MANUEL ALEJANDRO PAJOY TRUJILLO

METABOLOMICS AND SENSORIAL ANALYSIS: STRATEGIC TO SELECTE NEW YEAST TO IMPROVE COFFEE QUALITY

LAVRAS-MG
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METABOLÔMICA E ANÁLISE SENSORIAL: ESTRATÉGIAS PARA SELECIONAR NOVAS LEVEDURAS PARA MELHORAR A QUALIDADE DO CAFÉ

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do curso de Pós-graduação em Microbiologia Agrícola, área da concentração em Microbiologia Agrícola, para obtenção do título de Mestre

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“Depois de escalar uma grande montanha apenas se descobre que existem muitas outras montanhas para escalar”

(Nelson Mandela)

RESUMO GERAL

O monitoramento dos processos na produção de cafés desde o cultivo até a torra garante melhoria na qualidade final da bebida. A fermentação anaeróbica induzida pelo metabolismo microbiano (SIAF), com ou sem a adição de culturas iniciadoras auxiliam na produção de cafés especiais. As culturas iniciadoras depende de altitude e região de produção, variedade do café, assim como o método de processamento devem ser consideradas. O presente trabalho objetivou avaliar a performance fermentativa de potenciais culturas iniciadoras nos processos natural e despolpado da cultivar Acaiá por meio de metodologias metabolomicas, e sensoriais. Os frutos inteiros e descascados foram sanitizados com ácido peracético (520 ppm). As fermentações foram conduzidas inoculando as leveduras *Meyerozyma caribbica* (CCMA 1993, CCMA 1950, CCMA1992, CCMA 1617, CCMA1635), *Hanseniaspora uvarum* (CCMA 1944), *Pichia kluyveri* (CCMA1658), *Meyerozyma guillermondii* (CCMA1737), *Cystofilobasidium ferigula* (CCMA1647) e controle em biorreatores (5L) por duplicata. A fermentação foi conduzida por 168 horas para o processo natural e 144 horas para o processo despolpado. Nos controles de cada processo se realizo isolamentos de leveduras em ágar YEPG com pH 3.2. Os isolados foram identificados atravéz de MALDI-TOF. A cromatografia líquida de alta eficiência (HPLC) detectou os ácidos cítrico, tartárico, succínico, lático, e acético, além de etanol, nos dois processos. Os compostos voláteis foram analisados por cromatografia gasosa – espectrometria de massa (GC-MS), onde foram detetados 97 compostos no café despolpado e 118 no café natural, destacandose a produção de alcools, esteres, pyrazinas e furanos. O processo despolpado apresento notas a laranja, frutas tropicais e amadeirado, e o processo natural apresento notas achocolatadas. Nesta investigação se podem indicar as cepas como *Hanseniaspora uvarum* (CCMA 1944), *Pichia kluyveri* (CCMA1658), *Meyerozyma guillermondii* (CCMA1737) and *Meyerozyma caribbica* (CCMA1735) como apropiadas para fermentação do processamento natural, enquanto *Hanseniaspora uvarum* (CCMA 1944) and *Meyerozyma caribbica* (CCMA1735) como apropiadas para a fermentação do processamento despolpado.

Palavras–chave: Cafés especiais. Culturas iniciadoras. Fermentação. Levedura. Processo natural. Processo despolpado.

GENERAL ABSTRACT

Monitoring coffee production processes from cultivation to roasting guarantees an improvement in the final quality of the beverage. Anaerobic fermentation induced by microbial metabolism (SIAF), with or without the addition of starter cultures, helps in the production of specialty coffees. The starter cultures depend on the altitude and region of production, the coffee variety, as well as the processing method. The aim of this study was to evaluate the fermentation performance of potential starter cultures in the natural and pulped processes of the Acaiá cultivar using metabolomic and sensory methodologies. The whole, peeled fruit was sanitized with peracetic acid (520 ppm). Fermentations were carried out by inoculating the yeasts *Meyerozyma caribbica* (CCMA 1993, CCMA 1950, CCMA1992, CCMA 1617, CCMA1635), *Hanseniaspora uvarum* (CCMA 1944), *Pichia kluyveri* (CCMA1658), *Meyerozyma guillermondii* (CCMA1737), *Cystofilobasidium ferigula* (CCMA1647) and control in bioreactors (5L) in duplicate. Fermentation was carried out for 168 hours for the natural process and 144 hours for the pulped process. In the controls for each process, yeast isolations were carried out on YEPG agar at pH 3.2. The isolates were identified using MALDI-TOF. High performance liquid chromatography (HPLC) detected citric, tartaric, succinic, lactic and acetic acids, as well as ethanol, in both processes. The volatile compounds were analyzed by gas chromatography-mass spectrometry (GC-MS), where 97 compounds were detected in the pulped coffee and 118 in the natural coffee, highlighting the production of alcohols, esters, pyrazines and furans. The pulped process has notes of orange, tropical fruit and woodiness, while the natural process has chocolatey notes. In this research, strains such as *Hanseniaspora uvarum* (CCMA 1944), *Pichia kluyveri* (CCMA1658), *Meyerozyma guillermondii* (CCMA1737) and *Meyerozyma caribbica* (CCMA1735) can be indicated as suitable for fermentation in the natural process, while *Hanseniaspora uvarum* (CCMA 1944) and *Meyerozyma caribbica* (CCMA1735) are suitable for fermentation in the pulped process.

Keywords: Specialty coffees. Starter cultures. fermentation. Yeast. Natural process. Pulped process.

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO

O café é uma bebida preparada de grãos de café torrados e moídos, caracterizada pela cor marrom escura e sabor ligeiramente amargo. A bebida é uma das mais consumidas mundialmente, e, portanto, um dos produtos mais comercializados do mundo. O café é cultivado na América do Sul, América Central, Caribe e Ásia (Batista et al., 2016; Martinez et al., 2017). Brasil, Vietnã e Colômbia são líderes na produção de café (Conab, 2022).

Exitem três métodos para o processamento do café, os quais apresentam fermentation espontânea, sendo o processamento úmedo, seco, e semiseco (Schwan et al., 2012). Na fermentação espontanea podem ocurrir interações entre bactérias, leveduras e fungos, sendo os microrganismos comumente encontrados durante a fermentação do café (Martins et al., 2020; Silva et al., 2000). Na fermentação ocorrem mudanças físico-químicas nos grãos de café como redução do conteúdo de água, açúcares simples e formação de precursores de aroma e sabor. Isso é devido às interações dos microrganismos que ajudam na degradação de compostos da polpa e mucilagem liberando metabólitos que podem melhorar a qualidade do café (Rodarte et al., 2011; Schwan and Graham, 2015; Silva et al., 2000).

A sanitization dos grãos de café para o posterior uso de culturas inicidoras durante a fermentação não têm sido amplimente estudada, só se tem reporte da sanitização dos grãos de café antes da fermentação em autoclave (121°C, 5 min) e hipoclorito de sódio (porcentagem não reportado) (da Silva et al., 2022; Martinez et al., 2019), reduçendo a cargar microbiana epífita e ajudando na adeção das culturas inicidoras no grão de café (Martinez et al., 2019), assim como, uma adecuada uniformidade microbiana, e consecuentemente uniformidade na produção de metabolitos (Carvalho Ferreira et al., 2023).

O uso de culturas iniciadoras durante a fermentação tem demonstrado potencial na obtenção de café especiais de alta qualidade com atributos sensoriais desejáveis devido à produção de metabólitos que interferem na qualidade do café (Bressani et al., 2021b; Lee et al., 2015; Martinez et al., 2021a; Martins et al., 2019; Priscila. Pereira et al., 2021), não entanto muitos autores reportam à não sanitização dos grão antes da fermentação, presentando interferência de microrganismos epífitos e inteferendo na uniformidade microbiana e de metabolitos produzidos.

O processo fermentativo conduzido em biorreatores fechados permite a atividade metabólica das leveduras que consomem o oxigênio disponível e liberam gás carbônico e

compostos químicos (da Mota et al., 2022; Pereira et al., 2022). A inoculação de leveduras promove o controle do crescimento de microrganismos indesejáveis, incluindo os fungos toxigênicos, redução do tempo de fermentação e padronização dos processos que podem ser replicados durante diferentes safras (Evangelista et al., 2014a; Silva et al., 2013). As culturas iniciadoras como *Saccharomyces cerevisiae* (CCMA0543), *Torulaspora delbrueckii* (CCMA0684), *Candida parapsilosis* (CCMA0544), *Meyerozima caribbica* (CCMA0198; CCMA1738), *Picha kluyveri* (CCMA1743) demonstraram alterar o perfil sensorial da bebida comparada à amostra controle, sem cultura iniciadora (Bressani et al., 2021a, 2021c, 2020a; da Mota et al., 2020; da Silva et al., 2021; Martinez et al., 2017). Portanto, o presente trabalho teve como objetivo avaliar a performance fermentativa das potenciais culturas iniciadoras nos grãos de café previamente sanitizados com acido peracético nos processamentos natural e despolpado da cultivar Acaiá utilizando metodologias metabolomicas e sensorial.

2. REFERENCIAL TEÓRICO

2.1. Mercado cafeeiro

O Brasil exportou cerca de 50,92 milhões de sacas de 60 quilos de café verde no ano 2022 para 133 países, sendo os principais: Estados Unidos (18,3%), Alemanha (14,7%), Itália (8,1%), Bélgica (5,4%), e Japão (5,2%) tendo participação do porto de Santos com 76,9% e o porto do Rio de Janeiro com um 13,8% dos embarques de café brasileiro (Conab, 2023). No mesmo ano, foi observado uma alta de 4% nas exportações comparadas com o ano de 2021 (43,9 milhões de sacas de 60 quilos de café). No período 2022/23, o Brasil tem liderado o mercado internacional, seguido do Vietnã e Colômbia, e com expectativa de colheita para o ano 2023 de 34,1 milhões sacas de café, que representa 4,8% a mais que o ano 2022 (Conab, 2023).

Durante o ano 2022 o Brasil reportou uma área total de 2.241,158 hectares de café com uma área de produção de 1.841,528 hectares, os quais 1.815,897 hectares foram de café arábica e 437,339 hectares para café canéfora. Para o ano de 2022, 2.248.739 mil hectares na cafeicultura nacional com área de produção de 1.811,400 mil hectares. Na área de produção pode estar representada em 1.480.586 mil hectares para café arábica e 392,611 mil hectares para café canéfora. Na produção está estimada em 37.929,2 mil sacas de café para o café arábica e 16.813,2 mil sacas de café canéfora; representando um crescimento de 15,9% e 7,6%, respectivamente, comparado com a safra anterior (Conab, 2023).

2.2. Regiões produtoras de café

O primeiro levantamento do acompanhamento da safra brasileira de café arábica e canéfora do ano 2023 reporta nove estados produtores, os quais são Minas Gerais (MG), Espírito Santo (ES), São Paulo (SP), Bahia (BA), Rondônia (RO), Paraná (PR), Rio de Janeiro (RJ), Goiás (GO) e Mato Grosso (MT), como se apresenta na Tabela 1. (Conab, 2023).

Tabela 1. Produção de café durante o ano de 2022 e estimativa para o 2023 de acordo com o CONAB, 2023.

Estados produtores	**Produção 2022 (mil sacas beneficiadas)**	**Estimativa 2023 (mil sacas beneficiadas)**
Minas Gerais (MG)	21.960	27,831
Espírito Santo (ES)	16.721	13.650
São Paulo (SP)	4.387	4.935
Bahia (BA)	3.603	3.643
Rondônia (RO)	2.800	3.131
Paraná (PR)	497.9	686.7
Rio de Janeiro (RJ)	294.3	278.0
Goiás (GO)	277.7	254.1
Mato Grosso (MT)	227.9	239.5

Fonte: CONAB (2023).

O estado de Minas Gerais nas últimas safras produziu cerca da metade de todo volume colhido nacionalmente demostrando porque é reconhecido tradicionalmente como o maior produtor de café. Neste estado os cultivos se concentram em mesorregiões características, sendo o Sul e Centro–Oeste mineiro, o triângulo, o alto Paraíba, o Noroeste da Mata, o Vale do Rio Doce e a Zona Central, com uma destinação de área de 1,3 milhão de hectares para a cafeicultura na ordem de 338 mil hectares para lavouras de café em formação e 990 mil hectares para lavouras em produção. Em 2022 a produção foi de 21.960 mil sacas beneficiadas e a estimativa de produção para a temporada 2023 é de 27,831 mil sacas de café beneficiado, sendo a grande maioria de café arábica (Conab, 2023). Em comparação com a safra anterior, esta região tem uma estimativa para o ano 2023 de aumento de 15.9% do total de café colhido. Isto simboliza um crescimento de 26,7% (Conab, 2023).

O estado de Espírito Santo produziu 16.721 mil sacas de café beneficiado no ano 2022 e a expectativa para o ano de 2023 é de 13.650 mil sacas de café (Conab, 2023). O estado de São Paulo produziu 4.387 mil sacas de café beneficiado no ano 2022, tendo uma expectativa de produção de 4.935 mil sacas de café em 2023. Esta região se destaca-se pelo potencial tecnológico, com manejos mais drásticos nas podas, representando 60% das áreas com podas drásticas objetivando melhor recuperação vegetativa das plantas e maior vigor produtivo (Conab, 2022). No estado da Bahia é cultivado tanto café arábica como café canéfora, com

100% das operações de colheitas em caráter mecanizado. Esta região concentra sua produção em regiões como Atlântico (Sul da Bahia), Planalto (centro-sul e centro-norte da Bahia) e cerrado (extremo-Oeste da Bahia), que produziu 3.60.5 mil sacas de café beneficiado no ano 2022. Há expectativa de produção de 3.643 mil sacas de café para 2023 (Conab, 2023).

O estado de Rondônia produziu 2.800 mil sacas de café beneficiado no ano 2022, tendo uma expectativa para o ano 2023 de 3.131 mil sacas de café. No entanto, esta região está passando por um processo de transformação, como renovação do material genético com clones mais adaptados às condições climáticas (Conab, 2022). Estima-se maior produtividade e resistência a doenças nas lavouras novas comparadas com lavouras antigas instauradas com sementes com baixo padrão tecnológico (Conab, 2022). O estado do Paraná produziu 497.9 mil sacas de café beneficiado no ano 2022, tendo uma expectativa de aumento para o ano de 2023 de 686.7 mil sacas de café que representa 5.2% da área em produção (Conab, 2023). Isto é devido a substituição da área ocupada com café por cultivos de grãos, como a soja (Conab, 2022). Outro motivo da redução da área de produção é devido as podas drásticas de muitas lavouras que foram realizadas após as fortes geadas ocorridas em junho do ano 2021 afetando a área de produção.

O estado de Rio de Janeiro apresenta duas regiões produtoras de café, a região serrana carioca que possui temperaturas mais amenas e maior umidade e a região noroeste fluminense que tem clima mais seco com temperaturas mais altas, tendo a concentração dos cultivos nas partes mais altas que são propícias para o café arábica. Nesta região é predominante o cultivo de café arábica, mas também tem áreas com produção de café canéfora. O estado do Rio de Janeiro produziu 294.3 mil sacas de café beneficiado no ano 2022, tendo uma expectativa para o ano de 2023 de 278 mil sacas de café (Conab, 2023).

O estado de Goiás apresenta renovação ou substituição dos cultivos de café por cultivos anuais como soja e milho.A produção do estado pode ser aumentada sem aumento na área plantada adotandomelhorias no manejo nas áreas específicas de produção de café (Conab, 2022). O estado de Goiás produziu 277.7 mil sacas de café beneficiado no ano 2022, tendo uma expectativa o para e 2023 de 254,1 mil sacas de café (Conab, 2023).

O estado de Mato Grosso tem uma expectativa de aumento da área de produção de café para o ano 2023 com índices de condições pluviométricas favoráveis com bons volumes de chuva favorecendo a uniformidade da floração. Estima-se uma produção de 254.1 mil sacas de café, sendo maior que na safra do ano 2022 apresentado 227.2 mil sacas de café. Esta região se destaca por ter exclusivamente café de tipo canéfora (Conab, 2023).

Além de toda a produtividade registrada, o Brasil está adotando padrões de qualidade da matéria-prima, manutenção de sabor ao longo do tempo, boas práticas de fabricação com o Programa de Qualidade do Café (PQC) dirigido pela Associação Brasileira da Indústria de Café (ABIC), assegurando assim consistência da qualidade do café e do processo industrial, garantindo repetibilidade do padrão de qualidade em todos os lotes produzidos e exigindo uma melhor adequação da própria indústria cafeteira (BSCA, 2022).

2.3. Café arábica

O Café arabica é originário do sudoeste da Etiópia, sudeste do Sudão e norte do Quênia, tem um faixa de crescimento entre 600 e 2.000 metros sobre o nível do mar com temperaturas amenas entre 18 e 21 ºC (Carvalho, 2009; Veloso et al., 2020).

O café arabica é um alotetaploide (2n = 4x = 44 cromossomos), sendo autofértil com 90% de autopolinização de 10% de polinização cruzada (Carvalho, 2009; Hall et al., 2022). Tem material genético com elevada produtividade, é sensível as geadas e ao calor extremos tendo melhor desenvolvimento em terras com clima mais moderado. Contém mais concentração de lipídeos e sacarose comparado ao café canéfora (Carvalho, 2009; Toledo et al., 2016; Trejo et al., 2018).

