down sucrose into glucose and fructose, which could be utilized by yeast and LAB (Xiao *et al*., 2021). LAB, on the other hand, produced organic acids, creating a favorable environment for yeast growth. While yeast produced soluble nitrogen compounds and vitamins, which were beneficial for the growth of LAB and *Tatumella* sp. (Batista da Mota *et al*., 2022).

Alpha diversity was calculated using the Shannon-Weaver index. The values obtained were 2.167 and 2.117 for the whole coffee cherries SIAF sample and the pulped coffee cherries SIAF sample, respectively. Both values are in the low range. This indicates that each sample contained a low variety of microbes, with only one or two genera dominating, or the abundances of the genera were uneven.

These results were supported by beta diversity, calculated using the Bray-Curtis index. The beta diversity index was 0.26, also indicating low diversity. The genera and their abundances between the two samples are not significantly different. The low alpha and beta diversity index values suggest that the pulping process, or the removal of the skin and part of the flesh of the coffee cherries, results in similar microbial communities, albeit with varying abundances.

Upon further observation, these differences in genus abundance resulted in a higher relative abundance of chlorogenic acid, simple sugars, and carboxylic acids in the whole coffee cherries sample compared to the pulped coffee cherries sample after SIAF (Figure 2). This higher relative abundance of aroma precursors in the fermented whole coffee cherries contributed to the formation of various positive notes in the SIAF-Geothermal-brewed coffee.

Table 1. The tentatively detected species and their open reading frames (ORF) of enzymes

Microbial Species	ORF of Enzymes
Pantoea rodasii	Proteolytic, lipolytic
Rouxiella badensis	Proteolytic, lipolytic
Pseudomonas sp. M47T1	Amylolytic
Lactiplantibacillus paraplantarum	Proteolytic
Pantoea sp. A4	Proteolytic
Tatumella sp. UCD-D_suzukii	Proteolytic
Pantoea sp. Acro-835	Proteolytic
Tatumella sp. JGM130	Proteolytic, lipolytic
Tatumella ptyseos	Proteolytic, lipolytic
Leuconostoc citreum	Proteolytic, lipolytic
Tatumella citrea	Proteolytic
Serratia sp. M24T3	Amylolytic, lipolytic
Frateuria aurantia	Proteolytic
Kluyvera ascorbata	Proteolytic, lipolytic
Lactococcus lactis	Amylolytic, proteolytic, lipolytic
Lactiplantibacillus plantarum	Amylolytic, proteolytic, lipolytic
Hanseniaspora uvarum	Amylolytic, proteolytic
Leuconostoc pseudomesenteroides	Amylolytic, lipolytic
Tatumella sp. JGM118	Proteolytic

