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Sustainability Spotlight Statement

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The application of geothermal coffee processing, which involves Self-Induced Anaerobic Fermentation (SIAF) without water addition and geothermal drying, addresses the limitations of conventional coffee processing, including its dependence on weather conditions, time, water, and labor consumption. Water submergence is not applied during fermentation, resulted in less water being used. The geothermal heat facilitates coffee drying in the dry-house, allowing UV to pass through at higher temperatures, lower relative humidity, faster processing times and with less labour compared to direct sunlight drying. Moreover, the microbial community involved in the SIAF enhances the possibility of the aroma development of geothermal coffee drink.

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The Role of Microbial Community in the Self-Induced Anaerobic Fermentation on the Aroma Precursor Development of Geothermal Coffee

Zikrina Istighfarah [a], C. Hanny Wijaya* [a], Lilis Nuraida [a,b], Erliza Noor [c] and Wisnu Ananta Kusuma [d]

Coffee drying is an important stage for character development of green beans, which is usually conducted in direct sunlight. Thus, this method is very dependent on the weather conditions. Geothermal drying can be a substitute method to address the limitations of conventional drying. However, geothermal drying is very fast whereas the translocation of chemical compounds into the green bean is not ideal. Process adjustment was established by applying 5 days of Self-Induced Anaerobic Fermentation (SIAF) without addition of water before the geothermal drying to enhance the sensory quality. The whole process was further called 'geothermal coffee processing'. This study aimed to deliver knowledge about the role of microorganisms involved in the SIAF on the formation of chemical compounds responsible for geothermal coffee aroma. Samples were extracted to get the DNA of microorganisms and then analyzed with shotgun metagenomics. The species and microbial enzymes were identified by comparing the Open Reading Frame (ORF) with the databases. Further, non-volatile metabolites in the fermented green beans were analyzed using LC-MS/MS. The results showed that *Tatumella sp. JGM118*, *Hanseniaspora uvarum*, and *Leuconostoc pseudomesenteroides* were the dominant species. This microbial community secreted enzymes such as the glycoside hydrolases family (GH), transaminases, and L-lactate dehydrogenase which were detected by its ORF in the samples. Moreover, these enzymes catalyzed the production of non-volatile metabolites in the coffee beans. The non-volatiles were dominated by chlorogenic acid, amino acids, and carboxylic acids, which were not detected before SIAF. These metabolites were aroma precursors of the geothermal coffee drink after the roasting process. Therefore, it was confirmed that the microbial community in SIAF contributed to the production of geothermal coffee aroma precursors.

Sustainability spotlight

The application of geothermal coffee processing, which involves Self-Induced Anaerobic Fermentation (SIAF) without water addition and geothermal drying, addresses the limitations of conventional coffee processing, including its dependence on weather conditions, time, water, and labor consumption. Water submergence is not applied during fermentation, resulted in less water being used. The geothermal heat facilitates coffee drying in the dry-house, allowing UV to pass through at higher temperatures, lower relative humidity, faster processing times and with less labour compared to direct sunlight drying. Moreover, the microbial community involved in the SIAF enhances the aroma precursor development of geothermal coffee drink.

Introduction

Traditionally, green coffee beans are produced by either the wet or dry process. The wet process involves aerobic and submerged fermentation of pulped coffee to produce high-quality coffee beverages (1–3). Microbial activities eliminate coffee mucilage during this fermentation. The fermented coffees are then dried under the sun to obtain green beans. While in the dry process, fermentation occurs while drying. Whole coffee cherries are sun-dried on the drying floor and then peeled to get green beans (3–5).

Drying under direct sunlight is a common practice in coffee processing. However, this method has several limitations, including weather conditions. It causes variations in drying duration, as well as high workloads in the coffee industry (6). The long rainy season that occurs in tropical areas can create significant challenges for coffee farmers. The unpredictable weather in Indonesia makes this conventional coffee drying process less effective (7).

Mechanical drying using the excess heat from geothermal installation can be a substitute method that addresses the limitations of the conventional drying process (7). This excess geothermal energy can be utilized using Heat Pipe Heat Exchangers (HPHE) to increase the temperature in the dry-

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house, where the UV still can penetrate through, until 50°C with RH 30% (8,9). The business evaluation showed a significant surplus for coffee farmers due to the acceleration of the drying process (9). Conventional coffee drying generally requires up to 16 days, while geothermal drying only requires 2 to 4 days for pulped coffee and 7 to 9 days for whole coffee cherries.

Nevertheless, this acceleration of the drying process makes the metabolites' translocation into coffee beans incomplete. The water content evaporates very quickly with a reduction of more than 40% in the first three hours, causing water activity to decrease to 0.88 and reach 0.62 at the end of the drying process (7,10). This amount of water activity is insufficient for the survival of vegetative of microorganism cells, therefore, the fermentation process does not occur during the geothermal drying process.

SIAF is a new coffee fermentation technology that produces CO2 gradually as a result of microbial activities. Whole or pulped coffee is placed in a closed container to maintain anaerobiosis (11,12). This process produces metabolites that impact the quality of the coffee drink. Process adjustments are applied by employing Self-Induced Anaerobic Fermentation (SIAF) before geothermal drying to produce coffee that is favourable to consumers and Q-graders. This combination of SIAF and geothermal drying has not been studied before. Therefore, existing information on the role of microbiota involved in SIAF on the aroma precursor development of geothermal coffee is still limited.

SIAF can improve the sensory quality of coffee beverages by producing favourable chemicals compared to the conventional coffee fermentations (13). Chlorogenic acids, reducing sugars, organic acids, and amino acids are produced throughout coffee fermentation by yeasts and bacteria. These metabolites are precursors for pyrazines, aldehydes, ketones, furans, and esters during the roasting process. Sensory attributes resulting from the mentioned volatiles give roasted, nutty and fruity notes (14).

Microbial community involved in the SIAF was assumed to produce aroma precursors in the fermented green bean before geothermal drying. In this research, we analysed the relative abundance, diversity, and functional properties of the microbial community in the SIAF of Arabica coffee using Shotgun Metagenomics. LC-MS/MS was used to analyze the non-volatile metabolites resulting from the fermentation. Furthermore, the relations between the microorganisms, their enzymes and their produced non-volatile metabolites as the aroma precursors of geothermal coffee were analyzed by connecting the acquired data from the Shotgun Metagenomics and LC-MS/MS to the KEGG and NCBI databases. This knowledge aims to provide a theoretical basis for the development and research of Geothermal Coffee Processing.

Materials and Methods

Self-Induced Anaerobic Fermentation (SIAF) of Arabica Coffee

All coffee cherries samples were harvested on the same day from an Arabica coffee plantation in Kamojang, Indonesia. After being washed, 240 kg of whole coffee cherries and 300 kg of pulped coffee, respectively, were placed in three 200L fermentation barrels (Figure 1). Every barrel was then tightly closed and equipped with an air lock. The SIAF process was conducted for five days in room temperature. The precise temperature and pH were monitored daily. The geothermal drying was further conducted to get the final green coffee bean. However, in this study we only focus on the effects of SIAF process on the aroma precursor development of geothermal coffee.

After five days of fermentation, coffee of each 100 grams and the released water from coffee cherries of each 50 grams, respectively, were collected from the top, middle and bottom of each barrel and mixed thoroughly. The samples were then submerged with DNA Shield (Zymo Research, California, US) for shotgun metagenomics analysis. Coffee samples of each 100 grams for non-volatile metabolite analysis were also collected from the top, middle and bottom of each barrel and mixed thoroughly. The samples were then manually peeled to obtain the green coffee beans and freeze-dried with liquid nitrogen. All composite samples were further transported in a cool box containing ice gel from the coffee processing facility to our laboratory.

Shotgun Metagenomic

Shotgun metagenomics was employed to analyse the microbial community. This analysis applied the Next-Generation Sequencing (NGS) method which provided an overview of the abundance and diversity of various microbial species and enzymes involved in the SIAF of Arabica coffee. Firstly, the samples were thawed, and then DNA extraction was conducted using the CTAB method. The DNA concentration was measured with Qubit dsDNA HS Assay Kits (Thermos Scientific, Massachusetts, US) and the DNA integrity was measured with 4150 TapeStation (Agilent, California, US). Further, the NGS library preparation was accomplished using an xGen DNA Library Prep EZ UNI Kit (IDT, Coralville, US). The DNA concentration and DNA size of the samples were measured again to meet the requirements of the NGS instrument (Illumina NextSeq 2000, San Diego, US) (15).