O café arabica é avaliado de maneira sensorial pelo protocolo da Specialty Coffee Association (SCA). Essa associação fomentou a produção e o consumo de cafés especiais procurando satisfazer as demandas dos consumidores que cada vez mais exigem produtos de alto valor sensorial com adições de novas características específicas. Isto originou as boas práticas de processamento, inovação e esforços para alcançar a qualidade exigida dos consumidores finais que estão dispostos a pagar por uma melhor qualidade e origem do produto (Lourenzani et al., 2020). A origem do café pode ser um aliado na agregação de valor do produto, além disso, os consumidores estão mais preocupados com questões sociais e ambientais, que também remete ao apelo comercial. No entanto, se o café não tem qualidade reconhecida, os outros fatores se tornam menos influentes (Bote and Vos, 2017; Cheng et al., 2016; Lourenzani et al., 2020). No protocolo desenvolvido pela SCA é medido a qualidade do café baseado em 10 atributos (fragrância/aroma, sabor, gosto residual, sabor residual, acidez, corpo, uniformidade, equilíbrio, limpeza da xícara, doçura e classificação final do provador). A pontuação varia de 6 a 10 para cada atributo, formando uma escala de 60 a 100, e considerando os cafés especiais apenas os cafés que atingirem pontuações final maior ou igual a 80 pontos (SCAA, 2015).

O mercado brasileiro de cafés especiais implementou estratégias para a diferenciação dos cafés, permitindo aos consumidores perceber na procedência do café especial, como marca e registro de origem (Lourenzani et al., 2020).

2.3.1. Processamento do café

Durante a colheita deve-se coletar os frutos de café maduros caracterizados por serem de cor vermelha ou amarela dependendo da cultivar. No entanto, em uma colheita não seletiva, pode ser encontrado frutos de café verde, frutos super maduros, folhas, ramos, terra, paus e demais materiais que vieram da colheita. O café colhido deve ser processado com rapidez, pois uma vez retirado o fruto da planta, pode acontecer o processo de fermentação de maneira espontânea, sem controle, influenciando na qualidade da bebida final (Bressani et al., 2021b; Martinez et al., 2021; Pereira et al., 2021; Silva et al., 2000). O processo após a colheita vai depender da região e prática cultural local, onde podem seguir um dos três processamentos: natural (seco), cereja descascado (semi-seco) e úmido (Hall et al., 2022).

O processo chamado de processo natural ou seco consiste na desidratação (secagem) dos frutos sem retirar o pericarpo. Este processo é tradicionalmente usado desde a antiguidade, e implica na fermentação do fruto inteiro tendo uma interação microbiana mais complexa e variável (Evangelista et al., 2014b; Hall et al., 2022; Schwan and Graham, 2015; Silva et al., 2008, 2000). O processo úmido consiste em retirar o pericarpo dos frutos de café de maneira mecânica. Posteriormente o café é submerso em água dentro de tanques de fermentação por 48 até 72 horas para remover a mucilagem dos grãos. Após o processo de fermentação, o café é transportado para secagem (Evangelista et al., 2014b; Hall et al., 2022; Schwan and Graham, 2015; Silva et al., 2008, 2000). O método semi-seco é um intermediário entre o método seco e úmido, onde os grãos são descascados, mas a mucilagem não é retirada dos grãos. Estes são então transportado para secagem (Evangelista et al., 2014b; Hall et al., 2022; Schwan and Graham, 2015; Silva et al., 2008, 2000). Em todos os tipos de processamento têm-se interação microbiana, gerando fermentação diferente para cada um, destacando-se novamente que o processo vai variar de acordo com a prática cultural local (Hall et al., 2022; Schwan and Graham, 2015). Após fermentação, o café segue para o processo de secagem, que também afetará a qualidade final do café verde. Este processo é realizado com a exposição ao sol, seja em grandes pátios de concreto ou tijolos, ou terreiros suspensos na altura da cintura em cavaletes (Batista et al., 2016). Se a secagem é realizada em excesso, o café ficará quebradiço, produzindo grãos partidos durante o processo de descasque.

Se a secagem é insuficiente, terá umidade excessiva, sendo propenso a contaminação por fungos e bactérias durante o armazenamento (Batista et al., 2016).

2.4. Fermentação do café

Durante a fermentação do café, associam-se microrganismos pectinolíticos que fazem a degradação da polpa e mucilagem (rica em polissacarídeos) produzindo compostos metabólicos que interferem na qualidade final da bebida (Bressani et al., 2021c, 2020a; da Mota et al., 2020; Evangelista et al., 2014b).

A fermentação dos frutos de café é realizada de maneira espontânea, independentemente do método de processamento. Durante esta etapa ocorre reações exotérmicas pelos microrganismos onde a temperatura vai influenciar na degradação da mucilagem, no tempo de fermentação, e na qualidade da bebida. Também ocorrem alterações físico-químicas dos grãos, como redução de açúcares simples formando-se compostos como ácidos orgânicos, ésteres, álcoois, aldeídos, fenóis e lactonas que afetam as características de aroma e sabor (Batista et al., 2016; S. Martinez et al., 2021; Martins et al., 2020; P. V. Pereira et al., 2021; Silva et al., 2013).

Os compostos que se formam são gerados por microrganismos presentes na mucilagem que consomem carboidratos e/ou outros compostos orgânicos. Estes microrganismos mudam em resposta as condições climáticas relacionadas à umidade, pH e altitude de cultivo (Martinez et al., 2021a; Pereira et al., 2021) Apresenta-se grande variedade de microrganismos como leveduras, fungos filamentosos e bactérias Gram-positivas e Gram-negativas em diferentes momentos durante oprocesso de fermentação natural do café, assim também os microrganismos podem apresentar variação com o tipo de processamento (natural, despolpado, úmido) (Evangelista et al., 2015; Huch; Franz, 2014; Ribeiro et al., 2018). Os gêneros bacterianos mais comuns encontrados durante o processamento natural de café são *Lactobacillus, Arthrobacter, Acinetobacter, Klebsiella e Weissella*. Após um tempo do início da fermentação, as leveduras tendem a aumentar sua população, sendo reportados os gêneros *Saccharomyces, Pichia, Candida, Rhodotorula, Hanseniaspora e Kluyveromyces* como os mais comuns, independente do processamento do café. Os principais gêneros de fungos filamentosos encontrados são *Aspergillus*, *Penicillium* e *Fusarium*, especialmente durante a secagem e armazenamento (Evangelista et al., 2014a).

As interações entre altitude, temperatura, umidade e microrganismos influenciam no perfil das características sensoriais do café, sendo observados variações de ácidos graxos e ácidos clorogênicos em cafés processados em diferentes altitudes (Martins et al., 2020). Em

fermentações inadequadas podem ser formados compostos indesejáveis que levam a sabores indesejáveis. Se a fermentação é muito lenta e ineficiente irá acarretar a formação de ácido butírico e ácido propiônico, impactando negativamente na qualidade da bebida final (Schwan and Graham, 2015).

2.5. Sanitização dos grãos de café

A sanitização dos grãos de café antes da fermentação pode promover uma melhor uniformidade dos metabolitos genrados pelas culturas iniciadoras, mas, não existe bastante informação de praticas para à sanitização do café antes da fementation. Se tem implementado a sanitização dos grãs de café em autoclave a 121°C durante 5 min (Martinez et al., 2019), ademas de higienização da superfícies dos grão com hipoclorito de sódio (Concentração no informada) (da Silva et al., 2022) antes da fermentação, reducendo os pefies microbianos (da Silva et al., 2022; Martinez et al., 2019) e axuliando na adeção das culturas inicidoras no grão de café (Martinez et al., 2019).

2.6. Culturas iniciadoras

O uso culturas iniciadoras durante a fermentação por anaerobiose autoinduzida (SIAF), natural, despolpado e úmido foram descritos na literatura apresentando modificações dos compostos obtidos em comparação com os cafés fermentados espontaneamente (Bressani et al., 2021b, 2018; da Mota et al., 2022; Evangelista et al., 2014a; Lee et al., 2015; Martinez et al., 2017; Pereira et al., 2022; Silva et al., 2013). Além disso, também é descrita uma redução do tempo de fermentação tendo uma maior redução dos açúcares encontrados no grão e o controle do crescimento de microrganismos indesejáveis devido na redução do pH dentro do processo de fermentação (Pereira et al., 2021; Schwan;Wheals, 2003). Compostos como ésteres, álcoois superiores, cetonas e terpenóides são reportados como compostos gerados pela maioria das culturas iniciadoras proporcionando variações de precursores de sabor e aroma, ligados à qualidade da bebida final. Leveduras das espécies *S. cerevisiae, T. delbrueckii, C. parapsilosis, M. caribbica, Yarrowia lipolytica,* e *P. kluyveri* foram reportadas como microrganismos benéficos para o processo de fermentação de café arábica e canéfora (da Silva et al., 2021; Lee et al., 2017; Martins et al., 2019; P. V. Pereira et al., 2021; Silva et al., 2000). Estas leveduras foram apontadas como responsáveis pela produção de compostos voláteis como ácido fórmico, 2- metil-1,3-butadieno, 3,4-dimetil-2-pentanona e pantolactona. Estes compostos evidenciaram influência no corpo, acidez, doçura, resultando em notas sensoriais como chocolate, ameixa, pêssego e atributos florais na bebida final (Evangelista et al., 2014a; Martinez et al., 2021). Portanto a utilização de culturas iniciadoras pode

influenciar nos atributos sensoriais tornando uma alternativa economicamente viável ao agregar valor no produto, além de permitir padronizar o processo fermentativo. No entanto, os critérios para seleção de microrganismos fermentadores deve ter como foco atividades metabólicas relevantes, preferencialmente aqueles microrganismos (leveduras e bactérias) que geram alterações positivas agregando qualidade na bebida final (de Melo Pereira et al., 2019).

2.7. Considerações finais

A qualidade da bebida do café pode ser influenciada pelo o processamento pós-colheita. A atribuição de maior qualidade à bebida do café com o uso de culturas iniciadoras têm sido investigada amplamente apresentado novos benefícios na produção de compostos voláteis, ácidos e demais compostos metabólicos. Esses compostos podem modificar os perfis sensoriais além de proporcionar inocuidade e segurança alimentar com a inibição de microrganismos indesejáveis como fungos e bactérias deteriorantes.

Conhecer os microrganismos presentes durante a fermentação do café é importante, no entanto, se tem pouca informação sobre a sanitização dos grãos de café antes da fermentação como inhibidor de microrganismos epífitos tais como bactérias, fungos e leveduras que interferem na obteção de unifomidad metabólica no processo de fermentação do café com culturas inciadoras, e consecuentemente pode apresentar influencia na qualidade sensorial do café fermentado. Por isso se torna importante ter uniformidade na microbiota actuante dentro da fermentação do café.

REFERÊNCIAS

Batista, L.R.; Chalfoun de Souza, S.M.; Silva e Batista, C.F.; Schwan, R.F. 2016. Coffee: Types and Production, **in: Encyclopedia of Food and Health. Elsevier**, pp. 244–251. https://doi.org/10.1016/B978-0-12-384947-2.00184-7.

Bote, A.D.; Vos, J. 2017. Tree management and environmental conditions affect coffee (Coffea arabica L.) bean quality. **NJAS: Wageningen Journal of Life Sciences**. 39–46. https://doi.org/10.1016/j.njas.2017.09.002.

Bressani, A.P.P.; Batista, N.N.; Ferreira, G.; Martinez, S.J.; Simão, J.B.P.; Dias, D.R.; Schwan, R.F. 2021a. Characterization of bioactive, chemical, and sensory compounds from fermented coffees with different yeasts species. **Food Research International**, 110755. https://doi.org/10.1016/j.foodres.2021.110755.

Bressani, A.P.P.; Martinez, S.J.; Batista, N.N.; Simão, J.B.P.; Dias, D.R.; Schwan, R.F. 2021b. Co-inoculation of yeasts starters: A strategy to improve quality of low altitude Arabica coffee. **Food Chemistry**, 130133. https://doi.org/10.1016/j.foodchem.2021.130133.

Bressani, A.P.P.; Martinez, S.J.; Evangelista, S.R.; Dias, D.R.; Schwan, R.F. 2018. Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. **LWT,** 92, 212–219. https://doi.org/10.1016/j.lwt.2018.02.029.

Bressani, A.P.P.; Martinez, S.J.; Sarmento, A.B.I.; Borém, F.M.; Schwan, R.F. 2021c. Influence of yeast inoculation on the quality of fermented coffee (Coffea arabica var. Mundo Novo) processed by natural and pulped natural processes. **Int J Food Microbiology**. https://doi.org/10.1016/j.ijfoodmicro.2021.109107.

Bressani, A.P.P.; Martinez, S.J.; Sarmento, A.B.I.; Borém, F.M.; Schwan, R.F. 2020. Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. **Food Research International**. https://doi.org/10.1016/j.foodres.2019.108773.

BSCA, 2022. A BSCA- Café Especiais do Brasil. **A BSCA**. https://bsca.com.br/a-bsca.

Carvalho, C., 2009. Cultivares de café, **Embrapa**. ed.

Carvalho Ferreira, J.L.; de Souza Gomes, M.; Maciel de Oliveira, L.; Diniz Santos, L. 2023. Coffee fermentation process: A review. **Food Research International**. https://doi.org/10.1016/j.foodres.2023.112793.

Cheng, B., Furtado, A., Smyth, H.E., Henry, R.J., 2016. Influence of genotype and environment on coffee quality. **Trends Food Sci Technol**, 20–30. https://doi.org/10.1016/j.tifs.2016.09.003.

Conab, 2023. Acompanhamento da safra brasileira: 2° Levantamento. **Conab**. https://www.conab.gov.br/ultimas-noticias/5003-levantamento-da-conab-estima-producao-de-cafe-em-54-74-milhoes-de-sacas-na-safra-2023 (accessed 6.9.23).

Conab, 2022. Acompanhamento da safra brasilera. **Conab**. https://www.conab.gov.br/info-agro/safras/cafe

da Mota, M.C.; Batista, N.N.; Dias, D.R.; Schwan, R.F. 2022. Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality. **Food Bioscience**, 101640. https://doi.org/10.1016/j.fbio.2022.101640.

da Mota, M.C.B.; Batista, N.N.; Rabelo, M.H.S.; Ribeiro, D.E.; Borém, F.M.; Schwan, R.F., 2020. Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. **Food Research International**. https://doi.org/10.1016/j.foodres.2020.109482.

da Silva, B.; Pereira, P.V.; Bertoli, L.D.; Silveira, D.L.; Batista, N.N.; Pinheiro, P.F.; de Souza Carneiro, J.; Schwan, R.F.; de Assis Silva, S.; Coelho, J.M.; Bernardes, P.C. 2021. Fermentation of Coffea canephora inoculated with yeasts: Microbiological, chemical, and sensory characteristics. **Food Microbiology**, 103786. https://doi.org/10.1016/j.fm.2021.103786.

da Silva, M.C.S.; da Luz, J.M.R.; Veloso, T.G.R.; Gomes, W. dos S.; Oliveira, E.C. da S.; Anastácio, L.M.; Cunha Neto, A.; Moreli, A.P.; Guarçoni, R.C.; Kasuya, M.C.M.; Pereira, L.L. 2022. Processing techniques and microbial fermentation on microbial profile and chemical and sensory quality of the coffee beverage. **European Food Research and Technology**, 1499–1512. https://doi.org/10.1007/S00217-022-03980-6/FIGURES/7.

de Melo Pereira, G. V.; de Carvalho Neto, D.P.; Magalhães Júnior, A.I.; Vásquez, Z.S.; Medeiros, A.B.P.; Vandenberghe, L.P.S.; Soccol, C.R. 2019. Exploring the impacts of postharvest processing on the aroma formation of coffee beans – A review. **Food Chemistry**, 441–452. https://doi.org/10.1016/j.foodchem.2018.08.061.

Evangelista, S.; de Souza Cordeiro, C.; da Cruz Pedrozo, M.G.; Silva, C.F., Marques Pinheiro, A.C.; Schwan, R.F. 2014a. Inoculation of starter cultures in a semi-dry coffee (Coffea arabica) fermentation process. **Food Microbiology**, 87–95. https://doi.org/10.1016/j.fm.2014.05.013.

Evangelista, S.; Silva, C.F.; Miguel, M.G.P. da C.; Cordeiro, C. de S.; Pinheiro, A.C.M.; Duarte, W.F.; Schwan, R.F. 2014b. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. **Food Research International**, 183–195. https://doi.org/10.1016/j.foodres.2013.11.033.

Evangelista, S.R.; Miguel, M.G. da C.P.; Silva, C.F.; Pinheiro, A.C.M.; Schwan, R.F. 2015. Microbiological diversity associated with the spontaneous wet method of coffee fermentation. **Int J Food Microbiology**, 102–112. https://doi.org/10.1016/j.ijfoodmicro.2015.06.008.

Hall, R.D.; Trevisan, F.; de Vos, R.C.H. 2022. Coffee berry and green bean chemistry – Opportunities for improving cup quality and crop circularity. **Food Research International**, 110825. https://doi.org/10.1016/j.foodres.2021.110825.

Huch, M.; Franz, C.M.A.P. 2014. Coffee: Fermentation and microbiota. **in: Advances in Fermented Foods and Beverages: Improving Quality, Technologies and Health Benefits**, pp. 501–513. https://doi.org/10.1016/B978-1-78242-015-6.00021-9.

Lee, L.W.; Cheong, M.W.; Curran, P.; Yu, B.; Liu, S.Q. 2015. Coffee fermentation and flavor - An intricate and delicate relationship. **Food Chemistry**. https://doi.org/10.1016/j.foodchem.2015.03.124.

Lee, L.W.; Tay, G.Y.; Cheong, M.W.; Curran, P.; Yu, B.; Liu, S.Q. 2017. Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica: I. Green coffee. **LWT - Food Science and Technology**, 225–232. https://doi.org/10.1016/j.lwt.2016.11.047.

Lourenzani, A.E.B.S.; Watanabe, K.; Pigatto, G.A.S.; de Godoi Pereira, M.E. 2020. What fills your cup of coffee? The potential of geographical indication for family farmers' market access. **Coffee Consumption and Industry Strategies in Brazil,** pp. 149–165. https://doi.org/10.1016/B978-0-12-814721-4.00014-7.