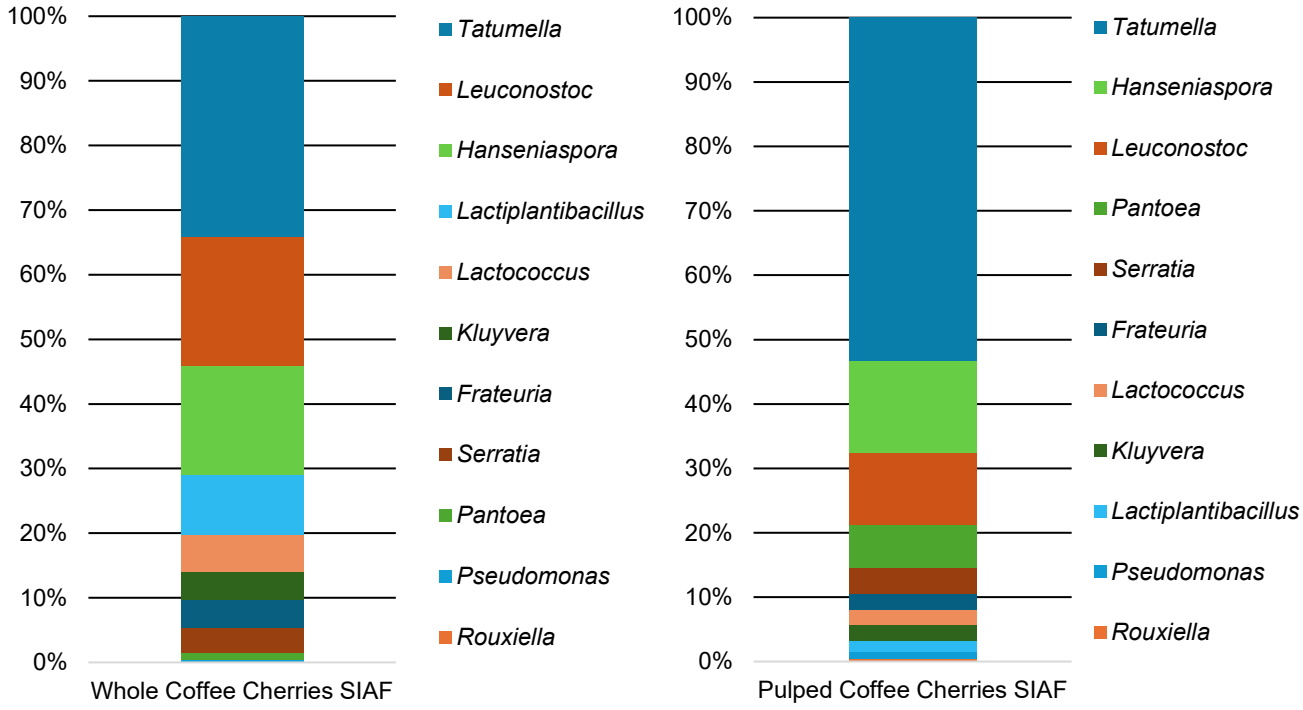

Figure 1. Relative abundance of tentatively identified microbial genera of Arabica coffee SIAF

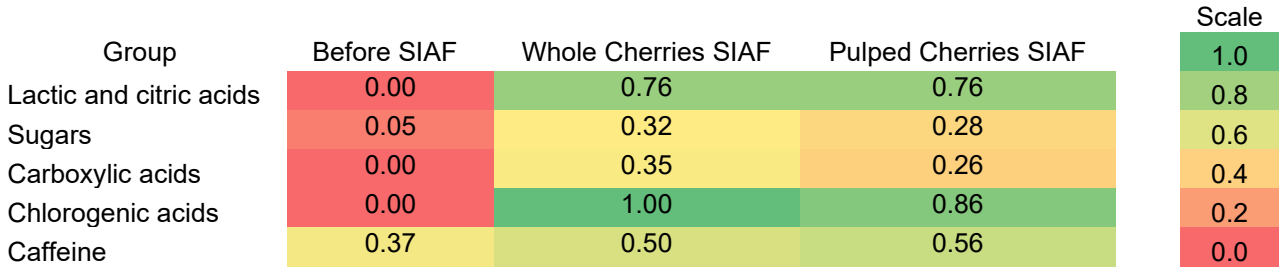

Figure 2. Relative abundance of tentatively detected non-volatile aroma precursors in Arabica coffee SIAF

There is a food safety concern in the coffee industry. Fungi pose a food safety threat in coffee processing due to their ability to produce ochratoxins (Attiya *et al*., 2021). This metabolite can survive high temperatures during the drying and roasting processes. Meanwhile, SIAF has the advantage of preventing the growth of mycotoxin-producing fungi due to anaerobic conditions. Another food safety concern is from *Tatumella ptyseos,* which is labelled as an opportunistic pathogen that causes human infections (Mardaneh *et al*., 2014; Sérgio Gonçalves da Costa *et al*., 2008). *Kluyvera ascorbata* has also been reported to be an opportunistic pathogen (Akiki *et al*., 2023), although its virulence has not been studied. However, the use of high temperatures during the coffee production process presumably could eliminate its safety risks.

Aroma compounds and attributes of SIAF-Geothermal coffee

The unique aroma attributes perceived by Q-graders of SIAF-Geothermal and conventional brewed coffees are available in Table 2. The whole SIAF-Geothermal coffee got the highest number of positive notes compared to other samples. These aroma attributes were compared to previous studies to predict the aroma-active compounds. Pineapple note might be affected by isobutyl isobutyrate and butyl butyrate, while pear note was predicted from *β*-damascenone. Grape note, which was also perceived in pulped SIAF-Geothermal coffee, was predicted from methyl caprate. The conventional coffees got negative notes, herb-like and under-ripe, which might be from furfuryl acetate and 1-furfuryl pyrrole, respectively.