The primary process that occurs in the NGS instrument was Surface Restricted Fluorescence Sequencing (SURF-seq). This process used the principle of sequencing by synthesis. After sequencing was completed, the resulted reads were filtered with fastp (v0.23.2) and quality checked using fastQC (v0.11.9) and MultiQC (v1.13). Further steps of bioinformatics analyses include assembling reads into contigs with de novo assembly using MEGAHIT (v1.2.9), quality control and visualization of contigs using QUAST (v5.0.2) and Bandage (v0.8.1).

Data transformation from FASTA file to SAM file and deletion of host DNA, coffee tree, was performed using SAMtools (v1.6). Contigs with coverage less than two and/or length shorter than 500 bp were filtered out using BBtools (BBMap v37.62). The complete genome prediction was conducted using MetaGeneMark (v3.38). The predicted ORFs with length shorter than 100 bp were filtered out using seqkit (v2.5.1). Filtered ORFs

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from each sample were merged and the redundant ORFs (percent identity = 100%) were filtered out using BBMap, generating the unique gene catalogues. Other parameters of all the tools were set to the application default.

Taxonomy assignment was performed using diamond v2.0.15 (blastx)-MEGAN v6.24.24 (LCA, MEGAN-LR) pipeline. Curated NCBI-nr database was used. Taxonomy results were filtered until species level. Functional binning was performed using various databases, including EC (MEGAN) and Cazy (diamond). Mapping to gene catalogues from each sample was performed using BWA (v0.7.17-r1188). Reads coverage was determined using bedtools (v2.30.0) and count table (Reads per Kilobases - RPK) was generated using SAMtools. Taxonomy visualization was performed using Krona (v2.8.1). Downstream taxonomy and functional analysis were performed using phyloseq (v1.44.0). Completeness check was performed using BUSCO (v5.4.4).

Moreover, Cazy database (cazy.org) was used to understand the mechanism of carbohydrate-active enzymes. The detected Enzyme Commission (EC) Numbers were compared to the KEGG database (kegg.jp) to recognize their cellular functions and metabolites. Alpha diversity was calculated with Shannon-Weaver index and beta diversity with Sørensen-Dice index. This metagenomic analysis was assisted by PT. Genetika Sains Indonesia.

Non-Volatile Metabolites Analysis

The non-volatile metabolites were analysed using UHPLC Q Orbitrap HRMS (Thermo Fisher Scientific, Bremen, Germany) (4,16). Sample preparation was carried out by lyophilizing and then grinding the green coffee beans. The ground green bean (8.25 g) was dissolved in 150 mL of boiling water. The solution was maintained at a temperature of 93°C while stirring using a magnetic stirrer for 1 minute. The sample solution was then cooled down and filtered using Whatman filter paper number 1. It was stored in a freezer at a temperature of -22°C before injection.

The filtrates of ground green beans were diluted with LC-MS grade methanol (1:1, v/v) and filtered through a 0.22 µm PTFE membrane (Merck, KGaA, Darmstadt, Germany). A 5 µL volume of sample was injected onto an Accucore C18+ column (100 × 2:1 mm, 1.5 µm) (Thermo Fisher Scientific, Bremen, 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 320nm.

Compound identification was performed by analysing raw data (chromatograms) using Compound Discoverer 3.2 software (Thermo Fisher Scientific) and comparing the data to online libraries (FoodDB, HMDB, NIST, Pubchem, and MzCloud). The mass spectral of the identified compounds were analysed using ThermoXcalibur 4.2 (Thermo Fisher Scientific). Identified compounds were screened using Annot (DeltaMass <5 ppm). Compound area data were normalized and displayed as heat maps.

Figure 1. Workflow of the experimental process shows that there are two different samples for Shotgun Metagenomics and non-volatile metabolite analysis using LC-MS/MS.

Results and discussion

Microbial Profile

The characteristics of reads and contigs in this study are shown in Table 1. Metagenomic dataset usually shows 5-23% duplicate reads (17). The whole coffee cherries SIAF sample gave slightly higher duplication rate which could be caused by PCR amplification during library preparation. Optimum GC content for metagenomic analysis was 50%. Lower or higher GC content than 50% was caused by lower relative sequencing coverage (18).

N50 for metagenomic assembly is usually 20kbp (19). Low N50 value might be due to low sequencing coverage, high diversity of microorganisms and high genome complexity. Moreover, default settings of the tools might not be optimal for the

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samples. However, low N50 value was allowed for diversity study since the contigs were aligned to the databases.

Microbial community of both samples showed low alpha diversity and no overlap for beta diversity. SIAF of whole coffee was dominated by *Tatumella sp.* JGM118 (28%), *Leuconostoc pseudomesenteroides* (18%) and yeast *Hanseniaspora uvarum* (17%), respectively. The succeeding highest species were *Lactiplantibacillus plantarum* (9%) and *Lactococcus lactis* (6%), respectively. The subordinates were species with relative abundance less than 5%, which were *Kluyvera ascorbata, Frateuria aurantia, Serratia sp.* M24T3*, Tatumella citrea, Leuconostoc citreum, Tatumella ptyseos, Tatumella sp.* JGM130*, Pantoe asp.* Acro-835, *Tatumella sp.* UCD-D_suzukii*, Pantoe asp.* A4*, Lactiplantibacillus paraplantarum, Pseudomonas sp.* M47T1*, Rouxiella badensis,* and *Pantoea rodasii.*

Meanwhile, microbial community in SIAF of pulped coffee was dominated by *Tatumella sp.* JGM118 (42%), *Hanseniaspora uvarum* (14%) and *Leuconostoc pseudomesenteroides* (9%), respectively. *Tatumella citrea* (5%) and *Serratia sp.* M24T3 (4%) were the following two most abundant species. The subordinates were species with relative abundance less than 4% which were *Pantoe asp.* Acro-835, *Tatumella ptyseos, Pantoe asp.* A4, *Frateuria aurantia, Tatumella sp.* JGM130, *Lactococcus lactis, Kluyvera ascorbate, Leuconostoc citreum, Lactiplantibacillus plantarum, Pseudomonas sp.* M47T1, *Tatumella sp.* UCD-D_suzukii, *Pantoea rodasii, Rouxiella badensis,* and *Lactiplantibacillus paraplantarum* (Figure 2).

The final temperature showed 20.2^{O}C for whole cherries SIAF and 21.2^{O}C for pulped cherries SIAF (Supplement Figure 1). The temperature of whole coffee cherries and pulped coffee cherries SIAF during 5 days increased 1.5^{O}C and 2.5^{O}C, respectively. This led to domination of mesophilic microbiota in the fermentation process. pH of whole cherries SIAF started at 5.5 and finished at 4.15, while pH of pulped cherries SIAF gradually decreased from 6.0 to 4.25 on the last day (Supplement Figure 2).

The temperature increase is a good sign of proper SIAF process due to the exothermic reaction of sugar conversion to organic acids and alcohols (13). pH monitoring showed that the coffee samples were not over-fermented since pH 4 was the estimated condition of complete mucilage degradation (20,21). SIAF occurs spontaneously by epiphytic microbial community. Their fermentative activity is affected by intrinsic and extrinsic factors which interfere the quality of final coffee beverages (11).

The low-level oxygen available in the SIAF bioreactors was utilized by yeast, Lactic Acid Bacteria (LAB) and facultative anaerobic bacteria like *Tatumella*. The symbiotic mutualism of yeast and LAB directly affects the aroma development of coffee drink. Therefore, self-induced anaerobic fermented coffee is associated with improved sensory attributes. LAB delivers acidic environment which is susceptible for yeast growth. While yeast provides soluble nitrogen compounds and vitamins that support the bacteria growth (13).

It was also recorded in a study from Brazil that *Hanseniaspora uvarum, Lactiplantibacillus plantarum,* and *Leuconostoc mesenteroides* dominated Arabica coffee SIAF at mesophilic condition (11). The notable difference here was the domination of *Tatumella sp. JGM118. Tatumella* was included in the family of *Erwiniaceae* (Figure 3). This family consists of several plant pathogens. *Tatumella ptyesos*, one of the species, was found to be the causative agent of pink disease in pineapple (12). However, as a member of *Enterobacterales* order, it has been mentioned that *Tatumella* have the capacity to decompose sucrose under anaerobic conditions during coffee fermentation. The intermediates of sugars degradation were further utilized through glycolysis and the TCA cycle to produce reducing sugars and organic acids, substrates of amino acid metabolism like fructose and other byproducts (22). These metabolites are the precursors of the Maillard reactions (23).