Martinez, S.; Rabelo, M.H.S.; Bressani, A.P.P.; da Mota, M.C.B.; Borém, F.M.; Schwan, R.F. 2021. Novel stainless steel tanks enhances coffee fermentation quality. **Food Research International**, 109921. https://doi.org/10.1016/j.foodres.2020.109921.

Martinez, S.J.; Bressani, A.P.P.; Dias, D.R.; Simão, J.B.P.; Schwan, R.F. 2019. 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. **Front Microbiology**. https://doi.org/10.3389/fmicb.2019.01287.

Martinez, S.J.; Bressani, A.P.P.; Miguel, M.G.; Dias, D.R.; Schwan, R.F. 2017. Different inoculation methods for semi-dry processed coffee using yeasts as starter cultures. **Food Research International**, 333–340. https://doi.org/10.1016/j.foodres.2017.09.096.

Martinez, S.J.; Simão, J.B.P.; Pylro, V.S.; Schwan, R.F. 2021a. The Altitude of Coffee Cultivation Causes Shifts in the Microbial Community Assembly and Biochemical Compounds in Natural Induced Anaerobic Fermentations. **Front Microbiology**. https://doi.org/10.3389/fmicb.2021.671395.

Martins, P.; Ribeiro, L.S.; Miguel, M.G.; Evangelista, S.R.; Schwan, R.F. 2019. Production of coffee (Coffea arabica) inoculated with yeasts: impact on quality. **J Science of Food Agriculture**, 5638–5645. https://doi.org/10.1002/jsfa.9820.

Martins, P.M.M.; Batista, N.N.; Miguel, M.G.; Simão, J.B.P.; Soares, J.R.; Schwan, R.F. 2020. Coffee growing altitude influences the microbiota, chemical compounds and the quality of fermented coffees. **Food Research International**, 108872. https://doi.org/10.1016/j.foodres.2019.108872.

Pereira, Priscila.; da Silveira, D.L.; Schwan, R.F.; de Assis Silva, S.; Coelho, J.M.; Bernardes, P.C. 2021. Effect of altitude and terrain aspect on the chemical composition of Coffea canephora cherries and sensory characteristics of the beverage. **J Science of Food Agriculture**, 2570–2575. https://doi.org/10.1002/jsfa.10885.

Pereira, P.V.; Bravim, D.G.; Grillo, R.P.; Bertoli, L.D.; Osório, V.M.; da Silva Oliveira, D.; Miguel, M.G.; Schwan, R.F.; de Assis Silva, S.; Coelho, J.M.; Bernardes, P.C. 2021. Microbial diversity and chemical characteristics of Coffea canephora grown in different environments and processed by dry method. **World J Microbiol Biotechnol**, 51. https://doi.org/10.1007/s11274-021-03017-2.

Pereira, T.S.; Batista, N.N.; Santos Pimenta, L.P.; Martinez, S.J.; Ribeiro, L.S.; Oliveira Naves, J.A.; Schwan, R.F. 2022. Self-induced anaerobiosis coffee fermentation: Impact on microbial communities, chemical composition and sensory quality of coffee. **Food Microbiology,** 103962. https://doi.org/10.1016/j.fm.2021.103962.

Ribeiro, L.S.; Evangelista, S.R.; Miguel, M.G.; van Mullem, J.; Silva, C.F.; Schwan, R.F. 2018. Microbiological and chemical-sensory characteristics of three coffee varieties processed by wet fermentation. **Annals Microbiology**, 705–716. https://doi.org/10.1007/s13213-018-1377-4.

Rodarte, M.P.; Dias, D.R.; Vilela, D.M.; Schwan, R.F. 2011. Proteolytic activities of bacteria, yeasts and filamentous fungi isolated from coffee fruit (Coffea arabica L.). **Acta Scientiarum Agronomy**. https://doi.org/10.4025/actasciagron.v33i3.6734.

SCAA, 2015. SCAA Protocols Cupping Specialty Coffee. **Specialty Coffee Association of America,** pp 1–10.

Schwan, R.; Graham, F.H. 2015. Cocoa and Coffee Fermentations.
Schwan, R.; Silva, C.; Batista, L. 2012. Coffee Fermentation. **Handbook of Plant-Based Fermented Food and Beverage Technology**, Second Edition. CRC Press, pp. 677–690. https://doi.org/10.1201/b12055-49.

Schwan, R.F.; Wheals, A.E. 2003. Mixed microbial fermentations of chocolate and coffee. **Yeasts in Food**, pp. 429–449. https://doi.org/10.1533/9781845698485.429.

Silva, C.F; Batista.L.R; Abreu, L.; Dias, E.; Schwan, R. 2008. Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation. **Food Microbiology**, 951–957. https://doi.org/10.1016/j.fm.2008.07.003.

Silva, C.F.; Schwan, R.F.; Sousa Dias, Ë.; Wheals, A.E. 2000a. Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. **Int J Food Microbiology**, 251–260. https://doi.org/10.1016/S0168-1605(00)00315-9.

Silva, C.F.; Vilela, D.M.; de Souza Cordeiro, C.; Duarte, W.F.; Dias, D.R.; Schwan, R.F. 2013. Evaluation of a potential starter culture for enhance quality of coffee fermentation. **World J Microbiol Biotechnology**, 235–247. https://doi.org/10.1007/s11274-012-1175-2.

Toledo, P.R.A.B.; Pezza, L.; Pezza, H.R.; Toci, A.T. 2016. Relationship Between the Different Aspects Related to Coffee Quality and Their Volatile Compounds. Compr Rev **Food Sci Food Saf**, 705–719. https://doi.org/10.1111/1541-4337.12205.

Trejo, L.; Gòmez, F.; Morales, V.; Marin, T.; Castañeda, O.; Pastelin, M. 2018. concentración de macronutrimentos y micronutrimentos en granos de café (coffea sp.) de diferentes orígenes. **AgroProductividad**. https://doi.org/10.32854/agrop.v11i4.263.

Veloso, T.G.R.; da Silva, M.; Cardoso, W.S.; Guarçoni, R.C.; Kasuya, M.C.M.; Pereira, L.L. 2020. Effects of environmental factors on microbiota of fruits and soil of Coffea arabica in Brazil. **Scientific Reports**, 1–11. https://doi.org/10.1038/s41598-020-71309-y

SEGUNDA PARTE – ARTIGOS

ARTIGO 1: STRATEGIES FOR SELECTING NEW YEAST STRAINS FOR THE PROCESSING OF NATURAL AND PULPED COFFEE

This article has been formatted according to the guidelines of the journal Food Microbiology

Abstract

The changes in the volatile composition of coffee influenced by the consumption of nutrients by the yeasts to produce metabolites during fermentation have been of great interest. Therefore, this research evaluated the impact of the SIAF method on the metabolomic compounds of strains *Meyerozyma caribbica* (CCMA 1993, CCMA1950, CCMA1992, CCMA1617, CCMA1735), *Hanseniaspora uvarum* (CCMA 1944), *Pichia kluyveri* (CCMA1658), *Meyerozyma guillermondii* (CCMA1737) and *Cystofilobasidium ferigula* (CCMA1647) inoculated in the natural and pulped coffee beans. The coffee beans were sanitization with peracetic acid before fermentation, then they were dosed in bioreactors with a capacity of 5L, Following the strains were inoculated each with duplicated and the control without inoculated with duplicated. The fermentation lasted 168 hours for the natural process and 144 hours for the pulped process. In the control treatment of each process, microbial isolation was performed on YEPG at pH 3.2 for the growth of mainly yeasts; however, bacterial growth was obtained. The isolates were identified through MALDI-TOF. The microorganisms most commonly found were *Candida glabrata, Candida carpophila, Candida guilliermondii, Torulaspora delbureckii e lactobacillus plantarum*. High-performance liquid chromatography (HPLC) detected citric, tartaric, succinic, lactic, and acetic acids in all fermented coffees. The production of lactic, citric, and succinic acids were detected in all treatments with inoculated strains. Volatile compounds (97) were detected by gas chromatography-mass spectrometry (GC-MS). The strains *Hanseniaspora uvarum (CCMA 1944), Pichia kluyveri (CCMA1658), Meyerozyma guillermondii (CCMA1737) and Meyerozyma caribbica (CCMA1735)* can be indicated as suitable for fermentation in natural process, due to they showed a better production of alcohols, esters, and pyrazines, and the strains *Hanseniaspora uvarum (CCMA 1944) and Meyerozyma caribbica (CCMA1735)* as suitable for pulped processing fermentation, due to they showed helped to production of pyrazines, and furans.

Keywords: Coffee fermentation, Coffee quality, SIAF, Yeasts.

1. Introduction

The alteration of the sensory profiles of coffee through fermentation by epiphytic microorganisms has been studied because it is associated with the degradation of mucilage to produce metabolites (Silva et al., 2008, 2000; Silva, 2015). The production of microbial metabolites can lead to beneficial (acetic acid, succinic acid, malic acid, esters, alcohols, sugars) or detrimental (acetic acid, butyric acids, toxins, etc) effects on the quality of coffee beans and the sensory characteristics of their beverages (Elhalis et al., 2021a; Hadj Salem et al., 2020; Wang et al., 2019). The impact of the production of metabolites by selected microorganisms as starter cultures has been extensively studied. These starter cultures providing changes in sensory profiles, generating process consistency, fermentation performance reliability, and food security (de Souza et al., 2017; Ferreira and Mendes-Faia, 2020; Gonzalez-Rios et al., 2007a; Martins et al., 2020; Ribeiro et al., 2020; Silva et al., 2008).

The use of yeasts as starter cultures for the production of the metabolite has highlighted the development of aromas and flavors such as caramel, chocolate, herbaceous, yellow fruits, and almonds, and reducing processing time (Bressani et al., 2021a, 2021b, 2020, 2018; Cassimiro et al., 2022; da Mota et al., 2022, 2020; Evangelista et al., 2014; Jimenez et al., 2023), which makes it an important microorganism in fermentation (Martins et al., 2019; Ribeiro et al., 2017a). These starter cultures can be selected according to the coffee varieties, nutritional conditions of the plants, production regions (altitude, ambient temperature), and processing methods, being important parameters in the coffee postharvest (Bressani et al., 2021c; Ribeiro et al., 2017b, 2017a).

Traditionally, three postharvest processes involve fermentation, the natural, pulped, and wet process, and each method has its specifications (Brando and Bando, 2015). However, in recent years, the fermentation method self-induced anaerobiosis fermentation (SIAF) has been implemented in each of the postharvest coffee processes, being the method with the greatest focus of studies in recent years and proving to be positive on the sensory characteristics of coffee (da Mota et al., 2022; Pereira et al., 2021).

Therefore, the objective was to evaluate the fermentative performance of potential starter cultures *M.caribbica* (CCMA 1993, CCMA1950, CCMA1992, CCMA1617, CCMA1735), *H. uvarum* (CCMA1944), *P. kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737), *C. ferigula* (CCMA1647) in the natural and pulped processes of the Acaiá cultivar using metabolomics and sensory methodologies.

2. Material and Methods

Coffee beans (*Coffea arabica*) of the Acaia variety were harvested at an altitude of 838 m at the farm Capoeira Coffee (S 21° 21' 27", W 46° 03' 26") in Areado, Minas Gerais, Brazil.

The beans from the natural process were sanitized, dosed in the bioreactors and inoculated in the farm space, and the beans from the pulped process were harvested and dosed in two high-density polyethylene tanks with a capacity of 50L. The beans from the two processes were transported to the Federal University of Lavras where the pulping process was pulped, sanitized, dosed into the bioreactors and inoculated.

2.1. Yeasts

The yeast strains used as starter culture for fermentation are presented in the table.1 belonging to the Agricultural Microbiology (CCMA) culture collection of the Department of Biology of UFLA, Lavras, MG, Brazil. These yeasts were selected isolated of arabica and canephora coffee, theses data can be seen in the table.1, in addition, these cultures were selected as starter cultures for their ability to produce polygalacturonase and pectin lyase enzymes in previously performed tests (data not shown). The yeasts were reactivated and cultivated according to (Martins et al., 2022). Cells were centrifuged (600 rpm for 5 min) and then resuspended in sterile filtered water (500 ml) and inoculated at 10^7-10^8 CFU /ml in coffee batter.

Table.1. Origin of the strains

ID of the CCMA	Strains	Isolated from	Isolated by
1993	*Meyerozyma caribbica*	*Coffea arabica L*	(Pereira et al., 2021)
1950	*Meyerozyma caribbica*		(Pereira et al., 2021)
1992	*Meyerozyma caribbica*		(Pereira et al., 2021)
1617	*Meyerozyma caribbica*		(Martins et al., 2019)
1737	*Hanseniaspora uvarum*		(Martins et al., 2019)
1658	*Pichia Kluyveri*		(Martins et al., 2019)
1737	*Meyerozyma guillermondii*	*Coffea Canephora P*	(Martins et al., 2019)
1647	*Cytofilobasidium ferigula*	*Coffea arabica L*	(Martins et al., 2019)
1735	*Meyerozyma caribbica*	*Coffea Canephora P*	(Martins et al., 2019)

2.2. Coffee fermentation processing

The natural and pulped coffee (4.5 L) was processed in cylindrical polyethylene bioreactors with a capacity of 5 L using the SIAF method. Before fermentation, the beans were placed in the bucket with a liquid solution of peracetic acid (PAA) at a concentration of 520 ppm for 15 min.

The Inoculated and control treatments were performed in duplicate. Samples were taken every 72 h until reached 144 h and 168 h of fermentation, the samples were frozen (-80) for later analysis. The temperature, pH and soluble solids was measured with a manual analog thermometer, pH tape and refractometer. The coffee was dried in the sun on suspended terraces until reaching 11% moisture.

2.3. Microbial analysis

The treatments inoculated were monitored by planting in the YEPG agar [in g/L: yeast extract 10 (HiMedia), glucose 20 (Dinamica), peptone 20 (HiMedia), and agar 20 (HiMedia)]. Coffee samples (2 g) were added to falcon tubes and homogenized with 18 mL peptone water (1 g/L bacteriological peptone, HiMedia), vortex during 3 min, and ten-fold dilutions were prepared, the sample were incubated for 24 h at 27°C (Martins et al., 2020).

In the treatments control of both processing were same plating process was carried out as in the inculcated treatments, however in the YEPG agar at pH 3.2 for bacteria inhibition. Morphological characteristics were evaluated for each morphotype, and the square root of the number of colonies was purified (Vilela et al., 2010). The phenotypic characterization was evaluated by microscope, and the bacterial colonies were performed using Gram staining (Holt, 1994). Purified isolates were stored in the same broth culture media used for plating at −80 °C containing 20% glycerol (w/w) (Martins et al., 2020).

2.4. Analysis of protein profile: MALDI-TOF

Microbial identification and statistical clustering of the isolates were evaluated by MALDI-TOF MS microflex LT spectrometer (Bruker Daltonics, Bremen, Germany). Bacteria and yeast were isolated from coffee in the 0 h, 72, 120, 144 and 168 h fermentation time. The isolates were grown for 24 h on plates using culture medium specific to each taxonomic group (YEPG agar for yeasts and nutrients Agar for bactria). For yeasts and bacteria, a cell mass from the culture plate was added to a tube containing 6 μL of an organic solution (ethanol/acetonitrile/trifluoroacetic acid (10%), 1:1:1).Both samples were vortexed for 30 s, and just yeasts were sonicated for 5 min. The supernatant (1 μL) was added onto the MALDI-TOF stainless steel plate. When the samples were almost dried, 1 μL of the matrix solution a-

cyano-4-hydroxycinnamic acid (CHCA) previously prepared to a final concentration of 10 mg CHCA/ml of organic solution was added and gently mixed. Samples were dried at room temperature and analyzed by MALDI-TOF MS. Each isolate was analyzed in triplicate to measure the quality and reproducibility of the spectra. Calibration was performed with the strain *Escherichia coli K12* as described by (Ferreira, 2014; Lima-Neto et al., 2014). E. coli were grown for 18 h in Luria-Bertani agar medium (LB) and incubated at 37 °C for 18 h. The mass spectra were processed with the MALDI Biotyper 3.0 software (Bruker Daltonics) (Lima-Neto et al., 2014).

2.5. Determination of carbohydrate and organic acids analysis by high-performance liquid chromatography (HPLC)

Organic acids (malic, lactic, acetic, citric, succinic, and tartaric acids), fermentable carbohydrates (sucrose, fructose, and glucose) and ethanol were analyzed during fermentation (0, 72, 120, and 144 h) by HPLC (Shimadzu, model LC-10Ai, Shimadzu Corp., Japan) equipped with a dual detection: UV detector (SPD 10Ai) and a refractive index detector (RID-10Ai).

The organic acid were analysed in Shimpack SCR – 101H column (7.9 mm x 30 cm) using UV detector at 210 nm. The operating conditions were according to (Evangelista et al., 2014). The carbohydrate were analysed in Shim-pack SCR-101C column operated at 80 °C, with ultrapure water used as the eluent at a flow rate of 0.6 mL/min (da Mota et al., 2022). The carbohydrates were detected by RID detector.

The chemical compound were extracted from three grams were mixed with 5 mL of Mili-Q water for 10 min, and the fluids were centrifuged 10,000 x g for 10 min at 4 °C two times. Only for acids analysis, the supernatant pH was adjusted to 2.11 with perchloric acid (200 mM) solution and re-centrifuged under the same conditions. After that, the supernatant was filtered through a 0.22 μm cellulose acetate membrane. The sample concentrations were determined using an external calibration method, and calibration curves were constructed by injecting different standards under the same conditions as the samples.

2.6. Volatile compounds

Headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME) was used to extract volatile compounds from samples of green coffee (final fermentation) and roasted coffee. For analysis were taken in vials, 2 gr of each sample previous crushed with presence of liquid nitrogen.

