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Article

Unlocking the Aromatic Potential of Native Coffee Yeasts: From Isolation to a Biovolatile Platform

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ABSTRACT: Postharvest processing of coffee has been shown to impact cup quality. Yeasts are known to modulate the sensory traits of the final cup of coffee after controlled fermentation at the farm. Here, we enumerated native coffee yeasts in a Nicaraguan farm during dry and semidry postharvest processing of Arabica and Robusta beans. Subsequently, 90 endogenous yeast strains were selected from the collected endogenous isolates, identified, and subjected to high-throughput fermentation and biovolatile generation in a model system mimicking postharvesting conditions. Untargeted volatile analysis by SPME-GC-MS enabled the identification of key aroma compounds generated by the yeast pool and demonstrated differences among strains. Several genera, including *Pichia*, *Candida*, and *Hanseniaspora*, showed both strain- and species-level variability in volatile generation and profiles. This fermentation platform and biovolatile database could represent a versatile opportunity to accelerate the development of yeast starter cultures for generating specific and desired sensory attributes in the final cup of coffee.

KEYWORDS: *coffee, microbiota, yeast, aroma, fermentation, starter culture*

INTRODUCTION

Coffee, belonging to the family Rubiacea, is produced from green beans originating from the *Coffea* tree fruits.[1] *Coffea arabica L.* and *Coffea canephora L.* yielding Arabica and Robusta coffee, respectively, represent the two main economically viable species. Coffee, as a worldwide commodity, implies the necessity to study its whole production process from plant to cup, in order to ensure appropriate sensory properties of the resulting coffee drink. Indeed, agricultural mastership followed by adequate harvesting and postharvest processing can prevent spoilage of the beans.[2] Additionally, appropriate roasting of the coffee beans and brewing will reveal the full aromatic potential of the variety of beans.[3]

Several variations of coffee postharvest processing exist based on the local knowledge, available infrastructure, and environmental resources (e.g., water). The processing methods applied will also impact the environment depending on the byproducts discharged, and the parameters are constantly being adapted due to the rising challenges of climate change and water availability. Three of the most widely applied methods of postharvest coffee processing are wet, dry, and semidry processing.[4,5] Wet processing usually requires large amounts of water and additional waste management (e.g., processing water and coffee cherry pulps). In comparison, the semidry and dry processes are more sustainable due to the lower amount of water used during processing and are usually applied at farms with limited access to water or with the need to further reduce the water consumption. The semidry process involves direct sun drying of the depulped beans without water addition.[6] Dry processing, mainly used for Robusta beans, involves a desiccation of the intact cherries by sun-drying. Subsequently, the outer layers of the dry cherries are mechanically removed, resulting in green coffee beans.[5] In all the above-mentioned processes, microorganisms thrive during processing and will produce specific metabolites that could diffuse into the green beans,[7] thus affecting the sensory quality of the final cup of coffee. However, the coffee beans, as intermediate seeds, are also metabolically active, and these endogenous activities will further define green bean chemistry. Consequently, those two key parameters will contribute, together with the genotypic background of the plant and various preharvest parameters, to the development of specific sensory traits.[2]

Previous studies have demonstrated diverse microbial communities in coffee postharvest processing, which could be affected by processing parameters, geographical origins, local conditions, and coffee varieties.[8−22] The use of starter cultures, especially the yeasts (e.g., *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Candida parasilosis*, *Pichia kudriavzevii*), was also tested to modulate the microbial community during processing, leading to differentiating sensorial traits in

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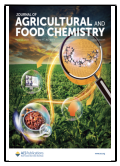

the final cup.[23−43] However, more studies on the coffee-related microorganisms are needed to further unlock their flavor generation potency and the potential to modulate coffee quality. In this study, we covered the evaluation of yeasts in a Nicaraguan farm with postharvest processes of higher sustainability potential, allowing us to go beyond wet processing. The evaluation included on-farm enumeration over processing methods (dry and semidry) and varieties (Arabica and Robusta), strain isolation and identification of selected isolates, and screening of volatile generation capability in a coffee fruit simulation medium (CFSM) at lab scale. This enabled the establishment of an *in vitro* fermentation and biovolatile platform of coffee-originated strains which could accelerate future selection of yeast strains as starter cultures for the development of a tailored fermented coffee, yielding different sensory experiences in-cup.

MATERIALS AND METHODS

Field Experiments. Field experiments were carried out at the farm La Cumplida (latitude and longitude coordinates, 13°00′05.4″N and 85°50′55.4″W, respectively, altitude 738 m) in Nicaragua. Coffee cherries or depulped coffees (45 kg in each case) were processed according to various postharvesting techniques. In brief, mature and healthy coffee cherries from the *Coffea arabica* var. Marsellesa were handpicked and either depulped and fermented without addition of water or dried directly on the patio, according to local farm practices. *Coffee canephora* var. Conillon (Robusta) was directly dried on a patio, based on the usual processing method of the farm. Semidry and dry processes were conducted following a total processing step of 96 h on a patio. An intermediate processing time of 48 h was set in accordance with local farm practices for the dry process. The prolongation of processing time to 96 h represents an extended duration that could potentially impact the microbial dynamics and final cup quality. No temperature control was applied. The temperature in the bean mass or in water ranged from 24.4 to 28.5 °C during the processing time. Samples were collected in triplicate at various time points during the processing, respectively, at 0, 48, and 96 h. Arabica and Robusta coffees undergoing dry and semidry processing types were handled at La Cumplida farm during 96 h before being transported to the drying station in Sebaco (Nicaragua) to allow proper drying of beans on the patio (latitude and longitude 12°51′04″N and 86°05′58W, respectively, altitude 472 m).

On-Farm Selective Plating and Enumeration. In order to evaluate yeast community dynamics of various coffee postharvest processes and coffee varieties, a selective agar medium was used. Plating was performed in biological triplicates. Yeasts were enumerated on YPD (yeast extract peptone dextrose) agar supplemented by 300 ppm of chloramphenicol to inhibit bacterial growth.[9] Original samples (20 g), consisting of coffee cherries or depulped coffees, were collected at various processing times. The sample was subsequently mixed with 20 g of sterile saline solution (0.85% sodium chloride) in a stomacher bag. Serial dilutions were performed on a 96-well microplate, by loading 20 μL of sample in 180 μL of sterile saline solution (0.85% sodium chloride). After mixing with a pipet, 20 μL of the latter sample was loaded to 180 μL of fresh saline solution and so on until the desired final dilution was obtained. Six dilutions of 20 μL each were spotted at regular intervals on agar plates and incubated for 48 h at room temperature. An inoculation loop was used to spread the six dilution spots on the plate to allow proper colony isolation, while ensuring no overlap between spots. Counts were expressed as log CFU/g of coffee cherries or depulped coffees ± standard deviation, as performed on triplicates. Culture media and their compounds were purchased from Merck (Darmstadt, Germany).

Yeast Isolate Recovery and Identification from Dry and Semidry Postharvest Processes. Subsequently to enumeration, 15 colonies of yeasts were isolated on YPD agar plate spots corresponding to high dilutions,[21] independently of their phenotype. Isolate recovery was performed for three time points, namely, 0, 48, and 96 h, for Arabica dry and semidry processes as well as Robusta dry processes. Importantly, yeast isolates from the 96 h time point for dry and semidry processes were subjected to temperature variation during transportation and could not be recovered in the lab, resulting in a total number of 90 isolates recovered from the isolation process. Thus, solely the yeast isolates from the first two time points, namely, 0 and 48 h, were processed for identification and aroma analysis. The 90 isolated colonies were recultured for 48 h at room temperature in liquid YPD medium. After incubation, 1 mL of culture was combined with 1 mL of 50% glycerol and stored at −20 °C until further analysis.