Table 1 Characteristics of the resulted reads and contigs

Sample Name (filtered)	Read duplication rate (%)	GC Read (%)	Read Length (bp)	Reads (M)	Largest contig (bp)	Total length contig (bp)	N50 (bp)	N75 (bp)	L50 (contigs)	L75 (contigs)	GC contig (%)
Whole coffee cherries SIAF	29.1	37	138	28.4	131338	421774401	1303	780	83512	190223	36.08
Pulped coffee cherries SIAF	18.3	40	132	26.3	290715	382692454	1175	742	84334	188740	38.39

GC read is the Guanine and Cytosine content in the reads. The N50 and N75 were the lengths of shortest contigs at 50% and 75% of the total assembly length. GC contig is the Guanine and Cytosine content in the contigs. The L50 and L75 were the counts of shortest contigs which their length sum makes up 50% and 75% of the genome size.

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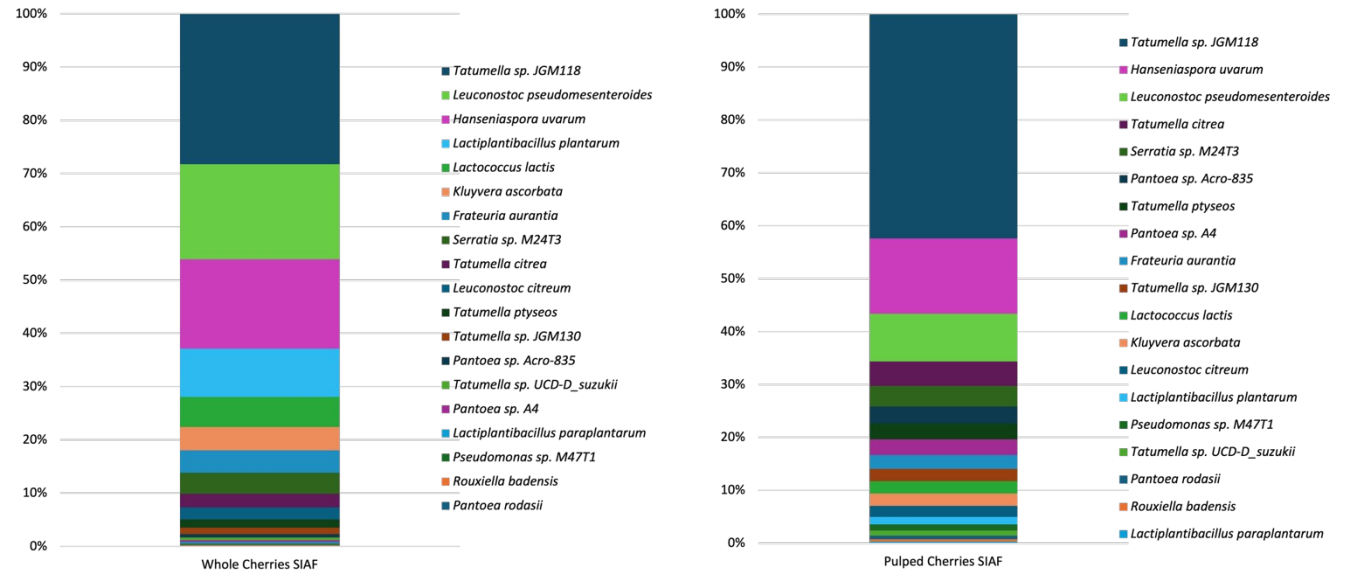

Figure 2 Relative abundance of microbial community in the whole (left) and pulped (right) coffee cherries SIAF. Tatumella sp. JGM118 dominated, followed by different microbial composition and relative abundance in both samples.

Yeasts are the key players of coffee fermentation. They hold a crucial role in the development of aroma precursors in the fermented green beans. They hydrolyze macromolecules to produce reducing sugars, citric acid, amino acids, and chlorogenic acids (24). These metabolites were translocated into the beans and affecting the coffee drink quality (20).

Besides, yeast is a volatile compound producer. The non-*Saccharomyces* yeasts deliver esters, alcohols, and fatty acids (25). *Hanseniaspora uvarum* exhibited higher activity of pectinase, amylase, cellulase, and protease compared to other yeast species (26). This yeast prefers fructose and glucose rather than sucrose (21).

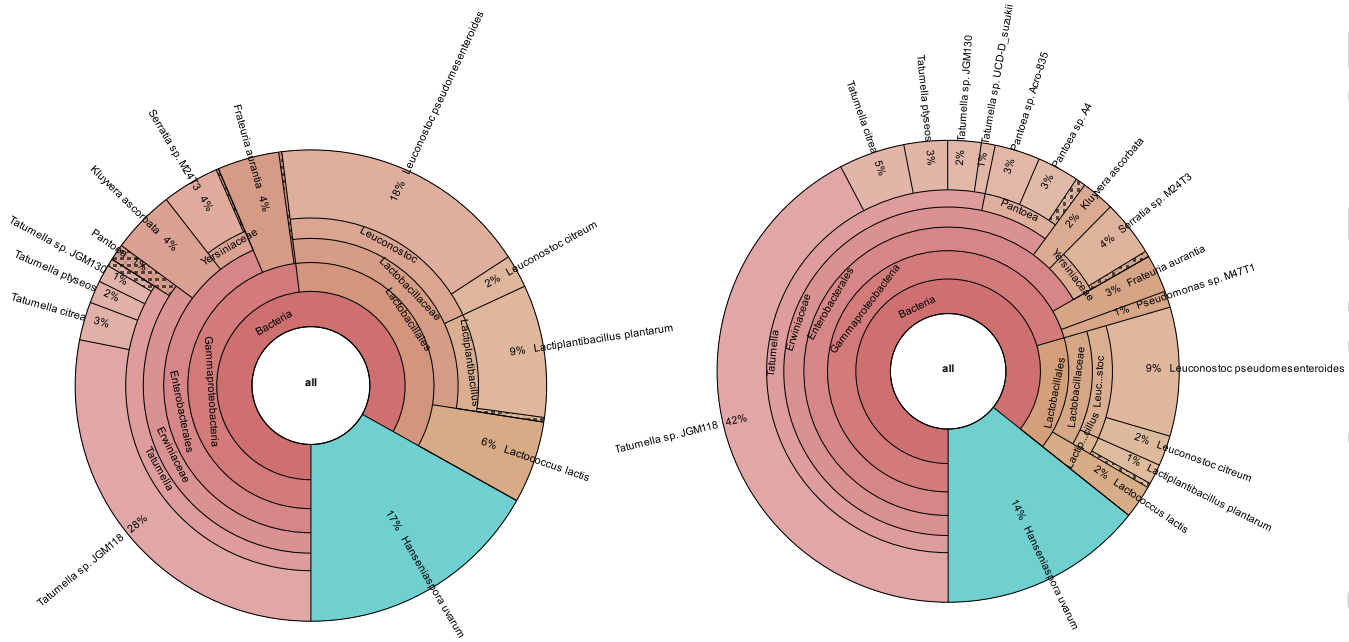

Figure 3 Microbial diversity of whole (left) and pulped (right) coffee cherries SIAF. The taxa showed species, genus, family, order, class and domain of the microbial community.

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The LAB were represented by *Leuconostocaceae* at the beginning of coffee fermentation. Further, the abundance of *Lactobacillaceae* increases at 48 and 72 h in SIAF of pulped coffee cherries (27). They are notable for organic acids producers especially lactic acid. Their activity is responsible for the degradation of coffee mucilage since pectic substances are soluble in organic acids. This acidification causes swelling on the mucilage cell wall (26). The present simple sugars were consumed first by them before polysaccharides, resulting in organic acids via Glycolysis and Pyruvate metabolism, producing d-lactate, ethanol and CO_2.

Frateuria aurantia (28), *Serratia* sp., (29) and *Pseudomonas* sp. strain M47T1 (30) are beneficial for plant as a potassium solubilizing bacteria, antifungal, and nematotoxic, respectively. While *Pantoea rodasii* (31), *Rouxiella badensis* (32), *Pantoea* sp. *A4* (33) are plant pathogens. *Kluyvera ascorbata* is an emerging pathogen and rare cause of urinary tract infection in immunocompetent patients (34). However, it will not have any effect on consumer health since high temperature processes applied before consumption. Aerobic groups of fungi that produce toxins like *Aspergillus* and *Penicillium* were not found in this anaerobic environment although their spores may still survive (25). Temperature and humidity control during coffee drying and storage are therefore needed.

Aroma Precursors

Analysis on the non-volatile metabolites using LC-MS showed that SIAF coffee is characterized by chlorogenic acid, carboxylic acid and amino acids (Figure 4). Before SIAF, these metabolites were not detected. Further, its concentration increased 7 until 10 folds after SIAF.

Chlorogenic acid (CGA) was the dominant metabolite in the green beans from SIAF process. CGA is an ester of caffeic acid and quinic acid. This metabolite is produced for plant protection (35). SIAF of whole cherries delivered a higher relative abundance of CGA compared to SIAF of pulped cherries, since CGA was concentrated in the mucilage. During roasting process, this phenolic compound is degraded back into quinic and caffeic acid, phenols derivates, and lactones (25).