Table 2. Unique aroma attributes and their predicted aroma-active compounds from SIAF-Geothermal and conventional coffees

Coffee Sample	Unique Aroma Attributes Perceived by Q-graders	Predicted Aroma-Active Compounds	Volatile Group	References
Pulped SIAF-geothermal coffee	Apple	*β*-Damascenone	Ketone	Liu *et al*. (2021, 2024); Wang *et al*. (2022)
	Grape	Methyl caprate	Ester	Elbarbary *et al*. (2025)
Whole SIAF-geothermal coffee	Pineapple	Isobutyl isobutyrate Butyl butyrate	Ester	Chen *et al*. (2022); Xin *et al*. (2019)
	Pear	*β*-Damascenone	Ketone	Gao *et al*. (2022)
	Grape	Methyl caprate	Ester	Elbarbary *et al*. (2025)
Pulped control coffee	Herb-like	Furfuryl acetate	Ester	Crnjar *et al*. (2023); Elhalis *et al*. (2023)
Whole control coffee	Under-ripe	1- Furfuryl pyrrole	Pyrrole	Elhalis *et al*. (2023); Obando and Figueroa (2024)

The predicted aroma-active compounds were indeed detected in the brewed coffee samples. Figure 3 shows the relative abundances of the predicted aroma-active compounds. However, relative abundance is not the sole reason for a volatile to be the character-impact compound in a sample. For example, isobutyl isobutyrate was the highest in pulped control coffee compared to the other samples. However, the pineapple note was not perceived in this sample. Therefore, it is apparent that the character-impact compound was affected not only by its relative abundance but also by its chemical structure, interaction between molecules, overall balance, aroma release, vapor pressure, and panelist (da Silva Vale *et al*., 2023).

Volatiles are not always aroma compounds, and aroma compounds are not always the aroma-active compounds or character impact compounds. Not all volatiles release aroma attributes. Chemical structures, interactions between molecules, and overall balance in the sample define this aroma release (Crnjar *et al*., 2023; da Silva Vale *et al*., 2023). Volatiles that deliver aroma attributes are aroma compounds. Aroma compounds need to be perceived by the panelists with an odor activity value (OAV) higher than 1 could be considered aroma-active compounds. OAV is the ratio between the concentration of a volatile and its threshold (Elbarbary *et al*., 2025). This threshold is affected by the determination method, panelists, and vapor pressure.

Many factors affect aroma-active compounds. A lot of studies need to be conducted to precisely define these compounds. Olfactometry and omics tests are necessary to define a certain aroma-active compound of an attribute from a sample. Moreover, coffee contains hundreds, even thousands, of volatiles and delivers a mixture of aromas from dozens of attributes (El Hosry *et al*., 2025). Therefore, this study can only predict these aroma-active compounds.

The predicted association between microbial community, precursors, and coffee aroma from SIAF-Geothermal coffee processing

The predicted association between microorganisms, enzymes, non-volatile metabolites, aroma-active compounds, and aroma attributes from the SIAF-Geothermal coffee processing could serve as a knowledge base for choosing a starter culture to enhance a specific desired aroma attribute in brewed coffee (Figure 4). The identified microbes are labelled as Generally Recognized as Safe (GRAS), opportunistic pathogens, Qualified Presumption of Safety (QPS), and plant pathogens. Each of these microbes possesses ORFs of enzymes that produce precursors.

The desired aroma attributes, such as apple and pear, are predicted to be perceived from ketones. This volatile group is formed through the Maillard reaction with sugar precursors and nitrogen-containing compounds (amino acids and peptides) (El Hosry *et al*., 2025). These precursors are produced by microbes possessing amylolytic and proteolytic enzymes. Pyrrole, which is also formed through the Maillard reaction, produces undesirable aroma attributes like unripe and earthy notes (Table 2) (El Hosry *et al*., 2025; Elhalis *et al*., 2023; Obando and Figueroa, 2024). This reaction is so complex that other volatile groups, like pyrazines, which deliver undesirable aroma attributes like burnt and nutty, are also produced from this reaction.

Other desirable aroma attributes, such as pineapple and grape, are predicted to be perceived from esters. Ester is produced via esterification of fatty acids or carboxylic acids with alcohols. Hence, microbes possessing lipolytic and amylolytic enzymes are predicted to contribute to the formation of esters. However, the undesirable aroma attribute, herb-like, might also be perceived from an ester. Therefore, it is difficult to choose a specific starter culture that contributes to the formation of positive aroma attributes.