Strain identification was performed via two complementary methodologies, i.e., proteome profiling and metabolic fingerprinting. Identification of yeasts and fungi by proteome profiling using Maldi-ToF was shown to represent an accurate, rapid, and cost-effective method, as compared to conventional genomic techniques using the ITS1 region of the 26S rRNA gene of yeasts.[44,45] Identification was first performed by proteome profiling with a matching score as the main outcome. In cases of low matching score, metabolic fingerprinting was conducted to obtain additional information on isolate identity. The metabolic fingerprinting technique was shown to allow rapid identification of yeasts as compared to conventional sequencing.[46]

Proteome Profiling by Maldi-ToF MS. Maldi-ToF MS (Biotyper Smart System GT, Bruker, Switzerland) was used to identify isolated yeasts. The reproducibility of the methodology is based on proteome profiling, by measuring constantly expressed high-abundant proteins such as ribosomal proteins. The observed mass range spectrum was set between 2000 and 20 000 Da. The database for yeast identification was provided by Bruker (Germany). First, protein and peptide extraction from yeast colonies was performed using formic acid. Using a 1 μL inoculation loop, freshly isolated colonies were transferred from the culture plate into 300 μL of HPLC-grade water and mixed thoroughly until the material was completely in suspension. Pure ethanol (900 μL) was added before mixing the suspension and spinned down by centrifugation at maximum speed (25 000*g*) for 2 min. The supernatant was discarded. To remove residual ethanol, the pellet was air-dried. The pellet was subsequently resuspended in 100% formic acid (25 μL) and mixed by pipetting up and down until fully suspended, followed by ultrasonication for 20 min. Acetonitrile (25 μL) was added, and mixing was carried out with a micropipette. Samples were centrifuged at 19 000*g* for 2 min, and the supernatant was subsequently deposited on a Maldi target plate with a HCCA matrix (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid). Maldi spectra were acquired, and data evaluation was carried out using BioTyper software for yeast identification. In the case of a low score value (0.000 to 1.999), a complementary technique (i.e., metabolic fingerprinting) was used for further identification of yeast isolates.

Metabolic Fingerprinting of Coffee Yeast Isolates. A pure culture was first prepared by streaking a glycerol stock of various isolated yeasts on a Biolog Universal Yeast (BUY) Agar Culture Media (Biolog, Ireland), followed by an incubation step at 26 °C in aerobic conditions for 48 h. The cells were subsequently picked from the agar surface with a sterile loop and suspended in sterile water at a specific cell density corresponding to 47% turbidity as measured on a GENIII Omnilog Turbimeter (Biolog, Ireland). Afterward, 100 μL of the cell suspension was inoculated into each of the 96 wells of the YT MicroPlate (Biolog, Ireland) and incubated at 26 °C for, respectively, 24, 48, and 72 h until a sufficient metabolic pattern was formed. MicroLog software (Biolog Europe) was used to identify the yeast from its metabolic pattern in the YT MicroPlate. A similarity score was obtained, and the identification with the score above 0.7 was used for the strain, as recommended by the supplier.

Volatile Compound Generation Screening of Yeast Strains in the Simulation Medium. *Growth and Screening of Yeast Strains.* All 90 identified yeast isolates were subjected to a fermentation step in a coffee fruit simulation medium, composed of 2 g/L sucrose, 2 g/L glucose, 2 g/L fructose, 5 g/L soy peptone, 0.1

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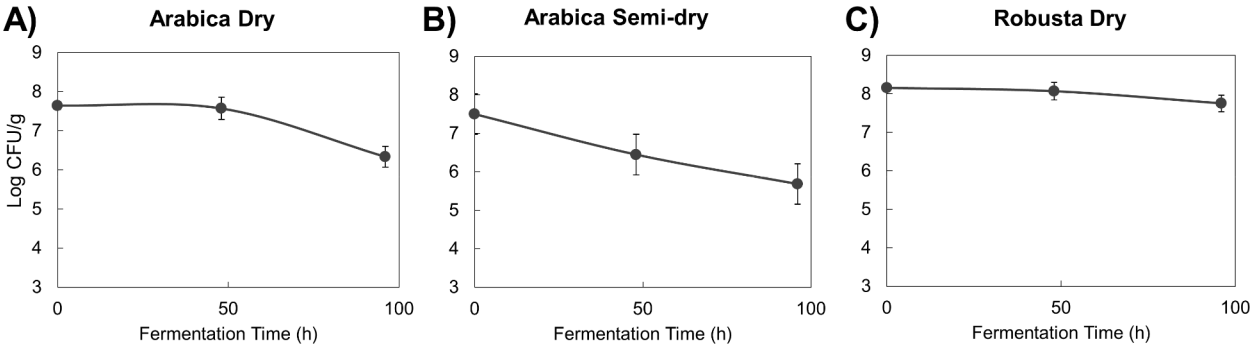

Figure 1. Enumeration of yeasts during Arabica dry (A), Arabica semidry (B), and Robusta dry (C) postharvest processing techniques, expressed as a function of time.

g/L citric acid, 0.1 g/L malic acid, 0.1 g/L quinic acid, 0.02 g/L caffeine, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L, 1 mL/L Tween 80,[47] and $MnSO_4 \cdot H_2O$.[48] The sugars in a 200 mL solution were autoclaved separately and mixed aseptically with the other compounds (800 mL) before use. The composition of CFSM was based on the metabolite analysis of fresh coffee fruit, mucilage, and fermentation water during coffee fermentations.[21,22] This medium aimed at providing a carbon and nitrogen source, organic acids, growth factors, and other coffee-related compounds in order to mimic the coffee fermentation matrix. Growth factors, such as $MnSO_4$, $MgSO_4$, and soy peptone, were included to support strain growth as a substitute to unknown growth agents present in coffee fruits.[48] Coffee-related compounds such as caffeine were included as potential microbial growth inhibitors to add a selective pressure representative of a coffee fruit environment.

To carry out fermentation experiments, glycerol stocks of the various isolates were streaked on YPD plates and incubated at 25 °C for 24 to 48 h. Inoculum was prepared by adding one colony to 10 mL of sterilized CFSM and incubated at room temperature until OD_{600} (optical density measured at 600 nm wavelength) ≥ 1.2. Subsequently, the sample was inoculated to obtain a starting OD_{600} of 0.1 in 100 mL of CFSM in PreSens flasks (BioPark Regensburg GmbH, Germany), enabling continuous and noninvasive monitoring of cell growth (based on turbidity measurements by the detection of 180°-scattered light correlated with OD), O_2 levels, and pH.[49] Fermentation was initiated at 30 °C under agitation at 200 rpm. A sample was taken at the beginning of incubation (0 h) for enumeration in YPD agar plates. After 16 h of incubation, three aliquots of 0.5 mL were taken and directly transferred into 2 mL amber-silanized crimp vials. These vials were immediately sealed with a magnetic cap (Agilent Technologies, Basel, Switzerland) and frozen prior to GC-MS evaluation. The fermentation experiments were terminated after 40 h of incubation at 30 °C. At this step, three aliquots of 0.5 mL were taken and directly transferred into 2 mL silanized amber vials and sealed with a magnetic cap. All these vials were placed in a freezer at −20 °C for further volatile analysis. Additionally, a sample of 1 mL was taken for yeast enumeration in YPD plates. Therefore, two fermentation times of, respectively, 16 and 40 h at 30 °C incubation were analyzed by CG-MS. The incubation time of 40 h was set to be close to the average fermentation time in accordance with local farm practices, while 16 h is considered as an intermediate point. This could potentially allow farmers to perform efficient controlled fermentations at the farm and save processing time, under the condition that selected yeasts would be competitive in this environment. Replication of the fermentation conditions was performed for a selection of strains from most prevalent species identified in this study (Table S1).