In both coffee SIAF samples, group of amino acids and peptides, group of lactic and citric acids, also caffeine from alkaloid group were the second, third and fourth highest relative abundance, respectively. Other metabolite groups such as sugars, quinic acids and coumarin were also identified in the fermented green beans. Lactic and citric acids are formed during SIAF. The high abundance of citric acid in the coffee SIAF was due to the high yield production of Krebs by the microbial community (35).

Coffee mucilage is a suitable substrate for microbial growth. It contains sugars like sucrose, also organic acids like citric and malic acids (13). During SIAF, microbial pectolytic enzymes catabolize pectin to release sugars. While microbial proteolytic enzymes also release amino acids and peptides.

Malic acid can be converted to lactic acid directly and citric acid through TCA Cycle (25). CO_2 influences the anaerobic conditions where glycerol is produced. Glycerol is the precursor of phospholipids which contribute to the formation of aldehydes and alcohols (13).

Steps of the coffee processing interfered the performance of microbial community in coffee fermentation although the calculated beta diversity was zero. Their performance was affected by the substrate and involved enzymes. The pulping process, for instance, changes the substrate composition and abundance. Therefore, the produced metabolites during SIAF may differ from the whole cherries sample (13).

Marked by the red colour in the heatmap (Figure 4), there were several amino acids and peptides which produced in the pulped cherries SIAF sample but not in the whole cherries, and vice versa. Hex-2-ulose was not found in the pulped cherries SIAF sample. This could be affected by the partial pulp removal and its transformation into Hexitol. bis-β-D-fructofuranose 1,2':2,3'-dianhydride might also not detected due to the pulping process. Lactic acid in this study was found to be higher in the sample of whole cherries SIAF, compared to the pulped cherries SIAF. Without pulping process, high concentration of carbohydrates, the substrate of lactic acid, was still intact in the whole coffee cherries. Lactic acid is mainly produced by LABs which favor glucose via Glycolysis or Pyruvate metabolism. It was relevant to the higher relative abundance of *Lactobacillales* in whole cherries SIAF sample (35%) compared to the pulped cherries SIAF sample (15%). The microaerophilic condition inside SIAF bioreactors also brought an ideal environment for LAB.

Citric acid is mainly produced by the yeast (13). Pyruvate as the result of sugar metabolism in the Kreb Cycle is converted into Acetyl-CoA and oxaloacetic acid, the precursors of citric acid. Citric acid was found to be higher in the pulped cherries SIAF. Although lower relative abundance of yeast was found in this sample, the relative abundance of *Tatumella* sp. JGM118 was higher.

Tatumella delivered a great amylolytic activity (23). The degraded sugars could be used for substrates of TCA cycle. The elimination of the coffee cherries outer skin provided direct access for the microbial community to the mucilage which delivered a variation of citric acid concentrations in between both samples. The pulping process also resulted in microbial enzyme alterations.

Citric acid contributes to sensory attributes of the coffee drink such as citrus note, fruity, floral, while lactic acid gave milky, creamy and tartness (36). The highlighted sensory attributes of SIAF coffee beverages are citrus, honey, caramel, chocolate, and nuts. Malic acid's associated aroma is green fruit like apple. Coffee drink from pulped cherries SIAF has aroma like cereal, nuts and coconut, corroborating the lactic acid content. While coffee drink from whole cherries SIAF delivers notes of rum, watermelon, chocolate, caramel, and brown sugar (36).

Proteins, polysaccharides, sugars and chlorogenic acids are naturally occurring metabolites of coffee pulp (20). These metabolites are coffee aroma precursors. However, fermentation alters the content of these metabolites. The mentioned metabolites are further involved in the formation of volatile compounds such as pyrazines, furans, esters, ketones, aldehyde and acids during coffee bean drying and roasting via a series of complex reactions. These volatiles are eventually generating aroma of the final coffee beverages (20).

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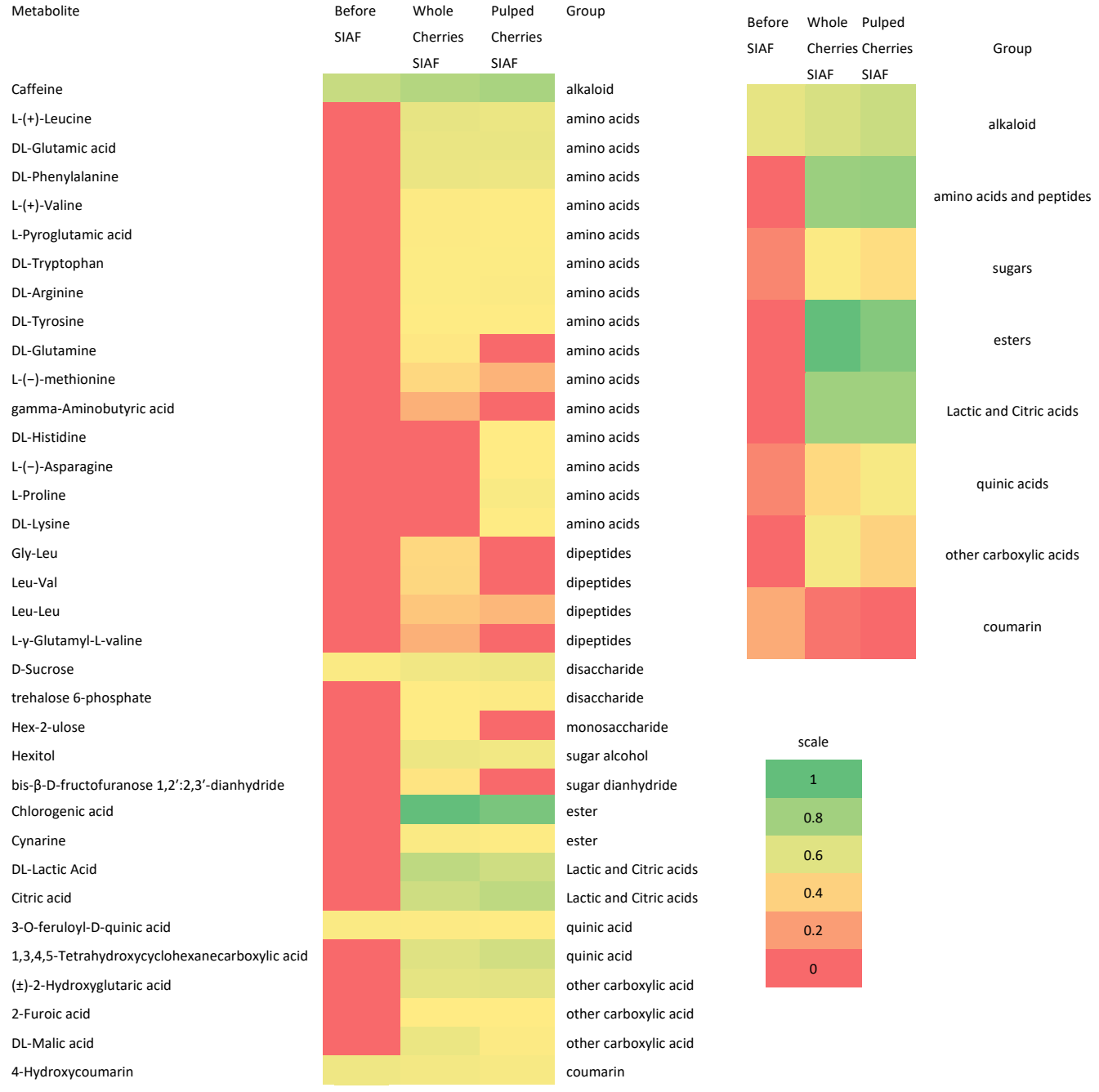

Figure 4 Heatmap of non-volatile metabolites in the green beans from whole cherries and pulped cherries SIAF that affecting the aroma development of geothermal coffee. Every compound group forms different aroma precursor.

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The series of complex reactions during roasting are caramelization, pyrolysis, dehydration, hydrolysis, enolization, cyclization, polymerization and mainly Maillard. Polysaccharides and sugars are the substrates of caramelization reaction. Phenols and guaiacol are composed via thermal reactions from chlorogenic acids and phenolics. Precursors of Maillard reactions are free amino acids and reducing sugars. Both metabolites interact and generate pyrazines and pyridines that giving the roasted and nutty aroma in the coffee beverages. Pyrazines and pyrroles are also produced via pyrolysis of hydroxyl amino acids like threonine and serine (20).