Tentatively Identified Aroma Compounds	Pulped Control Coffee	Pulped SIAF-Geothermal Coffee	Whole Control Coffee	Whole SIAF-Geothermal Coffee	Scale
β-Damascenone	0.01	0.01	0.01	0.01	1.0
Methyl caprate	0.16	0.37	0.29	0.31	0.8
Isobutyl isobutyrate	0.02	0.01	0.01	0.01	0.6
Butyl butyrate	0.02	0.02	0.01	0.02	0.4
Furfuryl acetate	0.61	0.47	0.49	0.72	0.2
1-Furfurylpyrrole	0.01	0.03	0.02	0.01	0.0

Figure 3. Relative abundance of volatiles tentatively detected in SIAF-Geothermal and control coffees

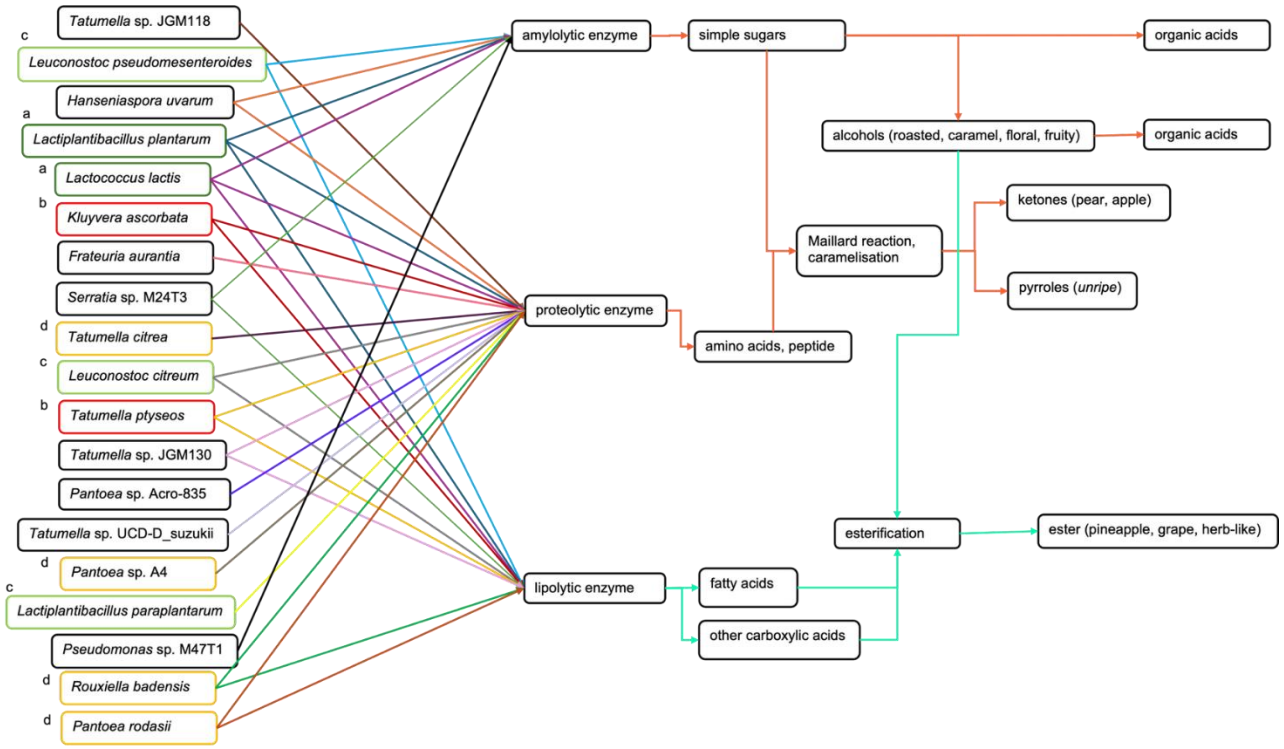

Figure 4. Predicted association of microbes, enzymes, precursors, and aroma attributes. [a]Generally Recognized as Safe (GRAS), [b]opportunistic pathogen, [c]Qualified Presumption of Safety (QPS), [d]plant pathogen

Since esters are known to deliver more positive notes than negative notes, it is easier to choose starter culture candidates that can produce precursors of esters. *Leuconostoc pseudomesenteroides* and *Serratia* sp. M24T3 possess lipolytic and amylolytic enzymes that can produce carboxylic acids and alcohols. Although *Ln. pseudomesenteroides* has not yet been granted GRAS status, it has been accorded QPS status. Furthermore, this species dominates the Arabica coffee SIAF (Istighfarah *et al.*, 2025; Pereira *et al.*, 2022).