Volatile Fingerprinting. Volatile compounds were analyzed using an adapted method[50] by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography (model 6890) and mass spectrometry (model 5973) (Agilent Technologies, Basel, Switzerland). An HTS Pal autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to sample the headspace with the SPME fiber (PDMS/DVB, 65 μm, 1 cm length, Merck & Cie, Schaffhausen, Switzerland). Each sample was analyzed in triplicate.

The frozen vials from the incubation step (i.e., 16 and 40 h) were thawed at room temperature prior to volatile analysis. Vials were placed in the sample tray cooled at 10 °C and then transported to the incubator and heated at 30 °C for 10 min for temperature stabilization. Subsequently, the SPME fiber was inserted in the vial and absorbed volatiles for 10 min at 30 °C. SPME fiber was then desorbed into the GC-MS inlet at 250 °C during 5 min.

The GC-MS was equipped with a DB-WAX capillary column (60 m long, 0.25 mm internal diameter, and 0.25 μm film thickness (Agilent Technologies, Basel, Switzerland). Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature was held for 5 min at 35 °C and then increased at 4 °C/min until 230 °C for 10 min. The inlet injector, heated at 250 °C, was in splitless mode for 3 min followed by split opening at 50 mL/min. The MS was operated in electron impact mode at a scanning range from m/z 29 to m/z 300 at 2.68 scans/s.

Untargeted data processing was performed using MS-DIAL ver. 4.00 (RIKEN, Japan)[51] for peak picking, peak alignment, and peak integration. Tentative peak identification was done with the NIST library (ver. 14) and verified with a retention index[52] (calculated based on a series of *n*-alkanes and comparison with the internal retention index database, Table S2).

Statistical Analysis. Regarding the on-farm manipulation, the standard deviation was calculated for enumeration results on the yeast community during the processing, as done in triplicate.

Regarding the yeast isolate screening outcome, the hierarchical clustering of the volatile data was performed in RStudio (ver. 1.2.1335, R Core Team, 2021) based on the peak height of each volatile as done in triplicate. The Euclidean distance matrix was first calculated based on the peak height, followed by the clustering of the samples using the method of "ward.D". In parallel, normalization of the volatile data was also done by calculating the average of peak height for each volatile compound across the samples.

RESULTS

Yeast Loads and Diversity during Dry and Semidry Postharvest Processing of Coffee. Yeast enumeration highlighted differences observed in various coffee postharvest processes (dry and semidry) and varieties (Arabica and Robusta). The yeast counts at the surface of coffee cherries and depulped coffees, respectively, ranged from log 5.68 to 8.16 (CFU/g) (Figure 1).

In all postharvest processing types, yeast loads decreased as a function of processing time. In the Arabica dry process, the yeast counts remained stable above log 7.0 (CFU/g) until 48 h of processing, followed by a drop to log 6.33 (CFU/g) after 96 h (Figure 1A). Regarding the Arabica semidry process, a continuous decrease of yeast counts from log 7.50 (CFU/g) to log 5.68 (CFU/g) was observed during processing (Figure

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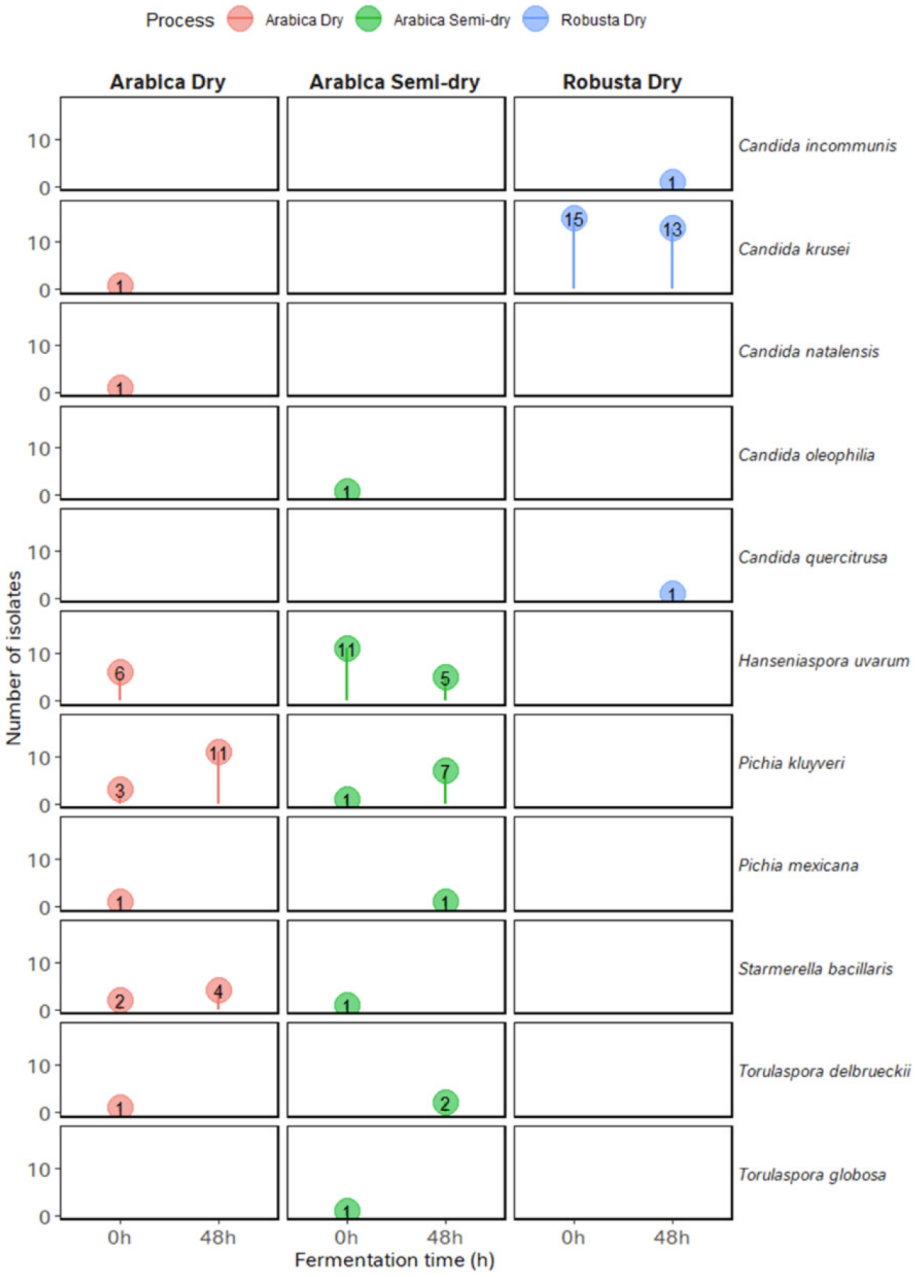

Figure 2. Yeast strain identification of various postharvest processing techniques and coffee varieties, namely, Arabica dry, Arabica semidry, and Robusta dry processing as a function of time, as determined by Maldi-ToF and metabolic fingerprinting. Identified yeast species after isolation and number of isolates with the same identification at each time point are displayed.

1B). In the Robusta dry process, high yeast loads persisted until the end of the process, with a concomitant slight decrease in counts from log 8.16 (CFU/g) to log 7.75 (CFU/g) after 96 h.