Fermentation also influences the aroma development in the coffee bean through translocation of microbial metabolites produced in this process into the green beans from the mucilage (20). Chlorogenic acid, citric acid, malic acid, quinic acids and other acids in the green beans not only contribute to the coffee acidity but also bring masking or synergistic effects to the coffee aroma. They create the Cross-modal aroma-taste interaction in coffee through sensory perception (37). Moreover, bitter tastant sub-qualities of coffee (caffeine, quinic acids, tryptophan, phenylalanine) also influence the retronasal perception of its aroma (38).

Microbial Enzymes

Coffee mucilage contains 47% wet basis of pectin (39). Carbohydrate content of the mucilage was measured to be sucrose (33.15 mg/g), glucose (49.24 mg/g), and fructose (76.26 mg/g). These numbers are much higher compared to the lipid (0.422 mg/g) and protein (0.996 mg/g) (40). Therefore, carbohydrate-active enzymes were significantly important in the coffee fermentation.

Since the coffee plant DNA had been removed using the bioinformatics analysis, only ORF of microbial enzymes that were further observed. Various ORF of carbohydrate active enzymes (Cazy) were detected. Samples of whole cherries SIAF and pulped cherries SIAF distributed different variations and relative abundance of Cazymes ORF (Figure 5). Coffee processing alters the microbial enzymes involved in coffee fermentation.

The main Cazymes that are directly involved in the degradation of carbohydrates are glycoside hydrolases (GH). While glycosyl transferases (GT) are involved in the carbohydrate biosynthesis. Carbohydrate esterases (CE) are the ones that remove acyl- or alkyl- group from carbohydrates. Auxiliary Activities (AA) breakdown lignin and non-catalytic carbohydrate-binding modules (CBM) do not have enzyme activity.

In the whole cherries SIAF sample, the ORF of GH1 family dominated with more than 45% relative abundance. The common enzyme activities of GH1 family are β-galactosidases and β-glucosidases which are possessed by *Lactococcus lactis, Lactiplantibacillus plantarum* and *Hanseniaspora uvarum* (41,42). β-galactosidases is one of the pectinases (43). Pectinases break down pectin of the coffee mucilage to produce sugars, organic acids, and alcohols. Hexitol is developed via Fructose and Mannose metabolism. The activity of this enzyme was confirmed in this study as sugar content increased in the green bean after SIAF.

The ORF of GH13 was the second most abundance of GH family in the whole cherries sample. Based on Cazy database, there are several Cazymes that included in this group. Enzyme α-amylase is produced by *Lactiplantibacillus plantarum*, *Pseudomonas sp*, and *Serratia* sp. Amylopullulanase is originated from *Lactiplantibacillus plantarum*. Oligo-α-1,6-glucosidase is associated with *Lactiplantibacillus plantarum* and yeast. Sucrose isomerase is originated from *Serratia sp* and sucrose phosphorylase from *Leuconostoc mesenteroides*. Enzyme α-glucosidase is possessed by yeast.

The following most abundance ORF of GH families in this sample were GH32, GH85, and G23. Invertase that breaks down sucrose into glucose and fructose is included in the GH32 family, produced by yeast. One of the GH85 family activities is endoglycosidase. While example of the GH23 is peptidoglycan lytic trans glycosylase which is associated with *Pseudomonas sp.*

In the pulped cherries SIAF sample, the ORF of GH 1, GH23, GH13, GH32, GH2, GH43, GH3, GH16, GH5, and GH73 families dominated the glycoside hydrolases, respectively. β-galactosidases and β-glucosidases are also included in the GH2, GH3 and GH5 families since both hydrolyse beta-glycosidic bonds. GH43 family exhibits activity like β-xylosidase or α-L-arabinofuranosidase. Activities of GH16 are reflected by β-porphyranase and β-1,3-glucanase which are originated from *Pseudomonas* sp*.,* also 1,6-glucanosyltransferas from yeast. While N-acetylglucosaminidase or autolysin from *Lactiplantibacillus plantarum* and *Lactococcus lactis* is one of the activities of GH73 family.

The enzymes contribute to aroma development detected from ORF in the coffee SIAF samples are shown in Table 2. The identified EC Numbers of enzymes detected from the ORF were compared to KEGG database to understand their molecular pathways and mechanisms related to coffee aroma development.

Several of their metabolites were detected in the previous non-volatile metabolite analysis results. While NCBI database was referred to predict the producers of the microbial enzymes. NCBI database provides information on genome and protein (enzymes) of microorganisms. However, the registered genomes are incomplete genomes which were sequenced by Whole Genome Shotgun (WGS). Therefore, the microbial

producers of aroma precursors can only be predicted. It is evident that all detected species in the samples participated in the production of metabolites that related to aroma development.

Amino acids are produced via various amino acid metabolisms. Activities of proteolytic enzymes like transaminases and dipeptidases were confirmed as amino acids were produced in the green beans after SIAF. Lactate is composed via glycolysis and malate via pyruvate metabolism. The detected microbial L-lactate dehydrogenase in the samples explain that microorganisms involved in the SIAF process played an important role in the formation of lactic acid.

As of August 2025, *Serratia sp.* M24T3, *Tatumella citrea, Pantoea asp.* Acro-835, and *Pseudomonas* sp. M47T1 have been not yet WGS-sequenced. Their ORF of enzymes are not available. The only available data are ORF that are common in the multiple species of their genus. Therefore, the predicted producers of several metabolites were mentioned as *Serratia* sp., *Tatumella* sp., *Pantoea* sp., and *Pseudomonas* sp..

The LAB belong to *Lactococcus* distribute proteolytic enzymes which enable the hydrolyzation of peptides and caseins. This group also provides transaminases that degrade amino acids and transform it into alfa-ketoacids which is precursor of aldehydes. *Lactiplantibacillus plantarum* has tyrosine kinases (44). Moreover, *L. lactis subsp. lactis* delivers arginine dehydrolase, which releases ammonia from arginine. *Hanseniaspora uvarum* enhances the abundance of D-lysine, Glutamine, Isoleucyl, Glycyl, Prolyl and Alanyl dipeptides. However, they decrease L-tyrosine, L-phenylalanine, Leucyl and Valyl di- and tripeptides. *H. uvarum* consumes intermediates of glycolysis and Kreb cycle (41).

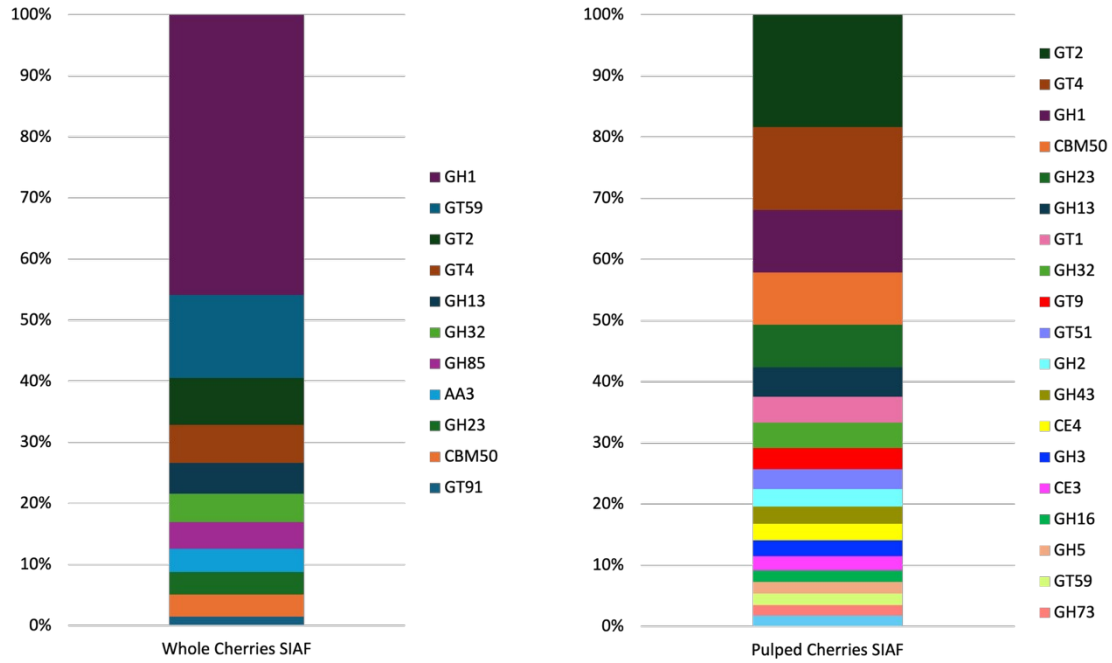

Figure 5 Carbohydrate-active Enzymes detected in the Whole (left) and Pulped (right) coffee cherries SIAF samples.