An example of a complete prediction in determining starter cultures was based on the desired aroma attributes (Table 3). Several microbes can produce the precursors of esters, which are alcohols and carboxylic acids. However, *Leuconostoc pseudomesenteroides* and *Serratia* sp. M24T3 are putative producers of both precursors. Thus, these microbes are the candidate taxa of ester producers. *Serratia* sp. M24T3 has not been fully sequenced, so the ORF of proteolytic enzymes may be present in its whole genome. Further, this species has not yet been labelled as GRAS or QPS. Therefore, *Leuconostoc pseudomesenteroides* is a good candidate for the culture starter. *Lactiplantibacillus plantarum* and *Lactococcus lactis* are GRAS-recognized species that possess amylolytic, proteolytic, and lipolytic enzymes. Thus, these two microbes are suitable options for starter cultures.

Table 3. Starter culture candidates to achieve the desired aroma attributes

Aroma Attribute	Predicted Aroma-Active Compounds	Aroma Precursors	Enzymes	Candidate Taxa Possessing Predicted Enzymatic Potential	Candidate Taxa for Single-Strain Culture
Pineapple	Isobutyl isobutyrate, Butyl butyrate (ester)	Fatty acids, alcohols (via esterification)	Lipolytic, amylolytic	*Hanseniaspora uvarum, Lactiplantibacillus plantarum, Leuconostoc pseudomesenteroides, Lactiplantibacillus paraplantarum, Pseudomonas* sp., *Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Tatumella ptyseos, Rouxiella badensis, Pantoea rodasii, Frateuria aurantia, Tatumella* sp. *JGM130, Serratia* sp.	*Leuconostoc pseudomesenteroides* dan *Serratia sp.* M24T3
Grape	Methyl caprate (ester)	Fatty acids, alcohols (via esterification)	Lipolytic, amylolytic	*Hanseniaspora uvarum, Lactiplantibacillus plantarum, Leuconostoc pseudomesenteroides, Lactiplantibacillus paraplantarum, Pseudomonas* sp., *Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Tatumella ptyseos, Rouxiella badensis, Pantoea rodasii, Frateuria aurantia, Tatumella* sp. *JGM130, Serratia* sp.	*Leuconostoc pseudomesenteroides* dan *Serratia sp.* M24T3

Besides the challenging selection of starter culture, its implementation to improve coffee sensory quality is not a simple matter. A competition between the starter culture and the natural microbial community may occur. The existing growth medium may not be sufficient for starter culture growth. Further research is needed to study the ability of starter cultures to improve the sensory quality of SIAF-Geothermal coffee.

The prediction delivered from this study should be further validated by conducting future studies. Microbial isolation, other metagenomics approaches like transcriptomics, and controlled SIAF using a starter culture are recommended to study the microbial community and its real contribution to the aroma formation. Olfactometry, odor value, and omics tests are suggested to understand the real aroma-active compounds. Further, the association of microbial communities with the produced precursors and aroma attributes could be used as a guide for future improvement of SIAF-Geothermal coffee processing.

CONCLUSION

A novel method, SIAF-Geothermal coffee processing, combines self-induced anaerobic fermentation (SIAF) and geothermal drying to produce green beans. The association between the microbial community, enzymes, non-volatile metabolites, aroma-active compounds, and aroma attributes from this method has not been discovered. Shotgun metagenomics, LC-MS/MS, SPME-GCMS,

the coffee taster's flavor wheel of the Specialty Coffee Association (SCA), and various databases were employed to predict the association. The microbial community in the whole coffee cherries SIAF was dominated by *Tatumella* with 34%, *Leuconostoc* with 20%, and *Hanseniaspora* with 17%. While the pulped coffee cherries SIAF was dominated by *Tatumella* with 53%, *Hanseniaspora* with 14%, and *Leuconostoc* with 11%. The beta diversity, as measured by the Bray-Curtis index, revealed low diversity between samples. The predicted association between microbial community, precursors, and coffee aroma from SIAF-Geothermal coffee processing could serve as a knowledge base for selecting a starter culture. The candidates for starter culture could be projected based on the desired aroma attributes. *Leuconostoc pseudomesenteroides* and *Serratia* sp. M24T3 are presumed to possess the enzymatic potential to deliver the desirable notes, pineapple and grape. Further, *Leuconostoc pseudomesenteroides* is preferred due to its QPS label. *Lactiplantibacillus plantarum* and *Lactococcus lactis* are other interesting candidates for starter culture, which are GRAS and possess all three types of enzymes. Microbial isolation, transcriptomics, controlled SIAF using a starter culture, olfactometry, odor value, and omics tests are the suggested approaches to validate this study and to comprehend the real association between the microbial community, precursors, and coffee aroma from SIAF-Geothermal coffee processing.

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