Subsequently to on-farm enumeration, the yeast community compositions also showed variations between the three processing variants, i.e., Arabica dry and semidry processes as well as the Robusta dry process (Figure 2 and Table S3).

Among the 90 isolates recovered from the coffee processing, 74 yeast isolates were identified through proteome profiling (Maldi-ToF) and 16 isolates through metabolic fingerprinting. These strains covered 5 genera and 11 species, including *Pichia* spp. (*Pichia kluyveri*, *Pichia mexicana*), *Candida* spp. (*Candida krusei*, *Candida quercitusa*, *Candida incommunis*, *Candida oleophila*, and *Candida natalensis*), *Hanseniaspora uvarum*, *Torulaspora delbrueckii*, and *Starmerella bacillaris*. Regarding the Arabica dry and semidry processing variants, similar trends were observed with *H. uvarum* shown to be prevalent during the first phase of processing, followed by a shift to *P. kluyveri* at a later stage (Figure 2). The presence of *S. bacillaris* appeared to be more dominant in Arabica dry processing, as compared to semidry processing. In contrast, the Robusta dry process showed a different pattern, with only *Candida* spp. being isolated, as well as a clear prevalence of *C. krusei* throughout the whole processing phase. No filamentous fungi were identified in the pool of the 90 isolates.

Overall, these strains were distributed evenly from the different processing variants at different processing time points, highlighting *Candida* spp., *Hanseniaspora* spp., and *Pichia* spp. as the main genera present in this selection.

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J. Agric. Food Chem. 2023, 71, 4665−4674

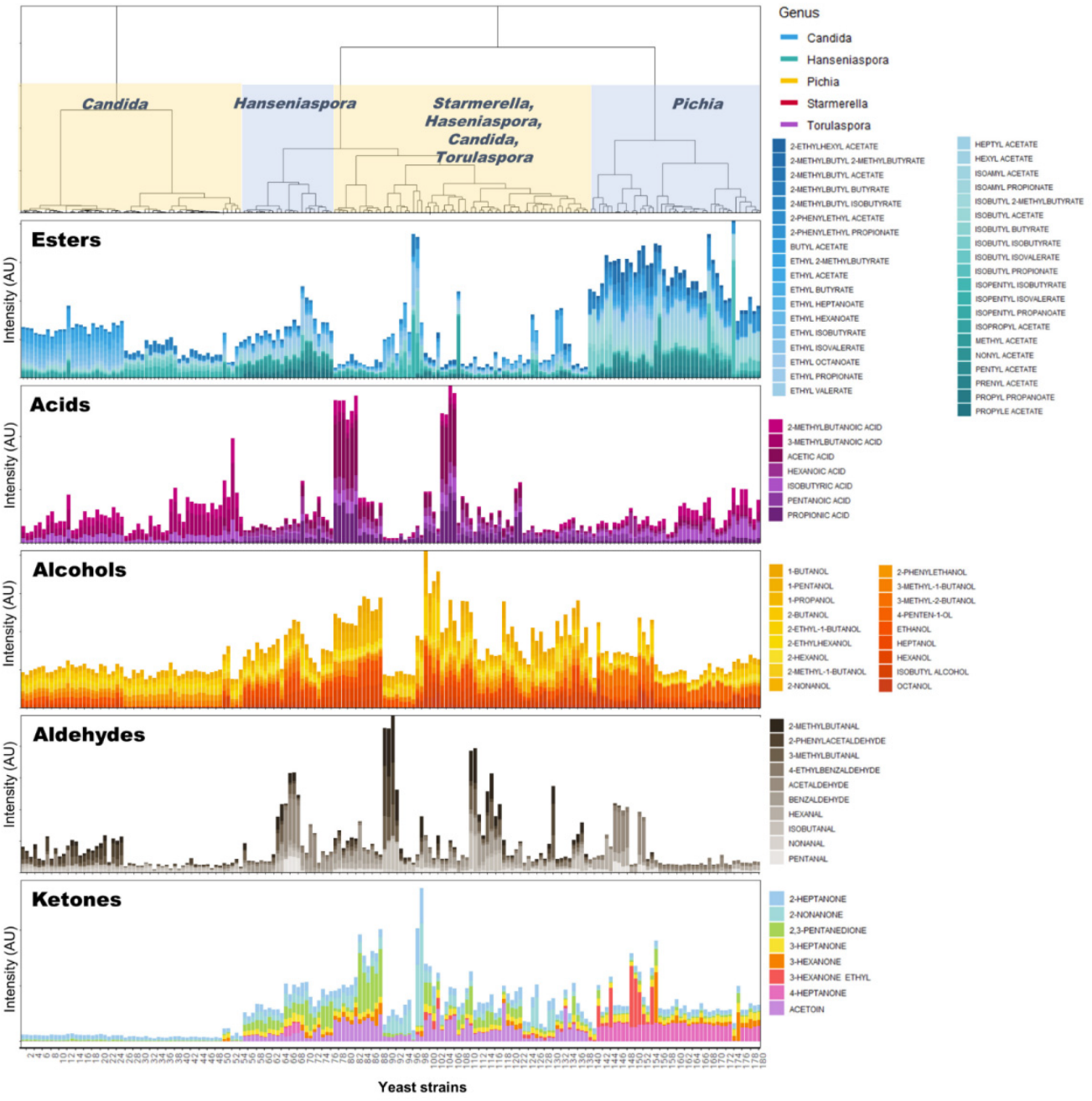

Figure 3. Volatile compound generation of the 90 yeast strains at two time points (T16 and T40) in the coffee fruit simulation medium (CFSM). Hierarchical clustering (top panel) based on the volatile intensity (peak height, AU) showed similarity among strains of certain species. The normalized volatile intensities of the five chemical classes were tabulated at the same order as the clustering. Normalization of the volatile data was also done by calculating the average of peak height for each volatile compound across the samples. The detailed information on the strain information corresponding to the clustering order was described in Table S5, and the peak height and experimental retention index of each compound were tabulated in Figures S1−S5 and Table S2, respectively.

***In Vitro* Fermentation of Yeast Strains and Biovolatile Fingerprinting.** The starting levels of the 90 strains, based on enumeration, ranged from log 5.81 to 6.70 (CFU/mL), showing effective control of the inoculation level using optical density at 600 nm (OD_{600}) (Table S4). After 40 h of incubation time, the ending enumeration levels ranged from log 7.48 to 8.74 (CFU/mL), showing growth in the defined simulation medium with limited nutrients as well as variations across the isolates (Table S4).

Regarding the aroma generation capabilities of the isolates, the yeast strains tested in a simulation medium produced a wide array of odor-potent volatiles, which were covered through the untargeted approach including esters, alcohols, aldehydes, ketones, and acids. The differentiations were reflected mainly at the species level within the collections of the yeast strains isolated (Figure 3 and Table S5).

The strains of *P. kluyveri* (numbers 139−180, Figure 3) were highlighted for the generation of odor-potent esters at a high level. Acetate esters (e.g., isoamyl acetate, 2-methylbutyl acetate, isobutyl acetate, butyl acetate, hexyl acetate, 2-phenylethyl acetate, etc.) were especially produced at higher proportions by *Pichia* strains in combination with low levels of

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J. Agric. Food Chem. 2023, 71, 4665−4674

ethanol and acids. In contrast, *Candida* strains, especially *C. krusei* (numbers 1−54, Figure 3), produced a higher proportion of ethyl esters (e.g., ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl butyrate, ethyl isobutyrate, ethyl hexanoate, etc.), and *Hanseniaspora* strains (numbers 55−76, Figure 3) were more balanced in the production of both ethyl and acetate esters. These esters could contribute to fruity (e.g., isoamyl acetate, 2-methylbutyl acetate, ethyl butyrate), floral (e.g., 2-phenylethyl acetate), and ethereal (e.g., ethyl acetate) notes with different odor thresholds leading to varying odor potentials.