Table 2 Microbial enzymes contributed to aroma precursors development identified in the whole and pulped coffee SIAF samples

EC Number Enzymes	Pathways	Metabolites	Reference	Predicted producers
2.6.1.42 Branched-chain-amino-acid transaminase	Valine, leucine, isoleucine metabolism	L-(+)-Leucine*		*Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Tatumella ptyseos, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii*
1.4.1.13 Glutamate synthase (NADPH)	Alanine, Aspartate, and Glutamate metabolism; Histidine metabolism	DL-Glutamic acid*		*Hanseniaspora uvarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Rouxiella badensis, Pantoea rodasii, Pseudomonas* sp.
3.5.3.8 Formimidoylglutamase				*Lactococcus lactis*
3.5.1.68 N-formylglutamate deformylase				*Rouxiella badensis, Pantoea rodasii, Pantoea* sp., *Serratia* sp., *Pseudomonas* sp.

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EC Number Enzymes	Pathways	Metabolites	Reference	Predicted producers
3.5.2.9 5-oxoprolinase (ATP-hydrolyzing)				*Hanseniaspora uvarum*
3.5.1.2 Glutaminase				*Hanseniaspora uvarum, Lactococcus lactis, Kluyvera ascorbata, Tatumella ptyseos, Tatumella* sp., *Rouxiella badensis, Pantoea rodasii*
2.6.1.9 Histidinol-phosphate transaminase	Phenylalanine metabolism	DL-Phenylalanine*		*Hanseniaspora uvarum, Leuconostoc pseudomesenteroides, Kluyvera ascorbata, Lactiplantibacillus paraplantarum, Rouxiella badensis*
2.6.1 Transaminases				*Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum, Lactococcus lactis, Lactiplantibacillus paraplantarum*
2.6.1.57 Aromatic-amino-acid transaminase				*Hanseniaspora uvarum, Lactococcus lactis, Kluyvera ascorbata, Tatumella* sp., *Pseudomonas* sp.,
2.6.1.1 Aspartate transaminase				*Tatumella* sp. JGM118, *Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Frateuria aurantia, Pseudomonas* sp.
2.6.1.66 Valine--pyruvate transaminase	Valine, Leucine, Isoleucine metabolism	L-(+)-Valine*		*Tatumella* sp. JGM118, *Kluyvera ascorbata, Tatumella* sp. *JGM130, Serratia* sp., *Pantoea* sp.
2.6.1 Transaminases	Tryptophan metabolism	DL-Tryptophan*		*Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum, Lactococcus lactis, Lactiplantibacillus paraplantarum*
2.6.1.9 Histidinol-phosphate transaminase	Tyrosine metabolism	DL-Tyrosine*		*Hanseniaspora uvarum, Leuconostoc pseudomesenteroides, Kluyvera ascorbata, Lactiplantibacillus paraplantarum, Rouxiella badensis*
2.6.1 Transaminases				*Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum, Lactococcus lactis, Lactiplantibacillus paraplantarum*
2.6.1.57 Aromatic-amino-acid transaminase				*Hanseniaspora uvarum, Lactococcus lactis, Kluyvera ascorbata, Tatumella* sp., *Pseudomonas* sp.,
2.6.1.1 Aspartate transaminase				*Tatumella* sp. JGM118, *Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Lactiplantibacillus paraplantarum, Frateuria aurantia, Pseudomonas* sp.
3.5.1.94 Gamma-glutamyl-gamma-aminobutyrate hydrolase	Alanine, Aspartate, and Glutamate metabolism; Arginine and Proline metabolism; Butanoate metabolism	gamma-Aminobutyric acid*		*Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp.
2.6.1.19 4-aminobutyrate--2-oxoglutarate transaminase				*Lactiplantibacillus plantarum, Kluyvera ascorbata, Tatumella ptyseos, Rouxiella badensis, Pantoea rodasii, Pseudomonas* sp.
1.1.1.23 Histidinol dehydrogenase	Histidine metabolism	DL-Histidine*		Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Tatumella ptyseos, Lactiplantibacillus paraplantarum , Rouxiella badensis, Pantoea rodasii
3.4.13.9 Xaa-Pro dipeptidase	Amino Acids metabolism	Proline*		*Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp.
3.4.13 Dipeptidases	Amino Acids metabolism	Gly-Leu*		*Tatumella* sp. JGM118, *Leuconostoc pseudomesenteroides, Lactiplantibacillus plantarum, Lactococcus lactis, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp., *Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii*
		Leu-Val*		
		Leu-Leu*		
		L-γ-Glutamyl-L-valine*		
1.1.1.289 Sorbose reductase	Fructose and Mannose metabolism	Hexitol*		*Hanseniaspora uvarum*

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EC Number Enzymes	Pathways	Metabolites	Reference	Predicted producers
1.1.1.14 L-iditol 2-dehydrogenase				*Lactiplantibacillus plantarum, Lactiplantibacillus paraplantarum, Pseudomonas* sp.
1.1.1.27 L-lactate dehydrogenase	Glycolysis	Lactate*		*Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Tatumella ptyseos, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii*
1.1.1.40 Malate dehydrogenase	Pyruvate metabolism	DL-Malic acid*		*Tatumella* sp. JGM118, *Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp. JGM130, *Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp.
2.5.1.18 Glutathione transferase	Glutathione metabolism	Glutathione**	(45)	*Tatumella* sp. JGM118, *Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Tatumella ptyseos, Tatumella* sp. JGM130, *Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp., *Pseudomonas* sp. *Pantoea* sp., *Pseudomonas* sp.
3.4.11.2 Membrane alanyl aminopeptidase	Glutathione metabolism	Glycine**	(46)	*Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Tatumella ptyseos, Lactiplantibacillus paraplantarum*
2.3.1.9 Acetyl-CoA C-acetyltransferase	Citrate cycle (TCA cycle)	Acetyl-CoA**	(47)	*Hanseniaspora uvarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Pantoea rodasii, Pseudomonas* sp.
1.1.1.37 Malate dehydrogenase	Citrate cycle (TCA cycle)	Oxaloacetate**	(47)	*Tatumella* sp. JGM118, *L. pseudomesenteroides, Hanseniaspora uvarum, L. plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp. JGM130, *Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp.
1.1.1 Alcohol dehydrogenases	Glycolysis	Ethanol**, Acetaldehyde**, Aldehyde**, Ketone**	(48,49)	*Tatumella* sp. *JGM118, Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp. *JGM130, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp.
	Pyruvate metabolism	Ethanol**, Acetaldehyde**	(48,49)	
3.5.1.4 Amidase	Phenylalanine metabolism	Phenylacetate**	(50)	*Tatumella* sp. JGM118, *Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Tatumella ptyseos, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Pseudomonas* sp.
	Aminobenzoate degradation	Benzoate**	(51)	
	Styrene degradation	Acrylate**	(51)	
2.2.1.6 Acetolactate synthase	Butanoate metabolism	Acetolactate**	(52)	*Tatumella* sp. JGM118, *Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp. JGM130, *Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp.,
1.1.1.47 Glucose 1-dehydrogenase	Pentose Phosphate pathway	D-glucono-1,5-lactone**	(20)	*Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp., *Pantoea* sp., *Pseudomonas* sp.

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EC Number Enzymes	Pathways	Metabolites	Reference	Predicted producers
1.1.1.6 Glycerol dehydrogenase	Glycerolipid metabolism	Glycerol**	(13)	*Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Leuconostoc citreum, Tatumella ptyseos, Rouxiella badensis, Pantoea rodasii*
3.1.4.46 Glycerophosphodiester phosphodiesterase	Glycerophospholipid metabolism	Glycerol 3-phosphate**	(13)	*Leuconostoc pseudomesenteroides, Hanseniaspora uvarum, Lactiplantibacillus plantarum, Lactococcus lactis, Kluyvera ascorbata, Frateuria aurantia, Leuconostoc citreum, Tatumella ptyseos, Tatumella* sp. *JGM130, Lactiplantibacillus paraplantarum, Rouxiella badensis, Pantoea rodasii, Serratia* sp.

The microbial enzymes were detected from their Open Reading Frame. Pathways were identified from KEGG database. *Metabolites related to aroma development detected by LC-MS/MS and matched to the enzymes based on the KEGG database. **Metabolites related to aroma development based on references and not detected by LC-MS/MS and matched to the enzymes based on the KEGG database. Microbial producers were predicted based on NCBI database.

Lactate dehydrogenase is possessed by *Lactococcus* and *Lactiplantibacillus plantarum* (44). This enzyme converts L-lactate from pyruvate. *L. lactis subsp. lactis biovar. diacetylactis* can produce diacetyl from citrate which brings buttery aroma (53). *Hanseniaspora uvarum* possess alcohol dehydrogenase (41,54).