The compounds were analyzed using the gas chromatograph-mass spectrometer QP2010 equipped with mass spectrometry (GC–MS) (Shimadzu) and a silica capillary Carbo-Wax 20 M (30 m × 0.25 mm × 0.25 mm) column. Operating conditions were performed as described by (Bressani et al., 2020).

The mass spectra of each compound detected were compared with the NIST11 library. The alkane series (C14–C22) was used to calculate the Kovats retention index (RI). The internal standard (4-Nonanol; 12.5 g/L) was added to each sample.

2.7. Sensory evaluation

The samples were prepared according to Specialty Coffee Association (SCA) (SCAA, 2015) methodology and were analyzed by four trained coffee tasters with Q-Grader certificates.

The data recording of the samples was carried out according to da Mota et al. (2020), where the following attributes were evaluated: flavor, taste, body, sweetness, acidity, defects, and final note, and an unstructured scale was used to evaluate the intensity of sweetness, acidity, body, and aftertaste.

2.8. Statistical Analysis

The experiment was conducted in a completely randomized design. The microbial growth, organic acids, and carbohydrates in each process was evaluated in a factorial arrangement with nine yeast and control by four fermentation times (0, 72, 120, and 144 h). Three repetitions were performed, totaling 20 experimental units. Data were analyzed statistically using variance analysis (ANOVA) followed by the Scott-Knott test ($p \leq 0.05$) to determine significant differences among treatments in Sisvar version 5.8.92 (Ferreira, 2014).

The principal component analysis was run between volatile compounds, green and roasted Berries, using R version 4.2.2.

3. Results

3.1. Physicochemical and microbial analysis

All treatments ranged between 20 and 22.25 °C during fermentation, and the highest temperatures occurred between 72 and 120 h in the two processes (Supplementary Material – Table. C1). The treatments inoculated that presented the highest temperature during fermentation were the treatments inoculated with *M. caribbica* (CCMA1993 and CCMA1992) and *C. ferigula* (CCMA1647) with value of 22.25 °C during 72 to 120 h of fermentation in both processes in compared to other treatments that present a temperature of 22° during the same fermentation time.

The highest consumption of soluble solids occurred in the pulped process (Supplementary material – Table C1). The soluble solids value at the final of the pulped process fermentation ranged between 6.65 and 8.70 °Brix by *M. caribbica* (CCMA 1992) and *M. caribbica* (CCMA 1993), respectively (Supplementary material-Table C1). In the natural process the final values oscillated between 11.60 and 8.2 °Brix represented in the *M. caribbica* (CCMA1735) and *C. ferigula* (CCMA1647).

The pH values did not show a difference between processes (Supplementary material – Table C1). In the pulped and natural process reached 3.68 and 3.72, respectively, in *Meyerozyma caribbica* (CCMA1950) fermentation.

Higher microbial population were detected to *P. kluyveri* (CCMA1658) (8.53 Log_{10} CFU/mL) and *M. guillermondii* (CCMA1737) (8.49 Log_{10} CFU/mL) (Fig 1) in pulped coffee and *H. uvarum* (CCMA 1944) (8.38 Log_{10} CFU/mL) in natural coffee (Fig 2).

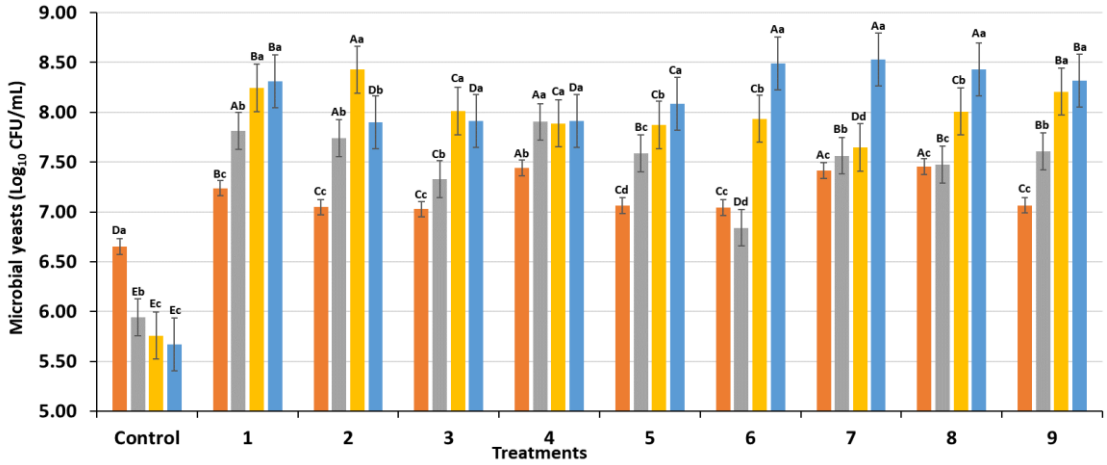

Figure 1. Mean for yeasts density during pulped processing; Followed by the same uppercase (Treatment) and lowercase (Fermentation time) letter did not differ from each other by the Scott-Knott ($p \leq 0.05$); Effect of strains ($p<0.001$); effect of times ($p<0.001$); Interation between strains and times ($p<0.001$); 1: *Meyerozyma caribbica* (CCMA1993); *2: Meyerozyma caribbica* (CCMA1950); *3: Meyerozyma caribbica* (CCMA1992); *4: Meyerozyma caribbica* (CCMA1617); *5: Hanseniaspora uvarum* (CCMA 1944); *6: Pichia Kluyveri* (CCMA1658); *7: Meyerozyma guillermondii* (CCMA1737); *8: Cystofilobasidium ferigula* (CCMA1647); *9: Meyerozyma caribbica* (CCMA1735). 0 hours ▭, 72 hours ▭, 120 hours ▭,144 hours ▭.

In general, all the strains showed adaptation during the fermentation processes, however each strain showed growth differentiation. This behavior can be seen in the graphs Fig 1 and Fig 2.

All the inoculated strains showed growth after the start of fermentation in the pulped process. In comparison to the others strains, *P. kluyveri* (CCMA1658) started growing after 72 hours. Only *M. caribbica* (CCMA1950 and CCMA1992) decreased at the final of

fermentation. *M. caribbica* (CCMA1617) was the strain that not exceeding 8.00 Log_{10} CFU/mL.

In the natural process, *M. caribbica* (CCMA1617), *H. uvarum* (CCMA 1944), *M. guillermondii* (CCMA1737) and *C. ferigula* (CCMA1647) showed a higher growth at 72 h of fermentation (Fig 2).

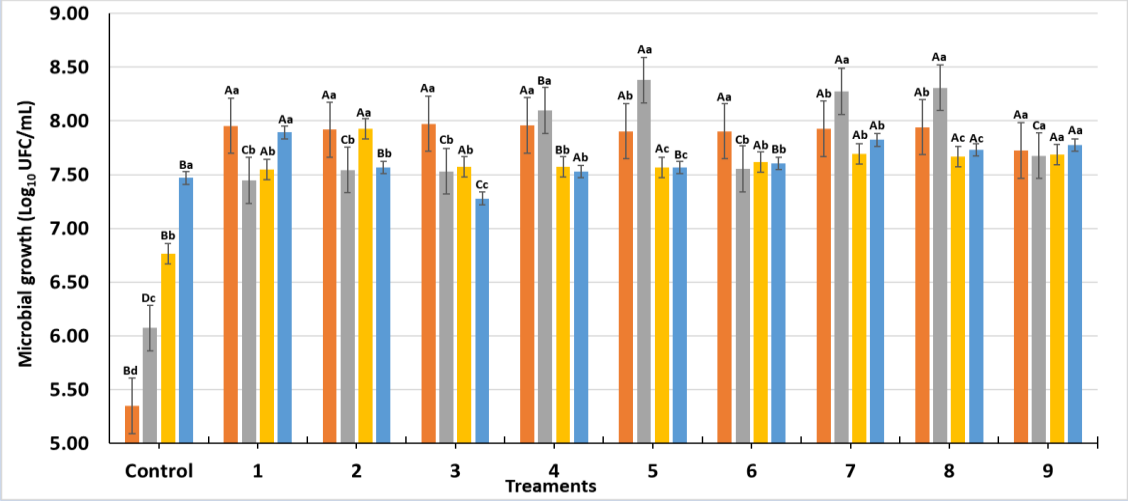

Figure 2. Mean for yeasts density microbial during natural processing. Followed by the same uppercase (Treatment) and lowercase (Fermentation time) letter did not differ from each other by the Scott-Knott ($p \leq 0.05$); Effect of strains ($p<0.001$); effect of times ($p<0.001$); Interation between strains and times ($p<0.001$); 1: *Meyerozyma caribbica* (CCMA-1993); *2: Meyerozyma caribbica* (CCMA1950); *3: Meyerozyma caribbica* (CCMA1992); *4: Meyerozyma caribbica* (CCMA1617); *5: Hanseniaspora uvarum* (CCMA 1944); *6: Pichia Kluyveri* (CCMA1658); *7: Meyerozyma guillermondii* (CCMA1737); *8: Cystofilobasidium* (CCMA1647); *9: Meyerozyma caribbica* (CCMA1735). 0 hours ▭, 72 hours ▭, 120 hours ▭,144 hours ▭.

3.2. Microbial isolation and Analysis of protein profile: MALDI-TOF

A total of 216 isolates were obtained, of which 151 were yeasts (47 in the natural process; 104 in the pulped process) and 65 bacteria (32 in the natural process; 33 in the pulped process).

The yeasts identified throughout the fermentation at different times were *Candida krusei, Candida orthopsilosis, Candida dubliniensis, Candida albicans, Saccharomyces cerevisiae, Wickerhamomyces anomalus, Candida guilliermondii, Torulaspora delbrueckii, Candida glabrata, Candida carpophila, Kazachstania unispora*.

The bacteria identified throughout the fermentation at different times were *Weissella cibaria, Lactobacillus plantarum, Streptococcus uberis, Enterococcus faecium, Lactobacillus mali, Cryptococcus liquefaciens, Klebsiella pneumoniae, Lactobacillus pentosus,*

Lactobacillus oris, Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus paralimentarius, Proteus mirabilis.

Yeasts showed a higher number of species at the beginning of fermentation in both processes. In the natural process, yeasts such as *Candida krusei, Candida orthopsilosis, Candida dubliniensis, Candida albicans, Saccharomyces cerevisiae, Wickerhamomyces anomalus, Candida guilliermondii, Torulaspora delbrueckii, Candida glabrata and Candida carpophila* were identified, and pulped process, yeasts such as *Wickerhamomyces anomalus, Candida guilliermondii, Torulaspora delbrueckii, Candida glabrata and Candida carpophila* were identified. In the 72 hours of fermentation the natural process not showed yeasts growth, but, the pulped process if showed growth of candida glabrata. During 120 hours of fermentation were identified species being *Kazachstania unispora, Wickerhamomyces anomalus, Torulaspora delbrueckii, Candida glabrata* were identified in the natural process, and in the pulped process were identified yeasts such as *Saccharomyces cerevisiae, Candida glabrata*. In the last fermentation time the natural process only showed *Saccharomyces cerevisiae* growth, and the pulped process only showed *Candida glabrata* growth.

The bacteria showed a lower number of species during the beginning of the fermentation. In the natural process, *Enterococcus faecium and Lactobacillus* mali were identified, and in the pulped process, only *Weissella cibaria* was identified. In the 72 hours of fermentation in the natural process, *Crytococcus liquefaciens, Lactobacillus plantarum and Klebsiella pneumoniae* were identified, and the pulped process did not show bacterial growth. During at 120 hours of fermentation in the natural process bacteria such as *Streptococcus uberis, Lactobacillus pentosus, Lactobacillus oris, Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum* was identified.

3.3. Evaluation of organic acids, ethanol, and carbohydrates

In both processes, carbohydrates such as sucrose, glucose and fructose were identified (Table.2). These compounds were slightly higher in the pulped process compared to the natural process.

Sucrose in both processes was the carbohydrate with the lowest concentration compared to glucose and fructose, as can be seen in the table.2. Glucose and fructose were consumed at different times in each process. The pulping process showed consumption of these compounds during 72 h, and the natural one during 168 h. Also, a slight increase in all carbohydrates can be highlighted during 144 h in the pulped process and 72 h in the natural process.

During fermentation, citric, malic, succinic, tartaric, and acetic acids were identified in both processes in all treatments (Table. 3). Tartaric was produced after 72 h of fermentation in natural coffee (Table. 3). Lactic and succinic acid were the compounds that increased during fermentation in both processes. The increase of these compounds varied between 72 and 144 h for the pulped coffee and 168 h for the natural coffee (Table.3). Acetic acid showed highest concentration at the beginning and decreased during fermentation in all treatments in both processes (Table. 3).

3.4. Analyzes of volatile compounds

Green and roasted coffee beans have different profiles of volatile compounds. In the pulped coffee, 97 volatile compounds were detected and 118 in the natural process (Supplementary Material – Table C2 and C3).

The volatile compounds were classified as acids (6 pulped, 7 natural), pyrroles (5 pulped, 6 natural), aldehydes (11 pulped, 14 natural), furans (14 pulped, 18 natural), esters (1pulped, 16 natural), ketones (8, pulped, 6 natural), pyrazines (13 pulped, 14 natural), phenols (10 pulped, 9 natural), lactones (4, pulped), pyrimidine (1 pulped, 1 natural), pyridine (7 pulped, 9 natural), pyrans (1natural), alcohols (10 pulped, 11 natural), thiophene (1 pulped, 1 natural), and other (6 pulped, 5 natural).

The natural process presented the highest number of alcohols, esters, pyrazines and furans compared to the pulping process (Supplementary Material – Table C2 and C3). Furthermore, Fig.3 shows the compounds that were only identified in the natural process and were not identified in the pulping process, as well as the compounds that were only identified in the pulping process and were not identified in the natural process. Fig.3 also shows the concentrations provided by each inoculated treatment and control.

The green beans of both processes showed correlation by aldehydes, alcohols, esters, and others and roasted beans by pyrazines and furans (Fig. 4). The principal component analysis explain 90.9% (Pulped) and 97.8% (Natural) of the variation of datas (Fig.4).

The aldehydes such as 2,4-heptadienal, (E,E)-, 2,4-decadienal, (E,E)-, 2,4-decadienal, (E,E)-2-thiophenecarboxaldehyde and phenylethyl alcohol were identified in the pulping process. Most of the aldehydes, alcohols and esters from both processes were identified in the green beans. Aldehyde such as 5-acetoxymethyl-2-furaldehyde was identified in roasted sample of the *Meyerozyma caribbica* (CCMA1617), *H. uvarum* (CCMA 1944), *P. kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737), *C. ferigula* (CCMA1647),

Table.2. Concentration of carbohydrates (g/L)

Natural					
Strains	Time	g/L			
		Sacarose	Fructose	Glicose	Ethanol
Contole	0	N/D	6.377Aa	7.138Aa	5.528Ab
	72	0.303Aa	4.259Bc	3.605Cc	5.493Cb
	120	N/D	5.317Ab	4.947Bb	11.084Aa
	144	N/D	1.284Ad	1.719Ad	10.906Aa
1	0	0.210Ab	0.557Bb	1.105Ba	3.703Bb
	72	0.294Aa	0.791Ga	1.162Ga	7.474Ba
	120	0.223Ab	0.312Dc	0.308Fb	6.583Ea
	144	0.209Ab	N/D	0.276Cb	7.130Ca
2	0	0.210Aa	0.557Bc	1.105Bc	3.703Bc
	72	N/D	5.346Aa	5.502Aa	5.725Cb
	120	N/D	5.324Aa	5.341Aa	7.349Ea
	144	N/D	1.127Ab	1.765Ab	8.001Ca
3	0	0.210Aa	0.557Bb	1.105Ba	3.703Bb
	72	0.202Ca	0.891Ga	1.176Ga	10.37Aa
	120	N/D	0.507Cb	0.615Eb	9.787Ba
	144	N/D	N/D	0.271Cc	9.273Ba
4	0	0.210Aa	0.557Bb	1.105Bb	3.703Bc
	72	N/D	1.667Ea	2.070Ea	7.258Bb
	120	0.218Aa	0.659Cb	0.745Ec	8.008Db
	144	N/D	N/D	0.281Cd	9.252Ba
5	0	0.210Aa	0.557Bb	1.105Bb	3.703Bc
	72	N/D	1.386Fa	1.937Ea	7.994Bb
	120	N/D	0.634Cb	0.778Ec	8.16Db
	144	0.187Aa	0.275Bc	0.295Cd	10.106Aa

Pulped					
Strains	Time	g/L			
		Sacarose	Glicose	Frutose	Ethanol
Controle	0	0.000Bd	1.411Aa	1.945Aa	3.266Bc
	72	0.362Bb	0.373Ab	0.247Cb	4.028Ab
	120	0.183Dc	0.211Ab	0.153Dc	4.493Aa
	144	0,388Ca	0.343Bb	0.189Cd	3.386Bc
1	0	0.210Ac	0.557Aa	1.105Ba	3.703Aa
	72	0.247Db	0.256Aa	0.169Eb	3.258Dc
	120	0.233Cb	0.289Aa	0.127Ed	3.542Cb
	144	0.354Da	0.298Ba	0.158Dc	3.291Bc
2	0	0.210Ad	0.557Ab	1.105Ba	3.703Aa
	72	0.310Cb	0.471Ab	0.435Bb	3.441Cb
	120	0.274Bc	0.340Ab	0.322Ac	3.533Cb
	144	0.419Ba	1.535Ba	0.303Ad	3.083Bc
3	0	0.210Ab	0.557Aa	1.105Ba	3.703Ab
	72	0.191Ec	0.296Aa	0.190Dc	3.965Aa
	120	0.186Dc	0.362Aa	0.223Bb	3.649Cb
	144	0.259Ga	0.357Ba	0.197Cc	3.352Bc
4	0	0.210Ad	0.557Aa	1.105Ba	3.703Ab
	72	0.465Ab	0.300Aa	0.172Ec	3.203Dc
	120	0.488Aa	0.347Aa	0.211Bb	3.489Cb
	144	0.280Fc	0.312Ba	0.167Dc	3.843Aa
5	0	0.210Ab	0.557Aa	1.105Ba	3.703Aa
	72	0.175Fc	0.224Ab	0.238Cb	3.270Db
	120	0.166Ec	0.227Ab	0.143Dd	3.287Db
	144	0.457Aa	0.297Bb	0.175Dc	3.239Bb