Different types of alcohols were produced by isolated coffee strains. *Pichia* and *Candida* strains could produce less ethanol as compared to other strains, while *Hanseniaspora*, *Starmerella*, and *Torulaspora* strains were strong alcohol producers with slight variants in the types of alcohol produced. *Hanseniaspora* and *Starmerella* (e.g., numbers 77−88, Figure 3) strains were especially effective in producing alkyl alcohols (e.g., 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-heptanol), while *Torulaspora* (e.g., numbers 131−134, Figure 3) were relatively more prolific in producing ethanol and branched-chain alcohols (e.g., 3-methyl-1-butanol, 2-phenylethanol). Different alcohols could contribute to fermented (e.g., 1-propanol, 1-butanol, 1-pentanol), green (e.g., hexanol, heptanol), fruity (e.g., 3-methylbutanol), and floral (e.g., 2-phenylethanol) notes and would lead to varying odor potentials depending on their odor thresholds and quantity present.

The strains of *Candida* spp. produced relatively higher levels of 2- and 3-methylbutanoic acids, potentially leading to sweaty, cheesy, and fermented notes,[53] while certain strains of *Hanseniaspora* and *Starmerella* produced a relatively higher amount of acetic acid (e.g., numbers 77−82 and 103−106, Figure 3), potentially leading to vinegar notes. The generation of higher aldehydes (e.g., 2-methylbutanal, 3-methylbutanal, and 2-phenylacetaldehyde) was mainly observed in strains of *Candida* and *Hanseniaspora* (e.g., numbers 63−68, 89−92, 110−117, and 130, Figure 3), which could contribute to the malty (e.g., 2-methylbutanal, 3-methylbutanal) and sweet (e.g., 2-phenylacetaldeyde) notes. Among the ketones, the *Starmerella* strains tended to generate a higher level of 2,3-pentanedione and acetoin, possibly contributing to a buttery note.[53]

Also, the *in vitro* fermentation time had an expected influence on the flavor intensity and bouquet, as shown by the two time points clustered separately for most genera tested (Figure 3 and Table S5). For example, the *Candida* strains with 16 h fermentation (e.g., numbers 1−25, Figure 3) were clustered separately from 40 h results (e.g., numbers 26−54, Figure 3 and Table S5), mostly due to the decreasing aldehyde and ester groups and a slight increase of the acid group.

DISCUSSION

A particular emphasis was set on coffee yeasts among other microorganisms, due to their aroma-generation capabilities[23,24] as well as their potential pectinolytic activity.[54] Based on the selective plating and isolate identification of native coffee yeasts, we have observed variability in communities and dynamics, at the same processing location and harvest time yet varied by the processing variants, processing duration, and coffee varieties.

The microbial loads of dry and semidry processing variants experienced decrement with extended processing time for both Arabica and Robusta trials. This could potentially be linked to a decrease of water activity with prolonged processing and higher temperature as also observed before.[6,9] The starting yeast levels in the Arabica semidry processing were relatively higher (7−8 log CFU/g) as compared to previous data.[6] Interestingly, the yeast counts reported by Vilela *et al.* during semidry processing of Bourbon coffees in Brazil remained stable between 0 and 96 h processing, unlike observations made in the present work. As the spontaneous fermentation can vary depending on the geographical and farm conditions, the difference could be attributed to parameters such as water activity, substrate availability, and varieties. Regarding Arabica dry processing, the yeast loads were similar to previous observations.[20] Indeed, while Silva and colleagues did not report on yeast enumeration during Arabica dry postharvest processing as a function of processing time, they evaluated yeast counts of coffee beans on the ground midprocess, with an average load of log 6.60 (CFU/g) over 8 farms, corroborating the observations of the present work (log 6.33 (CFU/g) after 96 h of processing), despite the varying conditions. The yeast loads of the Robusta dry process were significantly higher than those previously reported in various Brazilian farms, at different altitudes and terrain aspects, ranging from log 3.0 to log 6.0 (CFU/g).[17] This could be attributed to the higher altitude of the farm of the present work (738 m and, respectively, 300 and 600 m in the previous report), allowing a temperature favoring yeast growth on the top of the potential prefermentation step which might have occurred during transportation of the Robusta cherries to the farm, as explained later.[20]

Yeast enumeration of various coffee postharvest processing types and coffee varieties was followed by identification of the 90 colonies isolated from the highest dilutions, thought to represent the most competitive strains. The identification of yeast isolates enabled the reconstitution of dominant species as a function of time for dry and semidry processes. Even though this evaluation might not reflect the full complexity of yeast communities during postharvest processing, we hypothesized that this population might be representative of the most competitive species showing the ability to grow in diluted systems and thus represent good starter culture candidates.

The yeast community in both Arabica dry and semidry processing revealed the dominance of *P. kluyveri*, *H. uvarum*, and *S. bacillaris* during the first 48 h in both processes. The yeast community identified corroborated previous studies in different countries at the genus level but varied at the species level,[6,19,20] indicating a potential influence of geographical location and coffee varieties used. Indeed, while *Pichia*, *Hanseniaspora*, *Candida*, and *Torulaspora* genera were found in previous studies reporting on the isolation of yeasts in Arabica semidry processing in Brazil,[6] species largely differed from the present work, as exemplified by the presence of *Pichia kluyveri*, not found in the other study. Similar conclusions could be drawn for the Arabica dry process, with a larger yeast diversity observed in studies performed in Brazil, showing the presence of similar genera but various species.[19,20] Interestingly, *Saccharomyces* species were not detected in the current setup across the processing variants tested, despite their observation in previous reports.[6,19,20] While the *Candida* genus was similarly found to be prevalent in Robusta dry processing in Brazil (39% of isolates and, respectively, 100% in our study), a larger yeast diversity was observed with the presence of *Meyerozyma* (35%), *Hanseniaspora* (18%), and *Pichia* genera

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J. Agric. Food Chem. 2023, 71, 4665−4674

(8%), not found in the present work. This could be explained by the fact that the coffee cherries were sourced from a farm located at a lower altitude which is necessary for Robusta coffee growing. Thus, solid state fermentation may have occurred at the surface of the coffee cherries during transportation, with *Candida krusei* showing the highest competitiveness in this environment. Additionally, the ability of *Candida* species to tolerate low water activities (0.6−0.7)[55] might explain their prevalence in our study.

Overall, the current study supported the previous observations on yeast diversity but also highlighted the influence of geographical location and processing methods on the microbial community and the dominance.

The flavor generation capabilities of the yeast strains were evaluated in a model fermentation for dry and semidry processes. The selected 90 candidates were distributed evenly across different processing types (dry and semidry processes), on-farm processing time points (0 and 48 h, respectively), and coffee varieties (Arabica and Robusta). The coffee fruit simulation medium was designed to mimic the substrates and growth inhibitors present during the coffee fermentation process based on previous research.[48] Compared to a standard growth medium, the medium was formulated to select competitive strains in coffee fermentation systems.