The aforementioned microorganisms were present during SIAF of whole and pulped coffee cherries in relatively high abundance (Figure 2). *Lactococcus* group in this study was predicted to produce transaminase, amino acid hydrolase and dipeptidase. *Lactiplantibacillus plantarum* was found to have transaminase that produces tyrosine. *Hanseniaspora uvarum* was predicted to have ORFs that produce glutamate synthase, glutaminase and dipeptidases. Lactate dehydrogenase was indeed predicted to be produced by *Lactococcus* lactis, *Lactiplantibacillus plantarum* and other microbes. Alcohol dehydrogenase was also predicted to be possessed by *Hanseniaspora uvarum* and other microorganisms. Therefore, LABs and yeasts deliver a significant contribution in the coffee aroma development (Table 2).

Enzymes responsible for producing several metabolites found in the fermented green beans like caffeine, chlorogenic acid, and citric acid were not detected. This explains that several metabolites in the coffee were naturally occurring or produced by plant enzymes. As chlorogenic acid was present in the SIAF green beans but not before the fermentation, it was assumed that chlorogenic acid from coffee pulp was not yet translocated to the beans and complex matrix was not yet degraded by microbes so CGA could not be released. Nevertheless, enzymes which generate precursors of citric acid, Acetyl-CoA C-acetyltransferase for Acetyl-CoA and Malate dehydrogenase for Oxaloacetate, were identified.

Metabolites from the detected enzymes that were not available in the result of non-volatiles analysis could be volatiles, intermediates that had been further transformed during fermentation process or had not been translocated from mucilage into the green bean. Ethanol, Acetaldehyde, Aldehyde, Ketone from Alcohol dehydrogenases are volatiles. Therefore, volatile analysis is needed for further investigation.

Glutathione could be further decomposed into Glycine by the detected enzyme, Aminopeptidase. Other metabolites that were not found in the fermented green beans might be transferred during coffee drying. Thus, metabolite analysis for dried green beans is suggested for more comprehensive knowledge.

Conclusions

Geothermal coffee processing, employing SIAF, can be an alternative method which addresses the limitations of conventional coffee processing. This study delivered information about the role of microorganisms in the SIAF that affect the aroma development of the final coffee beverages. The dominant species in the coffee SIAF samples were found to be *Tatumella* sp. *JGM118*, *Hanseniaspora uvarum*, and *Leuconostoc pseudomesenteroides*. The concentration of non-volatile metabolites such as chlorogenic acid, amino acids and carboxylic acids which were not detected in the green bean before SIAF, increased 7 until 10 folds after SIAF. These metabolites are aroma precursors of coffee drinks. The ORF of microbial enzymes that contribute to the coffee aroma development were identified in the samples, such as glycoside hydrolases (GH), transaminases, and L-lactate dehydrogenase. Therefore, the role of microbial community in SIAF as the producer of geothermal coffee aroma precursors was confirmed. Further, it is recommended to detect volatiles in the self-induced anaerobic fermented green bean samples. Metabolite analyses and sensory evaluation by Q-graders on the geothermal dried green beans are also suggested to get more coherent information.

Author contributions

Zikrina Istighfarah, C. Hanny Wijaya, Lilis Nuraida, Erliza Noor and Wisnu Ananta Kusuma designed the experimentation. Zikrina Istighfarah performed the experiments and wrote the original manuscript. C. Hanny Wijaya, Lilis Nuraida, Erliza Noor and Wisnu Ananta Kusuma validated and reviewed the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting the findings of this study have been included on this article and as part of the Supplementary Information. No restrictions apply to the availability of these data.

References

1. Zhang SJ, De Bruyn F, Pothakos V, Torres J, Falconi C, Moccand C, et al. Following Coffee Production from Cherries to Cup: Microbiological and Metabolomic Analysis of Wet Processing of Coffea Arabica. Appl Environ Microbiol. 2019;85.
2. Zhang SJ, De Bruyn F, Pothakos V, Contreras GF, Cai Z, Moccand C, et al. Influence of Various Processing Parameters on the Microbial Community Dynamics, Metabolomic Profiles, and Cup Quality During Wet Coffee Processing. Front Microbiol. 2019 Nov 13;10.
3. de Sousa LHBP, da Luz JMR, da Silva M de CS, Moreli AP, Veloso TGR, Guarçoni RC, et al. Relationship between sensory and microbial profiles of fermented coffee by dry and washed methods. Food Chemistry Advances. 2023 Oct 1;2.
4. Yulianti Y, Adawiyah DR, Herawati D, Indrasti D, Andarwulan N. Detection of Markers in Green Beans and Roasted Beans of Kalosi-Enrekang Arabica Coffee with Different Postharvest Processing Using LC-MS/MS. Int J Food Sci. 2023;2023.
5. De Bruyn F, Zhang SJ, Pothakos V, Torres J, Lambot C, Moroni A V., et al. Exploring the impacts of postharvest processing on the microbiota and metabolite profiles during green coffee bean production. Appl Environ Microbiol. 2017;83(1).
6. Firdissa E, Mohammed A, Berecha G, Garedew W. Coffee Drying and Processing Method Influence Quality of Arabica Coffee Varieties (Coffee arabica L.) at Gomma I and Limmu Kossa, Southwest Ethiopia. J Food Qual. 2022 Apr 6;2022:1–8.
7. Gunawan Y, Margono KT, Rizky R, Putra N, Faqih R Al, Hakim II, et al. Enhancing the performance of conventional coffee beans drying with low-temperature geothermal energy by applying HPHE: An experimental study. Open Agric. 2021 Jan 1;6(1):807–18.
8. Gunawan Y, Putra N, Hakim II, Agustina D, Mahlia TMI. Withering of tea leaves using heat pipe heat exchanger by utilizing low-temperature geothermal energy. International Journal of Low-Carbon Technologies. 2021 Mar 1;16(1):146–55.
9. Farouq CU, Brilian VA, Fathurrodin AN, Pradipta MI, Dewi PP, Rahmadi A, et al. Technical and Business Evaluation of Geothermal Dryhouse for Coffee in Kamojang, West Java, Indonesia. In: IOP Conference Series: Earth and Environmental Science. Institute of Physics; 2024.
10. Pittia P, Nicoli MC, Sacchetti G. Effect of moisture and water activity on textural properties of raw and roasted coffee beans. J Texture Stud. 2007 Feb;38(1):116–34.
11. Pereira TS, Batista NN, Santos Pimenta LP, Martinez SJ, Ribeiro LS, Oliveira Naves JA, et al. Self-induced anaerobiosis coffee fermentation: Impact on microbial communities, chemical composition and sensory quality of coffee. Food Microbiol. 2022 May 1;103.
12. Marín-Cevada V, Caballero-Mellado J, Bustillos-Cristales R, Muñoz-Rojas J, Mascarúa-Esparza MA, Castañeda-Lucio M, et al. Tatumella ptyseos, an unrevealed causative agent of pink disease in pineapple. Journal of Phytopathology. 2010 Feb;158(2):93–9.
13. Batista da Mota MC, Batista NN, Dias DR, Schwan RF. Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality. Food Biosci. 2022 Jun 1;47.
14. Turan Ayseli M, Coskun I. Analyzing aroma-active compounds of traditional herbal coffee (Mırra) using the headspace-solid phase microextraction method combined with GC–MS/O. Microchemical Journal. 2024 Oct 1;205.
15. Schenk JJ, Becklund LE, Carey SJ, Fabre PP. What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. Appl Plant Sci. 2023 May 1;11(3).
16. Fernández-Alduenda MRoberto, Giuliano Peter. Coffee sensory and cupping handbook. Speciality Coffee Association; 2021. 130 p.
17. Niu B, Fu L, Sun S, Li W. Artificial and natural duplicates in pyrosequencing reads of metagenomic data. BMC Bioinformatics. 2010;11:187.
18. Browne PD, Nielsen TK, Kot W, Aggerholm A, Gilbert MTP, Puetz L, et al. GC bias affects genomic and metagenomic reconstructions, underrepresenting GC-poor organisms. Gigascience. 2020 Feb 1;9(2).
19. Eisenhofer R, Nesme J, Santos-Bay L, Koziol A, Sørensen SJ, Alberdi A, et al. A comparison of short-read, HiFi long-read, and hybrid strategies for genome-resolved metagenomics. Microbiol Spectr. 2024 Apr 2;12(4).
20. Elhalis H, Cox J, Zhao J. Coffee fermentation: Expedition from traditional to controlled process and perspectives for industrialization. Vol. 3, Applied Food Research. Elsevier B.V.; 2023.
21. Voce S, Passaghe P, Comuzzo P. Use of Hansensiaspora uvarum and managing growth conditions increase polysaccharides and antioxidants content in yeast autolysates for winemaking. LWT. 2024 Jul 1;203.
22. Xiao M, Huang T, Xu Y, Peng Z, Liu Z, Guan Q, et al. Metatranscriptomics reveals the gene functions and metabolic properties of the major microbial community during Chinese Sichuan Paocai fermentation. Food Microbiol. 2021 Sep 1;98.
23. Lee BH, Huang CH, Liu TY, Liou JS, Hou CY, Hsu WH. Microbial Diversity of Anaerobic-Fermented Coffee and Potential for Inhibiting Ochratoxin-Produced Aspergillus niger. Foods. 2023 Aug 1;12(15).
24. Góngora CE, Holguín-Sterling L, Pedraza-Claros B, Pérez-Salinas R, Ortiz A, Navarro-Escalante L. Metataxonomic Identification of Microorganisms during