Continution. Table.2. Concentration of carbohydrates (g/L)

Natural					
Strains	Time	g/L			
		Sacarose	Glicose	Frutose	Ethanol
6	0	0.210Aa	0.557Bc	1.105Bb	3.703Bc
	72	0.240Ba	1.526Ea	1.647Fa	9.638Ba
	120	N/D	1.080Bb	1.508Ca	7.279Eb
	144	N/D	0.215Bd	0.290Cc	7.611Cb
7	0	0.210Aa	0.557Bc	1.105Bc	3.703Bc
	72	N/D	2.496Da	2.966Da	6.880Bb
	120	N/D	1.052Bb	1.693Cb	8.965Ca
	144	0.122Bb	0.265Bd	0.267Cd	8.087Ca
8	0	0.210Aa	0.557Bb	1.105Bb	3.703Bc
	72	N/D	1.384Fa	1.646Fa	7.497Bb
	120	N/D	0.532Cb	0.791Ec	9.151Ca
	144	N/D	N/D	0.277Cd	9.225Ba
9	0	0.210Aa	0.557Bc	1.105Bb	3.703Bc
	72	N/D	3.706Ca	3.985Ba	5.051Cb
	120	N/D	0.851Bb	1.178Db	3.800Fc
	144	0.200Aa	0.367Bc	0.722Bc	7.543Ca

Pulped					
Strains	Time	g/L			
		Sacarose	Glicose	Frutose	Ethanol
6	0	0.210Ab	0.557Aa	1.105Ba	3.703Aa
	72	0.192Ec	0.288Aa	0.173Eb	3.315Db
	120	0.175Ec	0.203Aa	N/D	3.595Cc
	144	0.308Ea	0.375Ba	0.168Db	3.129Ca
7	0	0.210Ac	0.557Aa	1.105Ba	3.703Aa
	72	0.247Db	0.297Aa	0.149Fc	3.781Ba
	120	0.251Cb	0.250Aa	0.142Dc	3.535Cb
	144	0.371Da	0.310Ba	0.174Db	3.334Bc
8	0	0.210Ac	0.557Aa	1.105Ba	3.703Aa
	72	0.202Ec	0.223Aa	0.175Ec	3.561Cb
	120	0.236Cb	0.220Aa	0.178Cc	3.499Cc
	144	0.399Ca	0.355Ba	0.259Bb	3.105Cd
9	0	0.210Ac	0.557Aa	1.105BA	3.703Ab
	72	0.234Db	0.612Aa	0.512Ab	3.452Cc
	120	0.196Dc	0.429Aa	0.316Ac	3.93BCa
	144	0.313Ea	0.369Ba	0.171Dd	3.413Bc

Nota: Followed by the same uppercase (Treatment) and lowercase (Fermentation time) letter did not differ from each other by the Scott-Knott ($p \leq 0.05$); Effect of strains in the natural ($p<0.001$); effect of times in the natural ($p<0.001$); Interaction between strains and times in the natural ($p<0.001$); Effect of strains in the pulped ($p<0.001$); effect of times in the pulped ($p<0.001$); Interaction between strains and times in the pulped ($p<0.001$); 1: *Meyerozyma caribbica* (CCMA 1993); *2: Meyerozyma caribbica* (CCMA1950); *3: Meyerozyma caribbica* (CCMA1992); *4: Meyerozyma caribbica* (CCMA1617); *5: Hanseniaspora uvarum* (CCMA 1944); *6: Pichia Kluyveri* (CCMA1658); *7: Meyerozyma guillermondii* (CCMA1737); *8: Cystofilobasidium ferigula* (CCMA1647); *9: Meyerozyma caribbica* (CCMA1735).

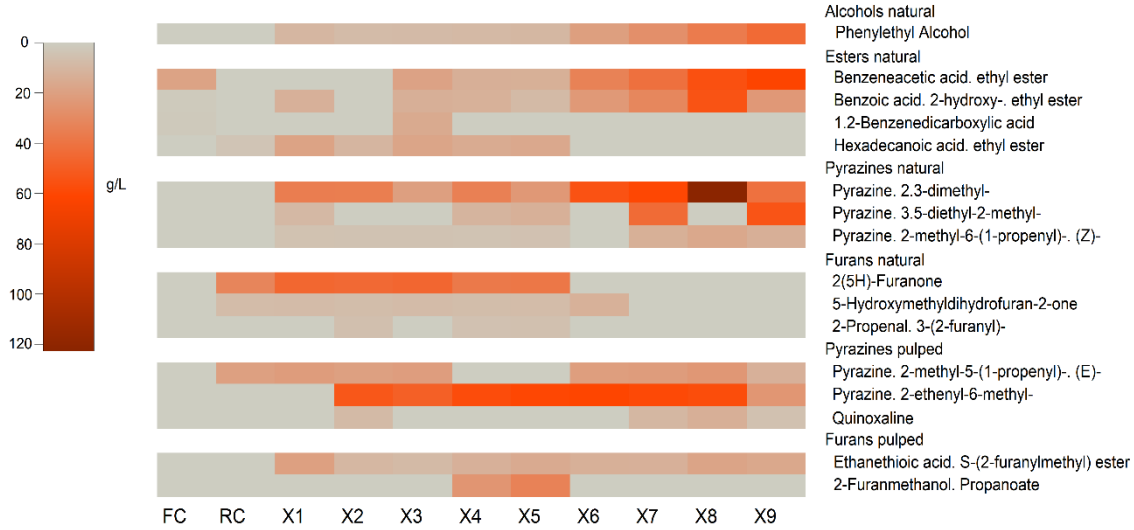

Figure.3. Volatile compounds identified only in each processing and their concentration in each treatment; FC: Green control; RC: toasted control; X1: *Meyerozyma caribbica* (CCMA 1993); X2*: Meyerozyma caribbica* (CCMA1950); X3*: Meyerozyma caribbica* (CCMA1992); X4*: Meyerozyma caribbica* (CCMA1617); X5*: Hanseniaspora uvarum* (CCMA 1944); X6*: Pichia Kluyveri* (CCMA1658); X7*: Meyerozyma guillermondii* (CCMA1737); X8*: Cystofilobasidium ferigula* (CCMA1647); X9*: Meyerozyma caribbica* (CCMA1735).

M. caribbica (CCMA1735) of the pulped process. On the other hand, benzeneacetaldehyde, .alpha.-ethylidene- was identified in both process in all treatment.

The alcohol such as 1,6-octadien-3-ol, 3,7-dimethyl-alcohol remained after roasting in all the samples. Phenylethyl Alcohol was only identified in the roasted sample, in the natural process.

The ester methyl salicylate ester was identified in the pulped process in *M. caribbica* (CCMA 1993), *M. caribbica* (CCMA1617); *H. uvarum* (CCMA 1944), *P. Kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737), *M. caribbica* (CCMA1735), also being identified in all treatments of the natural process. In addition, the esters such as benzeneacetic acid, ethyl ester; benzoic acid, 2-hydroxy-, ethyl ester; 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; 1,2-benzenedicarboxylic acid; bis(2-methylpropyl) ester; hexadecanoic acid, ethyl ester; hexadecanoic acid, methyl ester; cyclobutanecarboxylic acid, 4-tridecyl ester; and pentadecanoic acid, 14-methyl-, methyl ester only were detected in pulped coffee process (Supplementary Material – Table C2 and C3).

Pyrazines and furans are frequently found as compounds associated with roasted samples. The pyrazines with higher concentrations in green beans were pyrazine, 2-ethenyl-6-methyl. Pyrazine, 2-ethenyl-6-methyl- was detected in the treatments inoculated with *M. caribbica* (CCMA1950, CCMA1992, CCMA1617, CCMA1735), *H. uvarum* (CCMA 1944), *P. Kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737) and *C. ferigula* (CCMA1647) and

Table. 3. Organic acids concentration (g/L)

Natural					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
Control	Citric	0.173Ba	0.019Bb	0.027Cb	0.019Db
	Tartaric	0.501Aa	0.095Eb	0.109Db	0.024Ec
	Malic	0.418Aa	0.159Bb	0.084Ac	0.024Ad
	Succinic	0.999Ab	0.616Dc	1.274Da	1.185Da
	Lactic	0.365Ad	0.671Ec	1.017Fb	1.810Da
	Acetic	4.059Aa	1.115Bb	1.074Ab	0.462Bc
1	Citric	0.463Aa	0.095Ab	0.041Cc	0.067Dc
	Tartaric	N/D	0.093Ea	0.069Ea	0.085Da
	Malic	0.061Ba	0.008Db	N/D	N/D
	Succinic	0.306Bd	0.844Ca	0.463Gc	0.679Fb
	Lactic	0.142Ad	1.421Dc	1.567Eb	2.207Ca
	Acetic	0.975Ba	0.340Fb	0.095Gc	0.104Ec
2	Citric	0.463Aa	0.048Bc	0.085Bc	0.123Bb
	Tartaric	N/D	0.377Aa	0.422Aa	0.381Aa
	Malic	0.061Bc	0.153Ba	0.095Ab	0.025Ad
	Succinic	0.306Bd	0.869Cc	1.448Cb	2.552Ba
	Lactic	0.142Ad	1.327Dc	2.210Cb	3.234Ba
	Acetic	0.975Ba	0.837Cb	0.340Dc	0.208Dd
3	Citric	0.463Aa	0.044Bc	0.05Cc	0.140Bb
	Tartaric	N/D	0.234Ca	0.258Ba	0.181Cb
	Malic	0.061Ba	0.014Db	N/D	N/D
	Succinic	0.306Bd	0.771Cb	0.747Fb	0.964Ea
	Lactic	0.142Ac	1.600Cb	1.786Db	2.248Ca
	Acetic	0.975Ba	0.646Db	0.355Dc	0.340Cc

Pulped					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
Control	Citric	0,100Bb	0,167Ca	0,016Cb	0,212Da
	Tartaric	0,180Aa	N/D	0,005Cb	N/D
	Malic	0,124Aa	0,009Cb	N/D	0,014Db
	Succinic	0,267Aa	0,403Ca	0,306Ba	0,268Ba
	Lactic	0,076Ab	0,410Db	0,919Ca	0,709Ba
	Acetic	1,207Aa	0,089Cb	0,137Ab	0,129Cb
1	Citric	0,463Ab	0,029Dc	0,005Cc	0,888Aa
	Tartaric	N/D	0,008Fa	0,005Cb	N/Db
	Malic	0,061Ba	N/D	N/D	0,027Cb
	Succinic	0,306Aa	0,223Da	0,174Ca	0,154Ba
	Lactic	0,142Ab	0,583Ca	0,628Da	0,490Ba
	Acetic	0,975Ba	0,093Cc	0,102Ac	0,221Bb
2	Citric	0,463Aa	N/D	0,086Cb	0,163Db
	Tartaric	N/D	0,173Aa	N/D	N/D
	Malic	0,061Ba	N/D	0,008Cc	0,041Bb
	Succinic	0,306Ab	0,032Ec	0,592Aa	0,533Aa
	Lactic	0,142Ab	0,127Db	1,108Ca	0,746Ba
	Acetic	0,975Ba	0,404Ab	0,088Ac	0,075Cc
3	Citric	0,463Aa	0,001Db	0,044Cb	0,117Db
	Tartaric	N/D	0,023Ba	0,012Ab	0,003Cc
	Malic	0,061Ba	N/D	0,004Cc	0,017Db
	Succinic	0,306Aa	0,218Da	0,205Ca	0,294Ba
	Lactic	0,142Ad	0,636C	0,921Cb	1,394Aa
	Acetic	0,975Ba	0,108Cc	0,159Ac	0,248Bb

Continution. Table.3. Organic acids concentration (g/L)

Natural					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
4	Citric	0.463Aa	0.063Bb	0.074Bb	0.082Cb
	Tartaric	N/D	0.273Ca	0.129Dc	0.224Cb
	Malic	0.061Ba	0.040Cb	0.015Cc	0.005Bc
	Succinic	0.306Bc	0.891Ca	0.745Fb	0.682Fb
	Lactic	0.142Ac	1.234Db	1.426Eb	2.199Ca
	Acetic	0.975Ba	0.861Cb	0.402Cc	0.205Dd
5	Citric	0.463Aa	0.039Bc	0.052Cc	0.276Ab
	Tartaric	N/D	0.378Aa	0.218Cb	0.198Dc
	Malic	0.061Ba	0.037Cb	0.015Cc	0.006Bc
	Succinic	0.306Bd	1.487Ac	1.694Bb	3.692Aa
	Lactic	0.142Ad	1.709Cb	1.365Ec	2.174Ca
	Acetic	0.975Ba	0.428Eb	0.168Fc	0.221Dc
6	Citric	0.463Aa	0.089Ab	0.098Bb	0.087Cb
	Tartaric	N/D	0.320Ba	0.281Ba	0.192Cb
	Malic	0.061Ba	0.023Db	0.017Cb	0.001Bc
	Succinic	0.306Bc	1.242Ba	0.969Eb	1.382Ca
	Lactic	0.142Ad	2.582Ab	1.744Dc	3.056Ba
	Acetic	0.975Ba	0.357Fb	0.236Ec	0.103Ed
7	Citric	0.463Aa	0.090Ab	0.137Ab	0.119Bb
	Tartaric	N/D	0.266Ca	0.296Ba	0.176Cb
	Malic	0.061Ba	0.026Dc	0.038Bb	0.007Bd
	Succinic	0.306Bc	1.223Ba	1.278Da	0.766Fb
	Lactic	0.142Ad	2.244Bc	2.607Bb	3.043Ba
	Acetic	0.975Ba	0.393Eb	0.341Db	0.121Ec

Pulped					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
4	Citric	0,463Aa	0,110Cb	0,185Bb	0,164Db
	Tartaric	N/D	0,008Fa	N/D	N/D
	Malic	0,061Ba	0,016Cb	0,019Bb	0,017Db
	Succinic	0,306Ab	0,502Ba	0,410Ba	0,187Bb
	Lactic	0,142Ab	0,940Ba	1,075Ca	0,701Ba
	Acetic	0,975Ba	0,126Cb	0,203Ab	0,228Bb
5	Citric	0,463Aa	0,002Dc	0,019Cc	0,154Db
	Tartaric	N/D	0,020Ca	0,008Bb	N/D
	Malic	0,061Ba	0,003Dc	0,004Cc	0,024Cb
	Succinic	0,306Aa	0,316Ca	0,230Ca	0,215Ba
	Lactic	0,142Ac	0,780Cb	1,299Ca	0,794Bb
	Acetic	0,975Ba	0,043Cb	0,067Ab	0,083Cb
6	Citric	0,463Aa	0,137Cb	0,153Cb	0,338Ca
	Tartaric	N/D	0,015Da	0,007Bb	N/D
	Malic	0,061Ba	0,003Dc	N/D	0,02Cb
	Succinic	0,306Ab	0,604Ba	0,354Bb	0,227Bb
	Lactic	0,142Ac	1,411Ab	2,407Aa	1,188Ab
	Acetic	0,975Ba	0,06Cb	0,137Ab	0,146Cb
7	Citric	0,463Ab	0,117Cc	0,094Cc	0,653Ba
	Tartaric	N/D	0,013Ea	0,003Db	N/D
	Malic	0,061Ba	0,008Cc	0,005Cc	0,027Cb
	Succinic	0,306Aa	0,353Ca	0,213Cb	0,140Bb
	Lactic	0,142Ab	1,312Aa	1,012Ca	0,932Ba
	Acetic	0,975Ba	0,107Cb	0,113Ab	0,177Bb

Continution. Table.3. Organic acids concentration (g/L)

Natural					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
8	Citric	0.463Aa	0.042Bc	0.082Bb	0.088Cb
	Tartaric	N/D	0.175Da	0.112Db	0.120Db
	Malic	0.061Ba	0.041Cb	0.005Cb	N/D
	Succinic	0.306Bd	0.552Dc	1.038Eb	1.398Ca
	Lactic	0.142Ad	0.705Ec	1.083Fb	1.744Da
	Acetic	0.975Bb	1.388Aa	0.849Bc	0.519Ad
9	Citric	0.463Aa	0.060Bb	0.035Cb	0.040Db
	Tartaric	N/D	0.207Dc	0.259Bb	0.303Ba
	Malic	0.061Bb	0.218Aa	0.043Bc	0.003Bd
	Succinic	0.306Bc	1.507Ab	2.023Aa	1.525Cb
	Lactic	0.142Ad	1.507Cc	3.202Ab	3.909Aa
	Acetic	0.975Ba	0.897Cb	0.319Dc	0.227Dd

Pulped					
Strains	Compounds (g/L)	0 H	72 H	120 H	144 H
8	Citric	0,463Aa	0,541Ba	0,273Bb	0,409Ca
	Tartaric	N/D	N/D	N/D	N/D
	Malic	0,061Ba	0,027Bb	0,034Ab	0,057Aa
	Succinic	0,306Ab	0,463Ba	0,234Cb	0,105Bc
	Lactic	0,142Ab	0,313Db	0,637Da	0,570Ba
	Acetic	0,975Ba	0,237Bc	0,169Ac	0,506Ab
9	Citric	0,463Ab	0,800Aa	0,37Ab	0,552Bb
	Tartaric	N/D	N/D	N/D	N/D
	Malic	0,061Ba	0,043Ab	0,020Bc	0,024Cc
	Succinic	0,306Ac	0,793Aa	0,609Ab	0,406Ac
	Lactic	0,142Ac	0,960Bb	2,021Ba	1,392Ab
	Acetic	0,975Ba	0,143Cb	0,203Ab	0,124Cb

Nota: Followed by the same uppercase (Treatment) and lowercase (Fermentation time) letter did not differ from each other by the Scott-Knott ($p \leq 0.05$); Effect of strains in the natural ($p<0.001$); effect of times in the natural ($p<0.001$); Interaction between strains and times in the natural ($p<0.001$); Effect of strains in the pulped ($p<0.001$); effect of times in the pulped ($p<0.001$); Interaction between strains and times in the pulped ($p<0.001$); 1: *Meyerozyma caribbica* (CCMA 1993); *2: Meyerozyma caribbica* (CCMA1950); *3: Meyerozyma caribbica* (CCMA1992); *4: Meyerozyma caribbica* (CCMA1617); *5: Hanseniaspora uvarum* (CCMA 1944); *6: Pichia Kluyveri* (CCMA1658); *7: Meyerozyma guillermondii* (CCMA1737); *8: Cystofilobasidium ferigula* (CCMA1647); *9: Meyerozyma caribbica* (CCMA1735).