While *Saccharomyces cerevisiae* has been thoroughly studied regarding its flavor generation due to their wide applications in multiple foods and beverages, the nonsaccharomyces yeast strains' behavior at wet-lab and genetic levels still requires more extended studies due to their higher complexity and diversity. In the current study, we have demonstrated differentiating behaviors in the biovolatile generation that could lead to potential flavor directions in the final coffee drink, such as fruity, floral, acid, malty, or cheesy notes, based on the type of molecules and their relative intensities. Some of these flavor molecules, like esters, alcohols, acids, and aldehydes, are the low molecular mass secondary metabolites commonly considered as outcomes in yeasts' metabolisms from the Ehrlich pathway.[56] This pathway utilizes the branched-chain, aromatic, or sulfur-containing amino acids (e.g., valine, leucine, isoleucine, phenylalanine, and methionine) and converts them to α-keto acids through transamination. While these α-keto acids can be integrated into a central carbon metabolism, some can further be converted to higher aldehydes through decarboxylation and then be either oxidized to higher acids or reduced to higher alcohols. The generation of corresponding esters (either with acetyl-CoA or acyl-CoA) can be subsequently formed based on available enzymatic pathways. Even though these isolates were picked out from the same geographical origin, the behaviors of volatile production demonstrated various expression levels of the pathways at strain and species levels, indicating a diverse combination of enzymes present and their corresponding activities on the strain level in the model system of the coffee fruit simulation medium.

The fermentation time had an expected influence on the flavor intensity as the two time points were clustered separately for most genera tested. During the fermentation step, the flasks were not sealed to allow aeration and necessary pressure release. Therefore, we cannot help but speculate that aroma losses might have occurred after a long incubation period. However, such changes still indicated the dynamic conversion of the flavor molecules depending on the changes of aerobicity, substrate level, and endogenous enzyme activities as a function of fermentation duration.

This model fermentation setup could allow the selection of competitive strains which could generate relevant volatile molecules with a persisting active metabolism. By combining selected strains and coffee processing techniques, there will be a great opportunity to modulate the sensory profile of the final cup, as indicated in several field experiments[24,31,39,43] and model systems.[26,57] However, it is important to stress that the ability of those odor-potent molecules to diffuse into the bean[57] as well as the stability of biovolatiles during coffee roasting and brewing remain key to draw a correlation between coffee microbiota and sensory properties of the final cup. Hadj Salem and colleagues reported on the mass transfer of key aroma compounds such as 2-phenylethanol, butanal, and isoamyl acetate from the outer layer of the coffee cherry to the endosperm.[7] Those compounds, known to influence coffee cup quality, are being produced by the epiphytic strains identified in the course of this study, suggesting a link between aroma biosynthesis and sensory properties of the final coffee drink. However, it is noteworthy that the latter study evaluated the diffusion of aromas in a submerged fermentation with depulped coffees. Therefore, the mass transfer of aromas through the exocarp during solid state fermentation remains to be confirmed. Importantly, some studies reported on the use of yeast starter cultures during dry and semidry processing of coffee, showing a positive impact on coffee quality correlating with the metabolites produced by the yeast during the fermentation process,[23,24] supporting the potential of a fermentation and biovolatile screening platform for the development of novel yeast-based starter cultures. Nonetheless, it will be of utmost importance to determine whether selected yeasts will display the same metabolic behavior and competitiveness *in situ* during fermentation trials. However, native coffee yeasts are thought to thrive during postharvest processing due to their adaptation to such an environment.[23]

Overall, despite isolation from a single farm during the same harvest period, the diversity of volatile generation was clearly observed at both the species and strain level. The combinations of these yeast strains, influenced by the processing methods and coffee varieties, could lead to complex and diverse sensory differentiations in the final coffee beverage. Furthermore, the volatile generation capability defined in this study would allow a more accurate selection of starter cultures with specific flavor directions, and the effects with on-farm application will be validated and even extended to food matrices other than coffee. The biovolatile platform, covering isolation, identification, and aroma generation of native coffee yeasts, enabled the monitoring of growth and volatile production of numerous strains in the coffee fruit simulation medium using noninvasive optical sensors followed by SPME-GC MS analysis. This represents a tool for the selection of potential coffee starter cultures, enabling us to accelerate product development and increase the chances of sensorial diversification of the end coffee.

The current study tempted to take a deep dive into the impact of processing methods and coffee varieties on the aroma generation capability of the coffee yeasts in a Nicaraguan farm, using a combination of on-farm enumeration, isolation, identification, volatile analysis, and clustering. The processed Arabica beans had very different microbial communities during processing as compared to Robusta, which was dominated by the *Candida* species. Among the

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J. Agric. Food Chem. 2023, 71, 4665−4674

different Arabica processing variants, different identified species, namely, *Pichia*, *Hanseniapora*, *Torulaspora*, and *Starmerella*, were common to all processes; however, different evolution patterns throughout the process were observed. Through model fermentation in a simulation medium followed by volatile profiling, the yeast strains have demonstrated differentiating flavor generation capabilities at both species and strain levels across acids, esters, alcohols, and aldehydes. Their intensities were affected by the fermentation time, isolation sources, and genus. The combination of these strains might act collectively and translate into a complex flavor profile of the final coffee drink. In addition, the database generated on the biovolatile capabilities of coffee yeasts could allow a faster and better selection of starter cultures to be tested on-farm to create a controlled process and consistent quality. Thus, this platform could be applied in future research topics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c08263.

Table S1: Comparison of replicates (counts and sum of peak height of various compound classes) from representative strains during model fermentation in CFSM. Table S2: Calculated retention index of compounds described in this study. Table S3: Identified yeast isolates obtained from different coffee processing variants and processing times. Table S4: Yeast counts during model fermentations in CFSM. Table S5: Strain information and clustering based on the flavor generation in the simulation medium. Figure S1: Volatile intensity of acids for 90 yeast strains at two time points in the CFSM. Figure S2: Volatile intensity of aldehydes for 90 yeast strains at two time points in the CFSM. Figure S3: Volatile intensity of ketones for 90 yeast strains at two time points in the CFSM. Figure S4: Volatile intensity of alcohols for 90 yeast strains at two time points in the CFSM. Figure S5: Volatile intensity of esters for 90 yeast strains at two time points in the CFSM (PDF)

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Notes

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■ ABBREVIATIONS USED

SPME-GC-MS, solid-phase microextraction-gas chromatography-mass spectrometry; YPD, yeast extract peptone dextrose; Maldi-ToF MS, matrix-assisted laser desorption ionization-time of flight-mass spectrometry; CFSM, coffee fruit simulation medium; OD, optical density

■ REFERENCES

(1) Sakiyama, N.; Ferrão, M. A. G. Botany and production of coffee. *Cocoa and coffee fermentations*; Schwan, R. F., Fleet, G. H., Eds.; CRC Press: Boca Raton, FL, USA, 2015; pp 341−365.

(2) Waters, D. M.; Arendt, E. K.; Moroni, A. V. Overview on the mechanisms of coffee germination and fermentation and their significance for coffee and coffee beverage quality. *Critical reviews in food science and nutrition* **2017**, *57* (2), 259−274.

(3) López-Galilea, I.; Fournier, N.; Cid, C.; Guichard, E. Changes in headspace volatile concentrations of coffee brews caused by the roasting process and the brewing procedure. *J. Agric. Food Chem.* **2006**, *54* (22), 8560−8566.

(4) Brando, C. H.; Brando, M. Methods of coffee fermentation and drying. *Cocoa and coffee fermentations* **2014**, 367−396.

(5) Vincent, J., Green coffee processing. In *Coffee*; Springer: 1987; pp 1−33.

(6) Vilela, D. M.; Pereira, G. V. d. M.; Silva, C. F.; Batista, L. R.; Schwan, R. F. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (Coffea arabica L.). *Food Microbiology* **2010**, *27* (8), 1128−1135.