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the Coffee Fermentation Process in Colombian Farms (Cesar Department). Foods. 2024 Mar 1;13(6).
25. Martinez SJ, Batista NN, Bressani APP, Dias DR, Schwan RF. Molecular, Chemical, and Sensory Attributes Fingerprinting of Self-Induced Anaerobic Fermented Coffees from Different Altitudes and Processing Methods. Foods. 2022 Dec 1;11(24).
26. Shen X, Wang Q, Wang H, Fang G, Li Y, Zhang J, et al. Microbial Characteristics and Functions in Coffee Fermentation: A Review. Vol. 11, Fermentation. Multidisciplinary Digital Publishing Institute (MDPI); 2025.
27. da Silva Vale A, Balla G, Rodrigues LRS, de Carvalho Neto DP, Soccol CR, de Melo Pereira GV. Understanding the Effects of Self-Induced Anaerobic Fermentation on Coffee Beans Quality: Microbiological, Metabolic, and Sensory Studies. Foods. 2023 Jan 1;12(1).
28. Subhashini D V. Growth Promotion and Increased Potassium Uptake of Tobacco by Potassium-Mobilizing Bacterium Frateuria aurantia Grown at Different Potassium Levels in Vertisols. Commun Soil Sci Plant Anal. 2015 Jan 19;46(2):210–20.
29. Khanna A, Khanna M, Aggarwal A. Serratia marcescens- a rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. Journal of Clinical and Diagnostic Research. 2013 Feb 1;7(2):243–6.
30. Aziz RK, Bartels D, Best A, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics. 2008 Feb 8;9.
31. Brady CL, Cleenwerck I, van der Westhuizen L, Venter SN, Coutinho TA, De Vos P. Pantoea rodasii sp. nov., Pantoea rwandensis sp. nov. and Pantoea wallisii sp. nov., isolated from Eucalyptus. Int J Syst Evol Microbiol. 2012 Jul;62(7):1457–64.
32. Zhao M, Tyson C, Gitaitis R, Kvitko B, Dutta B. Rouxiella badensis, a new bacterial pathogen of onion causing bulb rot. Front Microbiol. 2022 Nov 30;13.
33. Crosby KC, Rojas M, Sharma P, Johnson MA, Mazloom R, Kvitko BH, et al. Genomic delineation and description of species and within-species lineages in the genus Pantoea. Front Microbiol. 2023;14.
34. Akiki M, Ali R, Jamil A, Slim J, Miller R. Kluyvera ascorbata: An Unusual Cause of Septic Shock in a Patient With Urothelial Cancer. Cureus. 2023 Dec 25;eCollection 2023 Dec.
35. Martinez SJ, Bressani APP, Simão JBP, Pylro VS, Dias DR, Schwan RF. Dominant microbial communities and biochemical profile of pulped natural fermented coffees growing in different altitudes. Food Research International. 2022 Sep 1;159.
36. Silva LCF, Pereira PVR, Cruz MAD da, Costa GXR, Rocha RAR, Bertarini PLL, et al. Enhancing Sensory Quality of Coffee: The Impact of Fermentation Techniques on Coffea arabica cv. Catiguá MG2. Foods. 2024 Mar 1;13(5).
37. Rune CJB, Giacalone D, Steen I, Duelund L, Münchow M, Clausen MP. Acids in brewed coffees: Chemical composition and sensory threshold. Curr Res Food Sci. 2023 Jan 1;6.
38. Paravisini L, Soldavini A, Peterson J, Simons CT, Peterson DG. Impact of bitter tastant sub-qualities on retronasal coffee aroma perception. PLoS One. 2019 Oct 1;14(10).
39. Haile M, Kang WH. The Role of Microbes in Coffee Fermentation and Their Impact on Coffee Quality. J Food Qual. 2019;2019.
40. Ferreira LJC, Casé IN, Bertarini PLL, Oliveira LM de, Santos LD. Impact of immature coffee fruits and water addition during spontaneous fermentation process: Chemical composition and sensory profile. Electronic Journal of Biotechnology. 2024 May 1;69:21–9.
41. Wang J, Wang Z, Gao H, Bai X, Li L, Wei R, et al. Metabolomics and flavor diversity in Cabernet Sauvignon wines fermented by various origins of Hanseniaspora uvarum in the presence and absence of Saccharomyces cerevisiae. LWT. 2024 Jul 1;203.
42. Plaza-Vinuesa L, Sánchez-Arroyo A, Moreno FJ, de las Rivas B, Muñoz R. Dual 6Pβ-Galactosidase/6Pβ-Glucosidase GH1 Family for Lactose Metabolism in the Probiotic Bacterium Lactiplantibacillus plantarum WCFS1. J Agric Food Chem. 2023 Jul 19;71(28):10693–700.
43. Ruiz HA, Rodríguez-Jasso RM, Hernandez-Almanza A, Contreras-Esquivel JC, Aguilar CN. Pectinolytic Enzymes. In: Current Developments in Biotechnology and Bioengineering: Production, Isolation and Purification of Industrial Products. Elsevier Inc.; 2016. p. 47–71.
44. Garcia-Gonzalez N, Battista N, Prete R, Corsetti A. Health-promoting role of lactiplantibacillus plantarum isolated from fermented foods. Vol. 9, Microorganisms. MDPI AG; 2021. p. 1–30.
45. Zhao J, Wang T, Xie J, Xiao Q, Cheng J, Chen F, et al. Formation mechanism of aroma compounds in a glutathione-glucose reaction with fat or oxidized fat. Food Chem. 2019 Jan 1;270:436–44.
46. Poisson L, Blank I, Dunkel A, Hofmann T. The Chemistry of Roasting-Decoding Flavor Formation. In: The Craft and Science of Coffee. Elsevier Inc.; 2017. p. 273–309.
47. do Rosário DKA, da Silva Mutz Y, Vieira KM, Schwan RF, Bernardes PC. Effect of self-induced anaerobiosis fermentation (SIAF) in the volatile compounds and sensory quality of coffee. European Food Research and Technology. 2024 Feb 1;250(2):667–75.
48. de Melo Pereira GV, Soccol VT, Pandey A, Medeiros ABP, Andrade Lara JMR, Gollo AL, et al. Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. Int J Food Microbiol. 2014 Oct 1;188:60–6.
49. Evangelista SR, da Cruz Pedrozo Miguel MG, de Souza Cordeiro C, Silva CF, Marques Pinheiro AC, Schwan RF. Inoculation of starter cultures in a semi-dry coffee (Coffea arabica) fermentation process. Food Microbiol. 2014;44:87–95.
50. de Melo Pereira GV, Neto E, Soccol VT, Medeiros ABP, Woiciechowski AL, Soccol CR. Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects. Food Research International. 2015 Sep 1;75:348–56.
51. Feng Y, Zhang A. A Floral Fragrance, Methyl Benzoate, is An Efficient Green Pesticide. Vol. 7, Scientific Reports. Nature Publishing Group; 2017.
52. Zhao T, Li Y, Yuan S, Ye Y, Peng Z, Zhou R, et al. Structure-Based Design of Acetolactate Synthase From Bacillus licheniformis Improved Protein Stability Under Acidic Conditions. Front Microbiol. 2020 Oct 27;11.
53. Kondrotiene K, Zavistanaviciute P, Aksomaitiene J, Novoslavskij A, Malakauskas M. Lactococcus lactis in Dairy Fermentation—Health-Promoting and Probiotic Properties. Vol. 10, Fermentation. Multidisciplinary Digital Publishing Institute (MDPI); 2024.
54. Langenberg AK, Bink FJ, Wolff L, Walter S, Von Wallbrunn C, Grossmann M, et al. Glycolytic Functions

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DOI: 10.1039/D5FB00673B

Are Conserved in the Genome of the Wine Yeast Hanseniaspora uvarum, and Pyruvate Kinase Limits Its Capacity for Alcoholic Fermentation. Appl Environ Microbiol. 2017;83.

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Data Availability Statement

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The data supporting the findings of this study have been included on this article and as part of the Supplementary Information. No restrictions apply to the availability of these data.