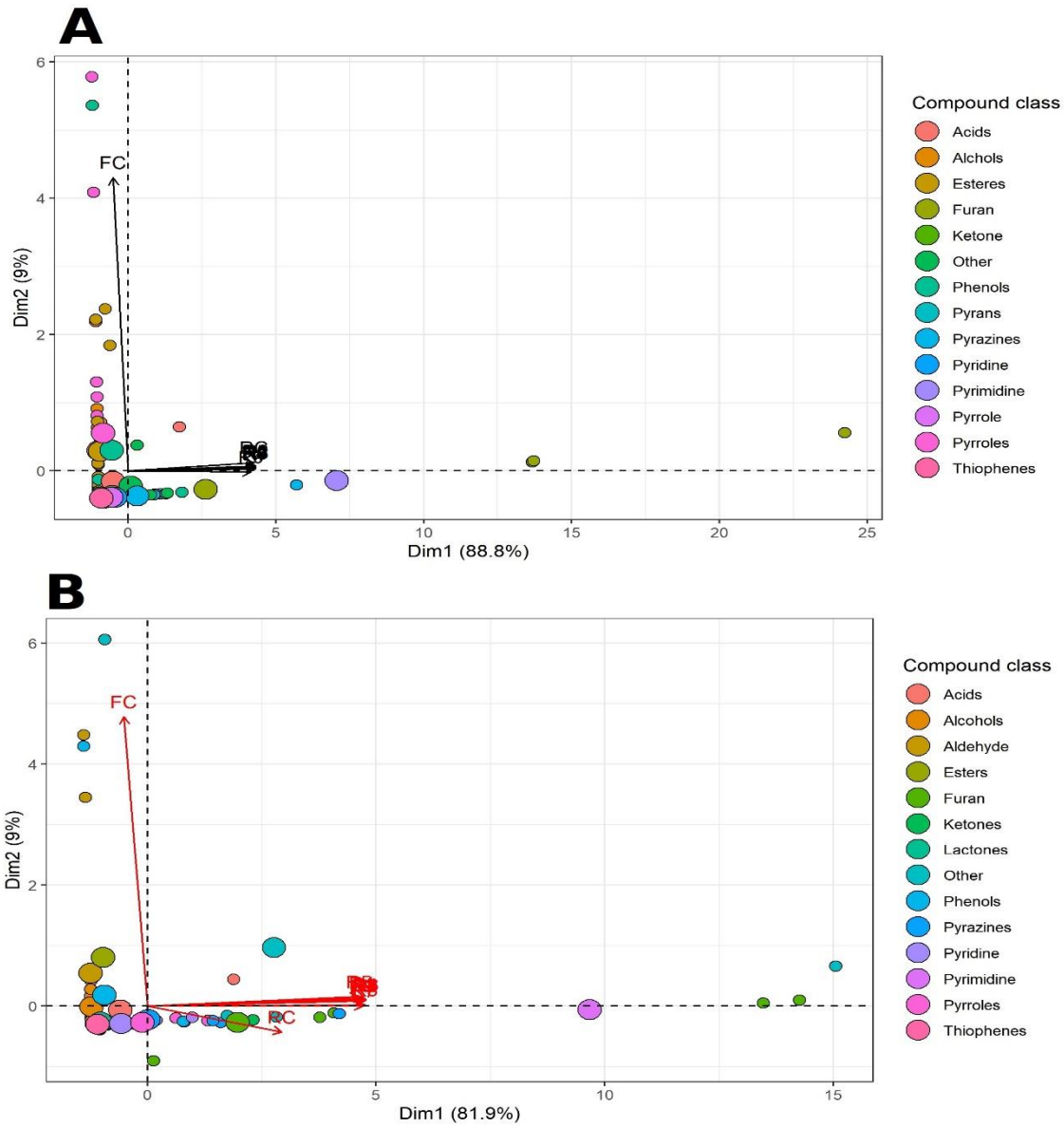

Figure 4. PCA analysis of the volatile group in green and roasted beans; A: Natural coffee process; B. Pulped coffee process. FC: Fermented green control; RC: Roasted control; R1: *Meyerozyma caribbica* (CCMA 1993); R2: *Meyerozyma caribbica* (CCMA1950); R3: *Meyerozyma caribbica* (CCMA1992); R4: *Meyerozyma caribbica* (CCMA1617); R5: *Hanseniaspora uvarum* (CCMA 1944); R6: *Pichia Kluyveri* (CCMA1658); R7: *Meyerozyma guillermondii* (CCMA1737); R8: *Cystofilobasidium ferigula* (CCMA1647); R9: *Meyerozyma caribbica* (CCMA1735).

Quinoxaline was detected in the treatments inoculated with *M. guillermondii* (CCMA1737), *C. ferigula* (CCMA1647), and *M. caribbica* (CCMA1735, CCMA1950).

3.4 Sensory analysis

H. uvarum (CCMA 1944) in pulped coffee showed greater flavor complexity while, *M. caribbica* (CCMA 1993) in pulped coffee and *M. caribbica* (CCMA1735) in natural coffee showed greater aroma complexity. In the pulped process presented sensory descriptors such as

honey, orange, tropical fruits, winey and woody (Fig.5 – Fig.6), while the natural process was described as honey, red fruits, caramelized (Fig.5 – Fig.6).

The pulped process, the aroma presented sensory descriptors such as honey, orange, tropical fruits, vinous and woody, while the natural process was describe as honey, red caramelized, red fruits, and chocolate.

4. Discussion

Not expected the bacteria growth in the treatments, due to the sanitization of beans coffee before of fermentation with peracetic acid, since, peracetic acid is used in different food matrices for inhibition of bacteria and fungal (Hasani et al., 2020; Jung et al., 2023; Lin et al., 2023; Shin et al., 2023; Zhang et al., 2020). In addition, the plating were used YEPG agar with pH at 3.2 for obtained only the number of yeasts population, however was obtained bacterial growth. The bacterial growth only was obtained in the control treatments of each processing, although, bacteria showed growth in YEPG agar with pH at 3.2, These showed lower species number in comparative with yeasts (Fig.3; Fig.4), which leads to suppose that the peracetic acid showed inhibition of bacterial or the bacterial did not growth in cultural medium with pH at 3.2.

In fermentation, microorganisms must adapt to physicochemical changes such as loss of water, decrease in nutrients and pH (Schwan et al., 2012; Silva, 2015), as well as the control of O2, limiting the coffee fermentation process to facultative aerobic microorganisms, due to self-induced anaerobiosis fermentation (SIAF) (da Mota et al., 2022; Pereira et al., 2022), which may explain decline of species yeasts and some bacteria, and the inhibitions of fungi during fermentation for both processes. However, the natural process showed an increase in the number of bacteria species up to 120 h of fermentation.

This result can be explained because the coffee beans in the natural process are not pulped, so they could have a higher microbial load, but this microbial load, it could have been altered during the beginning of fermentation, due to sanitization with peracetic acid, decreasing the microbial load, and it was not identified at 0 and 72 hours of fermentation, but it was at 120 hours of fermentation.

The natural process showed a higher concentration of carbohydrates. Sucrose showed a lower concentration than glucose and fructose in both processes, which can be explained since the concentration of carbohydrates is influenced by the level of fruit maturation and the processing method used (Knopp et al., 2006) However, carbohydrates from both processes showed increases at the final of fermentation and probably was related to energy metabolism

of the seeds in the face of changes in seed moisture and environmental humidity, (Y. Wang et al., 2023), influencing for fresh and floral sensory notes (Toledo et al., 2016).

Chemical compounds may vary according to genetic characteristics, postharvest method, and microbial species found in fermentation (Broissin-Vargas et al., 2018; Carvalho Ferreira et al., 2023; Evangelista et al., 2014; Yeager et al., 2023). Organic acids, such as citric, succinic and lactic acids, were detected in the fermentation as the main organic acids (Table. 3).

The natural process showed a higher concentration of organic acids in comparative with pulped process (Table. 2). *M. caribbica* (CCMA1950), *P. kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737) and *M. caribbica* (CCMA1735) stood out as the highest producers of succinic and lactic acid in this process. However, only *P. kluyveri* (CCMA1658) and *M. caribbica* (CCMA1735) showed production of succinic and lactic acids in the pulped coffee process, as shown in Table. 3.

Succinic acid can be produced by yeast by the tricarboxylic acid (TCA) cycle, the glyoxylate cycle, and amino acid catabolism, being limited by yeast strain genetics, aeration conditions, temperature of fermentation and chemical composition of the medium growth (Ferreira and Mendes-Faia, 2020; Y. Liu et al., 2022), which can explain the difference in the production of succinic acids by strains. Another limitation of the production of succinic acids by yeasts is the consumption of acetic acid as an energy source (Vasserot et al., 2010; Vilela-Moura et al., 2008).

It is known that lactic acid bacteria carry out the decarboxylation of l-malic in lactic acid, but this same process was evidenced in yeasts such as *M. caribbica* (Bressani et al., 2021c; del Mónaco et al., 2014; Sousa et al., 2012; van Wyk et al., 2022; Vicente et al., 2023, 2022), with reduction of malic acid and lactic acid production as also presented in this investigation (Table.3). However, sanitization with peracetic acid did not inhibit all bacteria as observed in the control treatment, and may present a low population of lactic acid bacteria in the inoculated treatments without being detected in the veneer, thus altering the concentration of lactic acid in the samples of the inoculated treatments.

In addition, the strains *M. caribbica* (CCMA1993) and *M. guillermondii* (CCMA1737) showed citric acid production only in the last fermentation time (Table 3). Citric acid is an organic compound that is present in the tricarboxylic cycle and can be consumed or produced by yeasts (Cavallo et al., 2017; Sayın Börekçi et al., 2022; Souza et al., 2014), which supports the production of citric acids by the inoculated yeasts.

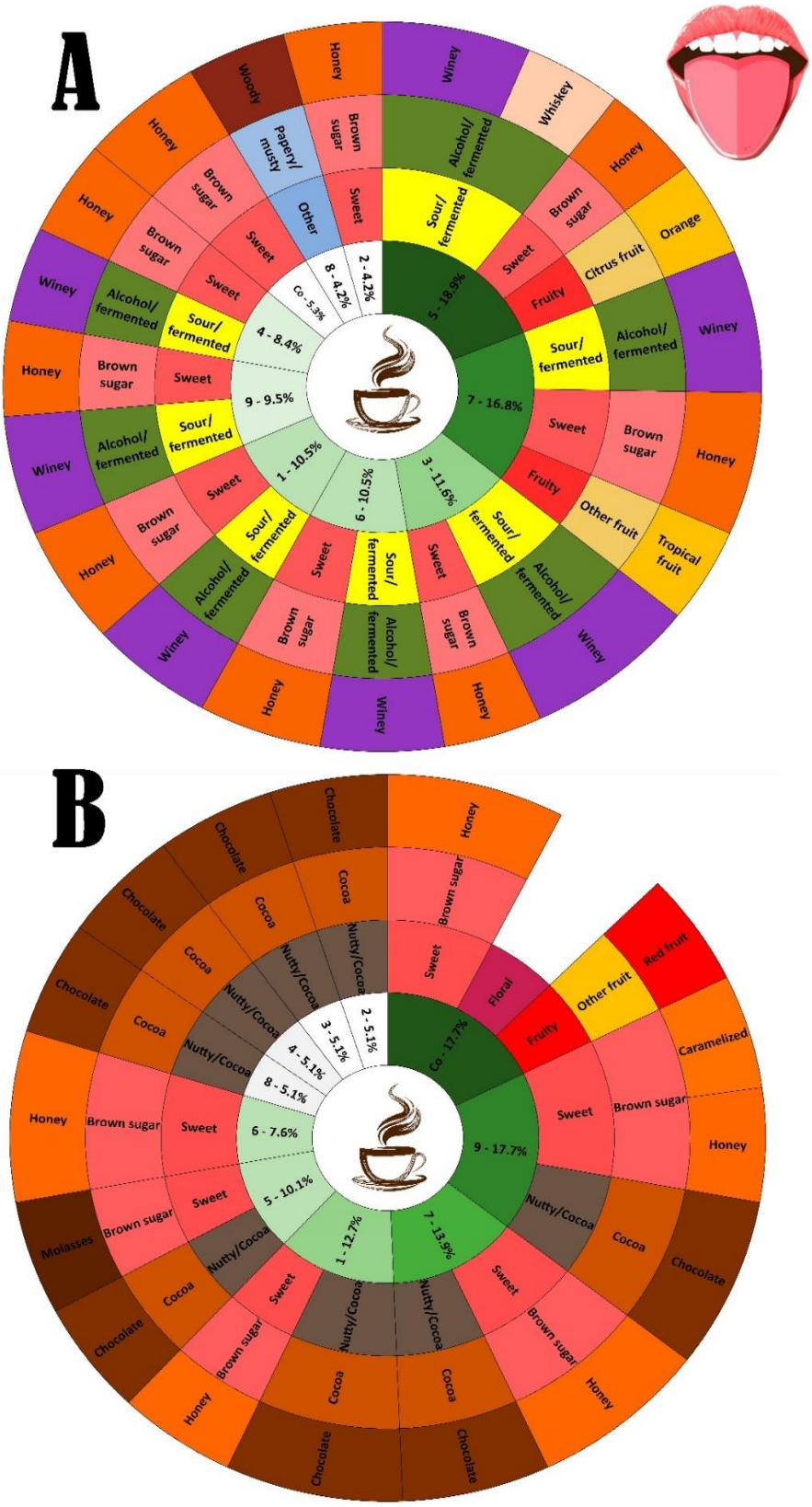

Figure 5. Description of flavor; A: pulped; B: Natural; 1: *Meyerozyma caribbica* (CCMA 1993); 2: *Meyerozyma caribbica* (CCMA1950); 3: *Meyerozyma caribbica* (CCMA1992); 4: *Meyerozyma caribbica* (CCMA1617); 5: *Hanseniaspora uvarum* (CCMA 1944); 6: *Pichia Kluyveri* (CCMA1658); 7: *Meyerozyma guillermondii* (CCMA1737); 8: *Cystofilobasidium ferigula* (CCMA1647); 9: *Meyerozyma caribbica* (CCMA1735).

This acids have a positive impact on the quality of the beverage (Silva et al., 2008; Sunarharum et al., 2014; Vilela et al., 2010) contributing to the acidity of the coffee beverage. The acidity integrated with other sensory parameters modifies beverage perceptions (Sunarharum et al., 2014).

The acid can be used as carbon sources by microorganism (Vasserot et al., 2010; Vilela-Moura et al., 2008) contributing to the decrease in the concentration of some acids such as acetic acid, which in high concentrations promote loss of quality of the beverage (Silva et al., 2008).

The volatile and non volatile composition modify the sensory perception of beverages and become a main component in the acceptance of coffee (Yeretzian et al., 2019). Some volatile compounds are generated from precursors formed during fermentation and transformed during thermally catalyzed reactions (Cao et al., 2023; Gonzalez-Rios et al., 2007b).

The fermentation processes shared some volatile compounds, but some compounds were identified in each process separately as shown in Fig.3. These compounds showed high concentrations of production in the inoculated treatments compared to the without inoculated treatment, which supports the production of volatile compounds by the microorganisms inoculated in each treatment.

Phenylethyl alcohol was the only alcohol identified different between the two process and produced only in the natural process This alcohol is generated by yeast through the metabolism of phenylalanine (Liu, 2015; Michel et al., 2016; Verhagen, 1994), but in the fig.3 can be seen observed that the treatments inoculated showed different concentrations between treatments. The concentration of Phenylethyl alcohol higher of 20 g/L was identified in the treatments inoculated with *P. Kluyveri (CCMA1658), M. guillermondii (CCMA1737), C. ferigula (CCMA1647), M. caribbica (CCMA1735)*. In rest of treatments showed concentration lower to 20 g/L, and the control treatment not showed production of this alcohol, which support the production of Phenylethyl alcohol in different concentrations by the yeasts inoculated. Furthermore, this compound remained in the toasted samples, being able to generate an olfactory sensory perception of rose-honey, being pleasant and imported into the consumer's sensory perception.

Some esters can be in relation with lipid and acetyl-CoA metabolism by the microorganisms, as well as natural compound of plant and formation or denaturation into roasted process.

The esters Benzeneacetic acid. ethyl este; benzoic acid, 2-hydroxy-, ethyl ester (ethyl salicylate); 1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester and the hexadecanoic acid. ethyl ester were differential compounds between the two processing, being produced only by the natural process (Fig.3).