(7) Hadj Salem, F.; Lebrun, M.; Mestres, C.; Sieczkowski, N.; Boulanger, R.; Collignan, A. Transfer kinetics of labeled aroma compounds from liquid media into coffee beans during simulated wet processing conditions. *Food Chem.* **2020**, *322*, 126779.

(8) Avallone, S.; Guyot, B.; Brillouet, J.-M.; Olguin, E.; Guiraud, J.-P. Microbiological and Biochemical Study of Coffee Fermentation. *Curr. Microbiol.* **2001**, *42* (4), 252−256.

(9) De Bruyn, F.; Zhang, S. J.; Pothakos, V.; Torres, J.; Lambot, C.; Moroni, A. V.; Callanan, M.; Sybesma, W.; Weckx, S.; De Vuyst, L. Exploring the Impacts of Postharvest Processing on the Microbiota and Metabolite Profiles during Green Coffee Bean Production. *Appl. Environ. Microbiol.* **2017**, DOI: 10.1128/AEM.02398-16.

(10) de Carvalho Neto, D.; de Melo Pereira, G.; Tanobe, V.; Thomaz Soccol, V.; da Silva, B.; Rodrigues, C.; Soccol, C. Yeast Diversity and Physicochemical Characteristics Associated with Coffee Bean Fermentation from the Brazilian Cerrado Mineiro Region. *Fermentation* **2017**, *3* (1), 11.

(11) Elhalis, H.; Cox, J.; Zhao, J. Ecological diversity, evolution and metabolism of microbial communities in the wet fermentation of Australian coffee beans. *Int. J. Food Microbiol.* **2020**, *321*, 108544.

(12) Evangelista, S. R.; Miguel, M. G. d. C. P.; Silva, C. F.; Pinheiro, A. C. M.; Schwan, R. F. Microbiological diversity associated with the spontaneous wet method of coffee fermentation. *Int. J. Food Microbiol.* **2015**, *210*, 102−112.

(13) Gonzalez-Rios, O.; Suarez-Quiroz, M. L.; Boulanger, R.; Barel, M.; Guyot, B.; Guiraud, J.-P.; Schorr-Galindo, S. Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted

coffee. *Journal of Food Composition and Analysis* **2007**, *20* (3), 297−307.
(14) Haile, M.; Kang, W. H. The Role of Microbes in Coffee Fermentation and Their Impact on Coffee Quality. *Journal of Food Quality* **2019**, *2019*, 4836709.
(15) Hamdouche, Y.; Meile, J. C.; Nganou, D. N.; Durand, N.; Teyssier, C.; Montet, D. Discrimination of post-harvest coffee processing methods by microbial ecology analyses. *Food Control* **2016**, *65*, 112−120.
(16) Masoud, W.; Cesar, L. B.; Jespersen, L.; Jakobsen, M. Yeast involved in fermentation of Coffea arabica in East Africa determined by genotyping and by direct denaturating gradient gel electrophoresis. *Yeast (Chichester, England)* **2004**, *21* (7), 549−56.
(17) Pereira, P. V.; Bravim, D. G.; Grillo, R. P.; Bertoli, L. D.; Osório, V. M.; da Silva Oliveira, D.; da Cruz Pedrozo Miguel, M. G.; Schwan, R. F.; de Assis Silva, S.; Coelho, J. M.; Bernardes, P. C. Microbial diversity and chemical characteristics of Coffea canephora grown in different environments and processed by dry method. *World J. Microbiol. Biotechnol.* **2021**, *37* (3), 51.
(18) Silva, C.; Batista, L.; Schwan, R. Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (Coffea arabica L.) beans. *Braz. J. Microbiol.* **2008**, *39*, 521.
(19) Silva, C. F.; Batista, L. R.; Abreu, L. M.; Dias, E. S.; Schwan, R. F. Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation. *Food Microbiol* **2008**, *25* (8), 951−7.
(20) Silva, C. F.; Schwan, R. F.; Sousa Dias, Ë.; Wheals, A. E. Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. *Int. J. Food Microbiol.* **2000**, *60* (2), 251−260.
(21) Zhang, S. J.; De Bruyn, F.; Pothakos, V.; Contreras, G. F.; Cai, Z.; Moccand, C.; Weckx, S.; De Vuyst, L. Influence of Various Processing Parameters on the Microbial Community Dynamics, Metabolomic Profiles, and Cup Quality During Wet Coffee Processing. *Frontiers in Microbiology* **2019**, *10*, 2621.
(22) Zhang, S. J.; De Bruyn, F.; Pothakos, V.; Torres, J.; Falconi, C.; Moccand, C.; Weckx, S.; De Vuyst, L. Following Coffee Production from Cherries to Cup: Microbiological and Metabolomic Analysis of Wet Processing of Coffea arabica. *Appl. Environ. Microbiol.* **2019**, *85* (6), e02635−18.
(23) Bressani, A. P. P.; Martinez, S. J.; Sarmento, A. B. I.; Borém, F. M.; Schwan, R. F. Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. *Food Research International* **2020**, *128*, 108773.
(24) da Mota, M. C. B.; Batista, N. N.; Rabelo, M. H. S.; Ribeiro, D. E.; Borém, F. M.; Schwan, R. F. Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. *Food Research International* **2020**, *136*, 109482.
(25) da Silva Vale, A.; de Melo Pereira, G. V.; de Carvalho Neto, D. P.; Rodrigues, C.; Pagnoncelli, M. G. B.; Soccol, C. R. Effect of Co-Inoculation with Pichia fermentans and Pediococcus acidilactici on Metabolite Produced During Fermentation and Volatile Composition of Coffee Beans. *Fermentation* **2019**, *5* (3), 67.
(26) De Carvalho Neto, D. P.; Vinícius De Melo Pereira, G.; Finco, A. M. O.; Rodrigues, C.; Carvalho, J. C. D.; Soccol, C. R. Microbiological, physicochemical and sensory studies of coffee beans fermentation conducted in a yeast bioreactor model. *Food Biotechnology* **2020**, *34* (2), 172−192.
(27) de Melo Pereira, G. V.; Neto, E.; Soccol, V. T.; Medeiros, A. B. P.; Woiciechowski, A. L.; Soccol, C. R. Conducting starter culture-controlled fermentations of coffee beans during on-farm wet processing: Growth, metabolic analyses and sensorial effects. *Food Research International* **2015**, *75*, 348−356.
(28) de Melo Pereira, G. V.; Soccol, V. T.; Pandey, A.; Medeiros, A. B.; Andrade Lara, J. M.; Gollo, A. L.; Soccol, C. R. Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. *Int. J. Food Microbiol.* **2014**, *188*, 60−6.
(29) Rodrigues Dias, S.; Serafim Coelho, V.; Brioschi, A.; Brioschi Junior, D.; Carvalho Guarconi, R.; Soares Cardoso, W.; Viana Freitas, V.; Renon Eller, M.; Louzada Pereira, L. Sensory Q-Grader evaluation of fermented arabica coffees by yeast (Saccharomyces cerevisiae) and lactic bacteria (Pediococcus acidilactici) cultures. *Coffee Science - ISSN 1984−3909* **2020**, *15*, e151690.
(30) Evangelista, S. R.; Miguel, M. G. d. C. P.; de Souza Cordeiro, C.; Silva, C. F.; Pinheiro, A. C. M.; Schwan, R. F. Inoculation of starter cultures in a semi-dry coffee (Coffea arabica) fermentation process. *Food Microbiology* **2014**, *44*, 87−95.
(31) Evangelista, S. R.; Silva, C. F.; Miguel, M. G. P. d. C.; Cordeiro, C. d. S.; Pinheiro, A. C. M.; Duarte, W. F.; Schwan, R. F. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. *Food Research International* **2014**, *61*, 183−195.
(32) Haile, M.; Kang, W. H. Isolation, Identification, and Characterization of Pectinolytic Yeasts for Starter Culture in Coffee Fermentation. *Microorganisms* **2019**, *7* (10), 401.
(33) Lee, L.; Tay, G.; Cheong, M. W.; Curran, P.; Yu, B.; Liu, S. Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica: I. Green coffee. *LWT - Food Science and Technology* **2017**, *77*, 225.
(34) Lee, L.; Tay, G.; Cheong, M. W.; Curran, P.; Yu, B.; Liu, S. Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica: II. Roasted coffee. *LWT - Food Science and Technology* **2017**, *80*, 32.
(35) Martinez, S. J.; Bressani, A. P. P.; Dias, D. R.; Simão, J. B. P.; Schwan, R. F. Effect of Bacterial and Yeast Starters on the Formation of Volatile and Organic Acid Compounds in Coffee Beans and Selection of Flavors Markers Precursors During Wet Fermentation. *Frontiers in Microbiology* **2019**, DOI: 10.3389/fmicb.2019.01287.
(36) Muzaifa, M.; Hasni, D.; Febriani; Patria, A.; Abubakar, A. Fermentation of coffee beans with inoculation of bacillus subtilis and its impact on coffee sensory quality. *IOP Conference Series: Earth and Environmental Science* **2019**, *364*, 012010.
(37) Pereira, G. V. d. M.; de Carvalho Neto, D. P.; Medeiros, A. B. P.; Soccol, V. T.; Neto, E.; Woiciechowski, A. L.; Soccol, C. R. Potential of lactic acid bacteria to improve the fermentation and quality of coffee during on-farm processing. *International Journal of Food Science & Technology* **2016**, *51* (7), 1689−1695.
(38) Ribeiro, L. S.; da Cruz Pedrozo Miguel, M. G.; Martinez, S. J.; Bressani, A. P. P.; Evangelista, S. R.; Silva, E. B. C. F.; Schwan, R. F. The use of mesophilic and lactic acid bacteria strains as starter cultures for improvement of coffee beans wet fermentation. *World journal of microbiology & biotechnology* **2020**, *36* (12), 186.
(39) Ribeiro, L. S.; Miguel, M. G. d. C. P.; Evangelista, S. R.; Martins, P. M. M.; van Mullem, J.; Belizario, M. H.; Schwan, R. F. Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage. *Food Research International* **2017**, *92*, 26−32.
(40) Silva, C. F.; Vilela, D. M.; de Souza Cordeiro, C.; Duarte, W. F.; Dias, D. R.; Schwan, R. F. Evaluation of a potential starter culture for enhance quality of coffee fermentation. *World J. Microbiol. Biotechnol.* **2013**, *29* (2), 235−247.
(41) Tang, V. C. Y.; Sun, J.; Cornuz, M.; Yu, B.; Lassabliere, B. Effect of solid-state fungal fermentation on the non-volatiles content and volatiles composition of Coffea canephora (Robusta) coffee beans. *Food Chem.* **2021**, *337*, 128023.
(42) Vinícius de Melo Pereira, G.; Soccol, V. T.; Brar, S. K.; Neto, E.; Soccol, C. R. Microbial ecology and starter culture technology in coffee processing. *Critical reviews in food science and nutrition* **2017**, *57* (13), 2775−2788.
(43) Wang, C.; Sun, J.; Lassabliere, B.; Yu, B.; Liu, S. Q. Coffee flavour modification through controlled fermentation of green coffee beans by Saccharomyces cerevisiae and Pichia kluyveri: Part II. Mixed cultures with or without lactic acid bacteria. *Food Research International* **2020**, *136*, 109452.
(44) Cassagne, C.; Normand, A. C.; l'Ollivier, C.; Ranque, S.; Piarroux, R. Performance of MALDI-TOF MS platforms for fungal identification. *Mycoses* **2016**, *59* (11), 678−690.