The esters Benzeneacetic acid. ethyl ester and benzoic acid, 2-hydroxy-, ethyl ester (ethyl salicylate) were compound with higher concentration in comparative as the esters 1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester and the hexadecanoic acid. ethyl ester.

The ester Benzeneacetic acid. ethyl ester has been positively correlated such as metabolic products of yeast such as *Wickerhamomyces anomalus, Candida intermedia, Trichosporon asahii, Pichia guilliermondii, Candida humilis, Candida tropicalis, Cyberlindnera jadinii, Hanseniaspora vineae, Metschnikowia spp, and Saccharomyces cerevisiae* (Shoubao et al., 2023). Though this ester was identified in the green control the higher concentration of this ester was identified in the treatments inoculated with *P. Kluyveri* (CCMA1658) *M. guillermondii* (CCMA1737); *C. ferigula* (CCMA1647); and *M. caribbica* (CCMA1735) which indicates production of this ester by the yeasts previous inoculated. While the ester benzoic acid, 2-hydroxy-, ethyl ester (ethyl salicylate) was not identified in the green and roasted control treatment and treatment inoculated with *M. caribbica* (CCMA1950), but if identified in the treatments inoculated with *M. caribbica, M. caribbica* (CCMA1992), *M. caribbica* (CCMA1617), *H. uvarum* (CCMA 1944); *P. Kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737); *C. ferigula* (CCMA1647); and *M. caribbica* (CCMA1735) in concentration between 10 until 60 g/L, not there is explain clear about this result, since this compound is synthesized from salicylic acid, which belongs to the plant and in the drink generates attributes tasting like warm, sweet, mint-like, and fruity-root (Flament and Bessière-Thomas, 2002; Kalaivani et al., 2016).

Like the ester benzoic acid, 2-hydroxy-, ethyl ester (ethyl salicylate) the ester hexadecanoic acid. ethyl ester (Ethyl palmitate) has been reported as compound plant natural, this ester has been identified in samples of palm oil, corn oil, soybean oil, canola oil, sunflower, linseed, coconut (Gunawan et al., 2017; Moura-Nickel et al., 2016), however has been positively correlated such as metabolic products of *S. cerevisiae*, and could add has fruity, candy, and perfume-like aromas in beverages (Chen et al., 2023).

The ester hexadecanoic acid. ethyl ester (Ethyl palmitate) was identified in the treatments inoculated with *M. caribbica* (CCMA 1993); *M. caribbica* (CCMA1950); *M. caribbica* (CCMA1992); *M. caribbica* (CCMA1617); and *H. uvarum* (CCMA 1944), and

treatment roasted control, however of the treatments inoculated showed high concentration in comparative with treatment roasted control (Fig.3), which indicates production of this ester by the yeasts previous inoculated.

On the other hand the ester 1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester (dibutyl phthalate) is a compound with category of organic pollutants frequently used in the production of plastics, It is commonly used as a plasticizer and additive in a number of applications such as agricultural mulch and medical devices (L. H. Liu et al., 2022; C. Wang et al., 2023; Wu et al., 2022).

The dibutyl phthalate are linked by unstable chemical bonds, it is easy to escape from plastics to the natural environment (Zhang et al., 2015), has been reported to be detected in air, water and soil, and its detection frequency and concentration are relatively high (Hongjun et al., 2013; Philip et al., 2018). Due to its low water solubility, dibutyl phthalate is readily adsorbed by soil (Yao et al., 2022). At the same time, the use of large quantities of agricultural films and sewage irrigation have made soil a major repository for dibutyl phthalate, being considered a pollutant in the samples identified, however this compound only was identified in the treatment inoculated with *M. caribbica* (CCMA1992), showing a concentration less than 20 g/L (Fig.3).

After roasting, some volatile compounds decrease, as well as the formation of other compounds due to the thermal decomposition of carbohydrates and some amino acids or the oxidation of polyunsaturated fatty acids (Cai et al., 2022; Cao et al., 2023; Galarza and Figueroa, 2022).

Although it is known that pyrazines and furans are produced during heat treatment, there is evidence that starter cultures can influence the formation of pyrazines and furans because yeast activities can impact internal components such as sugars and amino acids, which in turn can affect the synthesis of volatiles during roasting (Barea-Ramos et al., 2022; Elhalis et al., 2021a, 2021b). This explains the variations shown in Fig. 5 where the concentrations contributed by each inoculated treatment can be observed, also be observed that the large contribution in the concentration of pyrazines and furans was identified in the inoculated treatments in the comparison with control treatments. This contribution could indicate a good performance of the yeasts in the coffee dough during fermentation, as well as a good contribution for the sensory analysis because these groups may have contributed to the sensory perception of sweetness, acid, roasted, caramel, buttery, woody and earthy (Elhalis et al., 2020; Mayer et al., 2000), nd their levels in both bean types might explain the sensory

scores given to the sweetness, balance, clean up and uniformity of coffees brewed from the two types of bean.

On the other hand the retronasal and ortho-nasal perception interactions interpreted volatile compounds as odor, thus defining sensory descriptors (Sunarharum et al., 2014). For this reason, fermentation can lead to organic acids and volatile compounds, thus generating sensory descriptors desirable for better coffee acceptance.

5. Conclusion

The sanitization of the coffee beans before fermentation helped in the reduction of bacterial populations at the beginning of fermentation (72 and 120 h), due to the large population of microbes found at this time were yeasts (Fig. 3 and Fig. 4). This support the good action of sanitization of the peracetic acid on bacterial in the mass of coffee, converting to the acid peracetic a product ideal for sanitization due to that acquisition and used easy.

On the other hand the fermentation process generated ideal conditions for the growth of yeasts in the control treatments, as well as in the treatments inoculated, where the carbohydrates present in the coffee beans were consumed, and acetic acid became a non-sugar carbon source. However, the best populations growth (>8 $\log_{10}$ CFU/mL) in the natural process were for *M. caribbica* (CCMA1617), *H. uvarum* (CCMA 1944), *M. guillermondii* (CCMA1737), and *C. ferigula* (CCMA1647), and in the pulped process were Meyerozyma caribbica (CCMA1993), *M caribbica* (CCMA1950), *H. uvarum* (CCMA 1944), *P. Kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737), *C. ferigula* (CCMA1647), *M. caribbica* (CCMA1735).

In the natural process *H. uvarum* (CCMA 1944), *P. kluyveri* (CCMA1658), *M. guillermondii* (CCMA1737) and *M. caribbica* (CCMA1735) showed higher performance in the production of succinic acids and volatile compounds such as, alcohols, furan, esters, and pyrazines. Besides all the starter cultures provided sensory descriptors of chocolate in flavor and aromas.

In the pulped coffee process, the *H. uvarum* (CCMA 1944) and *M. caribbica* (CCMA1735) presented a higher concentration of lactic acid. This strains are ideal for use in this process influencing the formation of volatile compounds such as pyrazines and furans (supplementary material - Table.C3 and C4). Furthermore, *H. uvarum* (CCMA 1944), *M. guillermondii* (CCMA1737) and *C. ferigula* (CCMA1647) contributed with sensory descriptors of flavor as orange, tropical fruits, and woody respectively and *M. caribbica* (CCMA 1993, CCMA1950, CCMA1992, CCMA1735), *M. guillermondii* (CCMA1737) and

C. ferigula (CCMA1647) contributed with sensory descriptors of aromas as citrus fruit and chocolate.

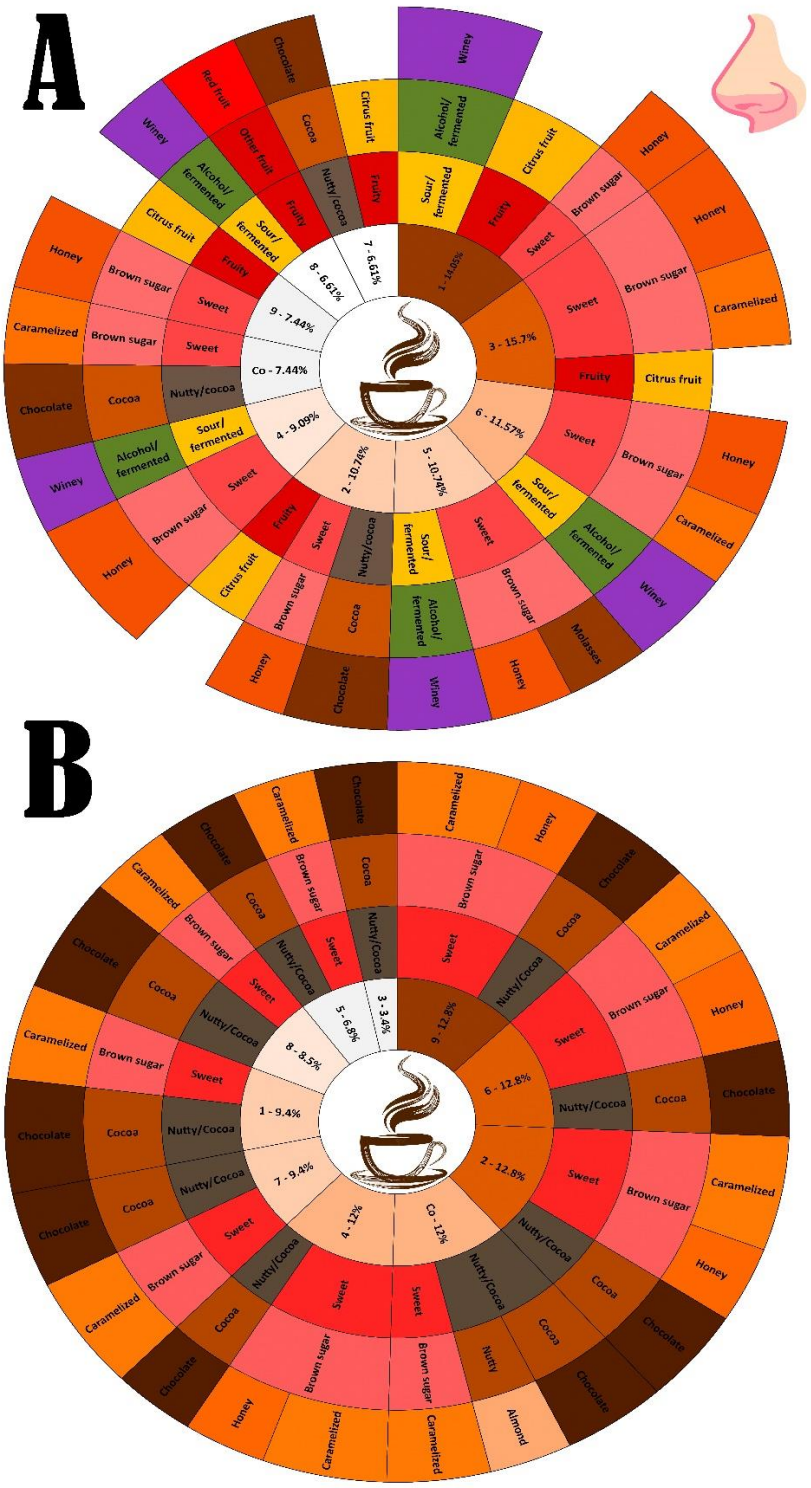

Figure 6. Description of aroma; A: pulped; B: Natural; 1: *Meyerozyma caribbica* (CCMA 1993); 2: *Meyerozyma caribbica* (CCMA1950); 3: *Meyerozyma caribbica* (CCMA1992); 4: *Meyerozyma caribbica* (CCMA1617); 5: *Hanseniaspora uvarum* (CCMA 1944); 6: *Pichia Kluyveri* (CCMA1658); 7: *Meyerozyma guillermondii* (CCMA1737); 8: *Cystofilobasidium ferigula* (CCMA1647); 9: *Meyerozyma caribbica* (CCMA1735).

6. References

Barea-Ramos, J.D., Cascos, G., Mesías, M., Lozano, J., Martín-Vertedor, D., 2022. Evaluation of the Olfactory Quality of Roasted Coffee Beans Using a Digital Nose. Sensors 2022, Vol. 22, Page 8654 22, 8654. https://doi.org/10.3390/S22228654.

Brando, C., Bando, M.F., 2015. Methods of coffee fermentation and drying, in: Cocoa and Coffee Fermentation.

Bressani, A.P.P., Batista, N.N., Ferreira, G., Martinez, S.J., Simão, J.B.P., Dias, D.R., Schwan, R.F., 2021a. Characterization of bioactive, chemical, and sensory compounds from fermented coffees with different yeasts species. Food Research International 150, 110755. https://doi.org/10.1016/j.foodres.2021.110755.

Bressani, A.P.P., Martinez, S.J., Batista, N.N., Simão, J.B.P., Dias, D.R., Schwan, R.F., 2021b. Co-inoculation of yeasts starters: A strategy to improve quality of low altitude Arabica coffee. Food Chem 361, 130133. https://doi.org/10.1016/j.foodchem.2021.130133.

Bressani, A.P.P., Martinez, S.J., Evangelista, S.R., Dias, D.R., Schwan, R.F., 2018. Characteristics of fermented coffee inoculated with yeast starter cultures using different inoculation methods. LWT 92, 212–219. https://doi.org/10.1016/j.lwt.2018.02.029.

Bressani, A.P.P., Martinez, S.J., Sarmento, A.B.I., Borém, F.M., Schwan, R.F., 2021c. Influence of yeast inoculation on the quality of fermented coffee (Coffea arabica var. Mundo Novo) processed by natural and pulped natural processes. Int J Food Microbiol 343. https://doi.org/10.1016/j.ijfoodmicro.2021.109107.

Bressani, A.P.P., Martinez, S.J., Sarmento, A.B.I., Borém, F.M., Schwan, R.F., 2020. Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. Food Research International 128, 108773. https://doi.org/10.1016/j.foodres.2019.108773.

Broissin-Vargas, L.M., Snell-Castro, R., Godon, J.J., González-Ríos, O., Suárez-Quiroz, M.L., 2018. Impact of storage conditions on fungal community composition of green coffee beans Coffea arabica L. stored in jute sacks during 1 year. J Appl Microbiol 124, 547–558. https://doi.org/10.1111/jam.13656.

Cai, Y., Xu, Z., Pan, X., Gao, M., Wu, M., Wu, J., Lao, F., 2022. Comparative Profiling of Hot and Cold Brew Coffee Flavor Using Chromatographic and Sensory Approaches. Foods 11, 2968. https://doi.org/10.3390/FOODS11192968/S1.

Cao, X., Wu, H., Viejo, C.G., Dunshea, F.R., Suleria, H.A.R., 2023. Effects of postharvest processing on aroma formation in roasted coffee – a review. Int J Food Sci Technol. https://doi.org/10.1111/ijfs.16261.

Carvalho Ferreira, J.L., de Souza Gomes, M., Maciel de Oliveira, L., Diniz Santos, L., 2023. Coffee fermentation process: A review. Food Research International. https://doi.org/10.1016/j.foodres.2023.112793.

Cassimiro, D.M. de J., Batista, N.N., Fonseca, H.C., Naves, J.A.O., Dias, D.R., Schwan, R.F., 2022. Coinoculation of lactic acid bacteria and yeasts increases the quality of wet fermented Arabica coffee. Int J Food Microbiol 369. https://doi.org/10.1016/j.ijfoodmicro.2022.109627.

Cavallo, E., Charreau, H., Cerrutti, P., Foresti, M.L., 2017. Yarrowia lipolytica: A model yeast for citric acid production. FEMS Yeast Res. https://doi.org/10.1093/femsyr/fox084.

Chen, L., Xiang, W., Liang, X., Liu, J., Zhu, H., Cai, T., Zhang, Q., Tang, J., 2023. Fungal Biomarkers in Traditional Starter Determine the Chemical Characteristics of Turbid Rice Wine from the Rim of the Sichuan Basin, China. Foods 12. https://doi.org/10.3390/foods12030585.

da Mota, M.C., Batista, N.N., Dias, D.R., Schwan, R.F., 2022. Impact of microbial self-induced anaerobiosis fermentation (SIAF) on coffee quality. Food Biosci 47, 101640. https://doi.org/10.1016/j.fbio.2022.101640.

da Mota, M.C.B., Batista, N.N., Rabelo, M.H.S., Ribeiro, D.E., Borém, F.M., Schwan, R.F., 2020. Influence of fermentation conditions on the sensorial quality of coffee inoculated with yeast. Food Research International 136. https://doi.org/10.1016/j.foodres.2020.109482.

de Souza, M.L., Passamani, F.R.F., Ávila, C.L. da S., Batista, L.R., Schwan, R.F., Silva, C.F., 2017. Uso de leveduras selvagens como agente de biocontrole contra fungos toxigênicos e produção de OTA. Acta Sci Agron 39, 349–358. https://doi.org/10.4025/actasciagron.v39i3.32659.

del Mónaco, S.M., Barda, N.B., Rubio, N.C., Caballero, A.C., 2014. Selection and characterization of a Patagonian Pichia kudriavzevii for wine deacidification. J Appl Microbiol 117, 451–464. https://doi.org/10.1111/JAM.12547.

Elhalis, H., Cox, J., Frank, D., Zhao, J., 2021a. Microbiological and biochemical performances of six yeast species as potential starter cultures for wet fermentation of coffee beans. LWT 137. https://doi.org/10.1016/j.lwt.2020.110430.

Elhalis, H., Cox, J., Frank, D., Zhao, J., 2021b. Microbiological and Chemical Characteristics of Wet Coffee Fermentation Inoculated With Hansinaspora uvarum and Pichia kudriavzevii and Their Impact on Coffee Sensory Quality. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.713969.

Elhalis, H., Cox, J., Frank, D., Zhao, J., 2020. The crucial role of yeasts in the wet fermentation of coffee beans and quality. Int J Food Microbiol 333. https://doi.org/10.1016/j.ijfoodmicro.2020.108796.