(45) Lacroix, C.; Gicquel, A.; Sendid, B.; Meyer, J.; Accoceberry, I.; François, N.; Morio, F.; Desoubeaux, G.; Chandenier, J.; Kauffmann-Lacroix, C.; Hennequin, C.; Guitard, J.; Nassif, X.; Bougnoux, M. E. Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of Candida species. *Clinical Microbiology and Infection* **2014**, *20* (2), 153−158.
(46) Greetham, D. Phenotype microarray technology and its application in industrial biotechnology. *Biotechnol. Lett.* **2014**, *36* (6), 1153−1160.
(47) Berbegal, C.; Benavent-Gil, Y.; Pardo, I.; Ferrer, S. A novel culture medium for Oenococcus oeni malolactic starter production. *LWT-Food Science and Technology* **2015**, *64* (1), 25−31.
(48) de Bruyn, F., Wet coffee fermentation: from ecosystem analysis to starter culture development, a microbiolgical approach. *PhD Thesis*, Vrije Universiteit Brussels 2019.
(49) Schmidt-Hager, J.; Ude, C.; Findeis, M.; John, G. T.; Scheper, T.; Beutel, S. Noninvasive online biomass detector system for cultivation in shake flasks. *Engineering in Life Sciences* **2014**, *14* (5), 467−476.
(50) Patel, S.; Tan, J.; Börner, R.; Zhang, S.; Priour, S.; Lima, A.; Ngom-Bru, C.; Cotter, P.; Duboux, S. A temporal view of the water kefir microbiota and flavour attributes. *Innovative Food Science & Emerging Technologies* **2022**, *80*, 103084.
(51) Lai, Z.; Tsugawa, H.; Wohlgemuth, G.; Mehta, S.; Mueller, M.; Zheng, Y.; Ogiwara, A.; Meissen, J.; Showalter, M.; Takeuchi, K.; et al. Identifying metabolites by integrating metabolome databases with mass spectrometry cheminformatics. *Nat. Methods* **2018**, *15* (1), 53−56.
(52) Molyneux, R. J.; Schieberle, P. Compound identification: a journal of agricultural and food chemistry perspective. *Journal of agricultural and food chemistry* **2007**, *55* (12), 4625−4629.
(53) Kreissl, J.; Mall, V.; Steinhaus, P.; Steinhaus, M. *Leibniz-LSB@ TUM odorant database*; Leibniz-Institute for Food Systems Biology at the Technical University of Munich, 2019.
(54) Masoud, W.; Jespersen, L. Pectin degrading enzymes in yeasts involved in fermentation of Coffea arabica in East Africa. *International journal of food microbiology* **2006**, *110* (3), 291−296.
(55) Barnett, J. A.; Payne, R. W.; Yarrow, D. *Yeasts: characteristics and identification*; University Press: Cambridge, 1990.
(56) Hazelwood, L. A.; Daran, J.-M.; Van Maris, A. J.; Pronk, J. T.; Dickinson, J. R. The Ehrlich pathway for fusel alcohol production: a century of research on Saccharomyces cerevisiae metabolism. *Applied and environmental microbiology* **2008**, *74* (8), 2259−2266.
(57) Hadj Salem, F.; Lebrun, M.; Mestres, C.; Sieczkowski, N.; Boulanger, R.; Collignan, A. Transfer kinetics of labeled aroma compounds from liquid media into coffee beans during simulated wet processing conditions. *Food Chem.* **2020**, *322*, 126779.

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