Evangelista, S., Silva, C.F., Miguel, M.G.P. da C., Cordeiro, C. de S., Pinheiro, A.C.M., Duarte, W.F., Schwan, R.F., 2014. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. Food Research International 61, 183–195. https://doi.org/10.1016/j.foodres.2013.11.033.

Ferreira, A.M., Mendes-Faia, A., 2020. The role of yeasts and lactic acid bacteria on the metabolism of organic acids during winemaking. Foods. https://doi.org/10.3390/foods9091231.

Ferreira, D.F., 2014. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. Ciência e Agrotecnologia 38, 109–112. https://doi.org/10.1590/S1413-70542014000200001.

Flament, Ivon., Bessière-Thomas, Yvonne., 2002. Coffee flavor chemistry 410.

Galarza, G., Figueroa, J.G., 2022. Volatile Compound Characterization of Coffee (Coffea arabica) Processed at Different Fermentation Times Using SPME–GC–MS. Molecules 27, 2004. https://doi.org/10.3390/molecules27062004.

Gonzalez-Rios, O., Suarez-Quiroz, M.L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.P., Schorr-Galindo, S., 2007a. Impact of "ecological" post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. Journal of Food Composition and Analysis 20, 289–296. https://doi.org/10.1016/j.jfca.2006.07.009.

Gonzalez-Rios, O., Suarez-Quiroz, M.L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.P., Schorr-Galindo, S., 2007b. Impact of "ecological" post-harvest processing on coffee aroma: II. Roasted coffee. Journal of Food Composition and Analysis 20, 297–307. https://doi.org/10.1016/j.jfca.2006.12.004.

Gunawan, E.R., Suhendra, D., Nurita, A.D., Komalasari, D., 2017. Four factor surface response optimization of ethyl palmitate from terminalia cattapa L. Kernel Oil. Asian Journal of Chemistry 29, 2107–2112. https://doi.org/10.14233/ajchem.2017.20580.

Hadj Salem, F., Lebrun, M., Mestres, C., Sieczkowski, N., Boulanger, R., Collignan, A., 2020. Transfer kinetics of labeled aroma compounds from liquid media into coffee beans during simulated wet processing conditions. Food Chem 322. https://doi.org/10.1016/j.foodchem.2020.126779.

Hasani, M., Wu, F., Hu, K., Farber, J., Warriner, K., 2020. Inactivation of Salmonella and Listeria monocytogenes on dried fruit, pistachio nuts, cornflakes and chocolate crumb using a peracetic acid-ethanol based sanitizer or Advanced Oxidation Process. Int J Food Microbiol 333. https://doi.org/10.1016/j.ijfoodmicro.2020.108789.

Holt, J., 1994. Bergey's manual of determinative bacteriology, 9th ed.

Hongjun, Y., Wenjun, X., Qing, L., Jingtao, L., Hongwen, Y., Zhaohua, L., 2013. Distribution of phthalate esters in topsoil: A case study in the Yellow River Delta, China. Environ Monit Assess 185, 8489–8500. https://doi.org/10.1007/S10661-013-3190-7/FIGURES/5.

Jimenez, E.J.M., Martins, P.M.M., Vilela, A.L. de O., Batista, N.N., Rosa, S.D.V.F. da, Dias, D.R., Schwan, R.F., 2023. Influence of anaerobic fermentation and yeast inoculation on the viability, chemical composition, and quality of coffee. Food Biosci 51. https://doi.org/10.1016/j.fbio.2022.102218.

Jung, S., Yeo, D., Wang, Z., Woo, S., Seo, Y., Hossain, M.I., Choi, C., 2023. Viability of SARS-CoV-2 on lettuce, chicken, and salmon and its inactivation by peracetic acid, ethanol, and chlorine dioxide. Food Microbiol 110, 104164. https://doi.org/10.1016/j.fm.2022.104164.

Kalaivani, K., Kalaiselvi, M.M., Senthil-Nathan, S., 2016. Effect of methyl salicylate (MeSA), an elicitor on growth, physiology and pathology of resistant and susceptible rice varieties. Sci Rep 6. https://doi.org/10.1038/srep34498.

Knopp, S., Bytof, G., Selmar, D., 2006. Influence of processing on the content of sugars in green Arabica coffee beans. European Food Research and Technology 223, 195–201. https://doi.org/10.1007/S00217-005-0172-1/TABLES/1.

Lima-Neto, R., Santos, C., Lima, N., Sampaio, P., Pais, C., Neves, R.P., 2014. Application of MALDI-TOF MS for requalification of a Candida clinical isolates culture collection. Brazilian Journal of Microbiology 45, 515–522. https://doi.org/10.1590/S1517-83822014005000044.

Lin, W., Zuo, J., Li, K., Hu, R., Xu, X., Huang, T., Wen, G., Ma, J., 2023. Pre-exposure of peracetic acid enhances its subsequent combination with ultraviolet for the inactivation of fungal spores: Efficiency, mechanisms, and implications. Water Res 229. https://doi.org/10.1016/j.watres.2022.119404.

Liu, L.H., Yuan, T., Zhang, J.Y., Tang, G.X., Lü, H., Zhao, H.M., Li, H., Li, Y.W., Mo, C.H., Tan, Z.Y., Cai, Q.Y., 2022. Diversity of endophytic bacteria in wild rice (Oryza meridionalis) and potential for promoting plant growth and degrading phthalates. Science of The Total Environment 806, 150310. https://doi.org/10.1016/J.SCITOTENV.2021.150310.

Liu, S.Q., 2015. Impact of yeast and bacteria on beer appearance and flavour. Brewing Microbiology: Managing Microbes, Ensuring Quality and Valorising Waste 357–374. https://doi.org/10.1016/B978-1-78242-331-7.00017-4.

Liu, Y., Chua, X.Y., Dong, W., Lu, Y., Liu, S.Q., 2022. Effects of sequential inoculation of Lachancea thermotolerans and Oenococcus oeni on chemical composition of spent coffee grounds hydrolysates. Curr Res Food Sci 5, 1276–1286. https://doi.org/10.1016/j.crfs.2022.08.002.

Martins, P., Ribeiro, L.S., Miguel, M.G. da C.P., Evangelista, S.R., Schwan, R.F., 2019. Production of coffee (Coffea arabica) inoculated with yeasts: impact on quality. J Sci Food Agric 99, 5638–5645. https://doi.org/10.1002/jsfa.9820.

Martins, P.M.M., Batista, N.N., Miguel, M.G. da C.P., Simão, J.B.P., Soares, J.R., Schwan, R.F., 2020. Coffee growing altitude influences the microbiota, chemical compounds and the quality of fermented coffees. Food Research International 129, 108872. https://doi.org/10.1016/j.foodres.2019.108872.

Martins, P.M.M., Batista, N.N., Santos, L.D., Dias, D.R., Schwan, R.F., 2022. Microencapsulation of epiphytic coffee yeasts by spray drying using different wall materials: Implementation in coffee medium. Int J Food Microbiol 379, 109839. https://doi.org/10.1016/j.ijfoodmicro.2022.109839.

Mayer, F., Czerny, M., Grosch, W., 2000. Sensory study of the character impact aroma compounds of a coffee beverage. European Food Research and Technology 211, 272–276. https://doi.org/10.1007/S002170000169/METRICS.

Michel, M., Kopecká, J., Meier-Dörnberg, T., Zarnkow, M., Jacob, F., Hutzler, M., 2016. Screening for new brewing yeasts in the non-Saccharomyces sector with Torulaspora delbrueckii as model. Yeast 33, 129–144. https://doi.org/10.1002/YEA.3146.

Moura-Nickel, C.D., Contarti Da Cruz, L.C., Igarashi-Mafra, L., Yamamoto, C.I., Rolemberg, M.P., Mafra, M.R., 2016. Determination of cloud point in binary and ternary mixtures containing biodiesel and diesel constituents. Part I – Ethyl palmitate, ethyl stearate and n-hexadecane. Fuel 180, 442–447. https://doi.org/10.1016/J.FUEL.2016.04.054.

Pereira, Priscila., da Silveira, D.L., Schwan, R.F., de Assis Silva, S., Coelho, J.M., Bernardes, P.C., 2021. Effect of altitude and terrain aspect on the chemical composition of Coffea canephora cherries and sensory characteristics of the beverage. J Sci Food Agric 101, 2570–2575. https://doi.org/10.1002/jsfa.10885.

Pereira, T.S., Batista, N.N., Santos Pimenta, L.P., Martinez, S.J., Ribeiro, L.S., Oliveira Naves, J.A., Schwan, R.F., 2022. Self-induced anaerobiosis coffee fermentation: Impact on microbial communities, chemical composition and sensory quality of coffee. Food Microbiol 103, 103962. https://doi.org/10.1016/j.fm.2021.103962.

Philip, J.M., Aravind, U.K., Aravindakumar, C.T., 2018. Emerging contaminants in Indian environmental matrices – A review. Chemosphere 190, 307–326. https://doi.org/10.1016/J.CHEMOSPHERE.2017.09.120.

Ribeiro, L.S., da Cruz Pedrozo Miguel, M.G., Martinez, S.J., Bressani, A.P.P., Evangelista, S.R., Silva e Batista, C.F., Schwan, R.F., 2020. The use of mesophilic and lactic acid bacteria strains as starter cultures for improvement of coffee beans wet fermentation. World J Microbiol Biotechnol 36, 186. https://doi.org/10.1007/s11274-020-02963-7.

Ribeiro, L.S., Miguel, M.G. da C.P., Evangelista, S.R., Martins, P.M.M., van Mullem, J., Belizario, M.H., Schwan, R.F., 2017a. Behavior of yeast inoculated during semi-dry coffee fermentation and the effect on chemical and sensorial properties of the final beverage. Food Research International 92, 26–32. https://doi.org/10.1016/j.foodres.2016.12.011.

Ribeiro, L.S., Ribeiro, D.E., Evangelista, S.R., Miguel, M.G. da C.P., Pinheiro, A.C.M., Borém, F.M., Schwan, R.F., 2017b. Controlled fermentation of semi-dry coffee (Coffea arabica) using starter cultures: A sensory perspective. LWT - Food Science and Technology 82, 32–38. https://doi.org/10.1016/j.lwt.2017.04.008.

Sayın Börekçi, B., Kaya, M., Kaban, G., 2022. Citric Acid Production by Yarrowia lipolytica NRRL Y-1094: Optimization of pH, Fermentation Time and Glucose Concentration Using Response Surface Methodology. Fermentation 8. https://doi.org/10.3390/fermentation8120731.

SCAA, 2015. SCAA Protocols Cupping Specialty Coffee. Specialty Coffee Association of America 1–10.

Schwan, R., Silva, C., Batista, L., 2012. Coffee Fermentation, in: Handbook of Plant-Based Fermented Food and Beverage Technology, Second Edition. CRC Press, pp. 677–690. https://doi.org/10.1201/b12055-49.

Shin, M., Kang, J.W., Kang, D.H., 2023. A study on antibiotic resistance gene degradation in fresh produce using peracetic acid combined with an ultraviolet-C light-emitting-diode. Food Control 145. https://doi.org/10.1016/j.foodcont.2022.109478.

Shoubao, Y., Jie, Y., TingTing, S., Jiaquan, G., Cuie, S., 2023. Yeast diversity in pit mud and related volatile compounds in fermented grains of chinese strong-flavour liquor. AMB Express 13, 1–14. https://doi.org/10.1186/S13568-023-01562-7/FIGURES/4.

Silva, C.F., Batista, L.R., Abreu, L., Dias, E., Schwan, R., 2008. Succession of bacterial and fungal communities during natural coffee (Coffea arabica) fermentation. Food Microbiol 25, 951–957. https://doi.org/10.1016/j.fm.2008.07.003.

Silva, C.F., 2015. Microbial activity during coffee fermentation, in: Cocoa and Coffee Fermentations.

Silva, C.F., Schwan, R.F., Sousa Dias, Ë., Wheals, A.E., 2000. Microbial diversity during maturation and natural processing of coffee cherries of Coffea arabica in Brazil. Int J Food Microbiol 60, 251–260. https://doi.org/10.1016/S0168-1605(00)00315-9.

Sousa, M.J., Ludovico, P., Rodrigues, F., Leão, C., Côrte-Real, M., Sousa, M.J., Ludovico, P., Rodrigues, F., Leão, C., Côrte-Real, M., 2012. Stress and Cell Death in Yeast Induced by Acetic Acid. Cell Metabolism - Cell Homeostasis and Stress Response. https://doi.org/10.5772/27726.

Souza, K.S.T., Schwan, R.F., Dias, D.R., 2014. Lipid and citric acid production by wild yeasts grown in glycerol. J Microbiol Biotechnol 24, 497–506. https://doi.org/10.4014/jmb.1310.10084.

Sunarharum, W.B., Williams, D.J., Smyth, H.E., 2014. Complexity of coffee flavor: A compositional and sensory perspective. Food Research International. https://doi.org/10.1016/j.foodres.2014.02.030.

Toledo, P.R.A.B., Pezza, L., Pezza, H.R., Toci, A.T., 2016. Relationship Between the Different Aspects Related to Coffee Quality and Their Volatile Compounds. Compr Rev Food Sci Food Saf 15, 705–719. https://doi.org/10.1111/1541-4337.12205.

van Wyk, N., Scansani, S., Beisert, B., Brezina, S., Fritsch, S., Semmler, H., Pretorius, I.S., Rauhut, D., von Wallbrunn, C., 2022. The Use of Hanseniaspora occidentalis in a Sequential Must Inoculation to Reduce the Malic Acid Content of Wine. Applied Sciences 2022, Vol. 12, Page 6919 12, 6919. https://doi.org/10.3390/APP12146919.

Vasserot, Y., Mornet, F., Jeandet, P., 2010. Acetic acid removal by Saccharomyces cerevisiae during fermentation in oenological conditions. Metabolic consequences. Food Chem 119, 1220–1223. https://doi.org/10.1016/j.foodchem.2009.08.008.

Verhagen, L.C., 1994. Beer flavour. Understanding Natural Flavors 211–227. https://doi.org/10.1007/978-1-4615-2143-3_14.

Vicente, J., Baran, Y., Navascués, E., Santos, A., Calderón, F., Marquina, D., Rauhut, D., Benito, S., 2022. Biological management of acidity in wine industry: A review. Int J Food Microbiol 375, 109726. https://doi.org/10.1016/J.IJFOODMICRO.2022.109726.

Vicente, J., Kelanne, N., Navascués, E., Calderón, F., Santos, A., Marquina, D., Yang, B., Benito, S., 2023. Combined Use of Schizosaccharomyces pombe and a Lachancea thermotolerans Strain with a High Malic Acid Consumption Ability for Wine Production. Fermentation 9. https://doi.org/10.3390/fermentation9020165.

Vilela, D.M., Pereira, G.V. de M., Silva, C.F., Batista, L.R., Schwan, R.F., 2010. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (Coffea arabica L.). Food Microbiol 27, 1128–1135. https://doi.org/10.1016/j.fm.2010.07.024.

Vilela-Moura, A., Schuller, D., Mendes-Faia, A., Côrte-Real, M., 2008. Reduction of volatile acidity of wines by selected yeast strains. Appl Microbiol Biotechnol 80, 881–890. https://doi.org/10.1007/s00253-008-1616-x.

Wang, C., Sun, J., Lassabliere, B., Yu, B., Zhao, Feifei, Zhao, Fangju, Chen, Y., Liu, S.Q., 2019. Potential of lactic acid bacteria to modulate coffee volatiles and effect of glucose supplementation: fermentation of green coffee beans and impact of coffee roasting. J Sci Food Agric 99, 409–420. https://doi.org/10.1002/jsfa.9202.

Wang, C., Yao, X., Li, X., Wang, Q., Wang, Jinhua, Zhu, L., Wang, Jun, 2023. Effects of dibutyl phthalate on microbial community and the carbon cycle in salinized soil. J Clean Prod 404, 136928. https://doi.org/10.1016/J.JCLEPRO.2023.136928.

Wang, Y., Wang, X., Hu, G., Al-Romaima, A., Peng, X., Li, J., Bai, X., Li, Z., Qiu, M., 2023. Anaerobic germination of green coffee beans: A novel strategy to improve the quality of commercial Arabica coffee. Curr Res Food Sci 6. https://doi.org/10.1016/j.crfs.2023.100461.

Wu, C., Ma, Yajie, Wang, D., Shan, Y., Song, X., Hu, H., Ren, X., Ma, X., Luo, J., Cui, J., Ma, Yan, 2022. Microbiology combined with metabonomics revealing the response of soil microorganisms and their metabolic functions exposed to phthalic acid esters. Ecotoxicol Environ Saf 233, 113338. https://doi.org/10.1016/J.ECOENV.2022.113338.

Yao, X., Zhang, J., Wang, C., Wang, Q., Li, X., Zhang, D., Wang, Jinhua, Zhu, L., Wang, Jun, 2022. Toxicity of dibutyl phthalate to pakchoi (Brassica campestris L.): Evaluation through different levels of biological organization. Science of The Total Environment 849, 157943. https://doi.org/10.1016/J.SCITOTENV.2022.157943.

Yeager, S.E., Batali, M.E., Guinard, J.X., Ristenpart, W.D., 2023. Acids in coffee: A review of sensory measurements and meta-analysis of chemical composition. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2021.1957767.

Yeretzian, C., Opitz, S., Smrke, S., Wellinger, M., 2019. Coffee Volatile and Aroma Compounds – From the Green Bean to the Cup, in: Coffee: Production, Quality and Chemistry . Royal Society of Chemistry, pp. 726–770. https://doi.org/10.1039/9781782622437-00726.

Zhang, T., Wang, T., Mejia-Tickner, B., Kissel, J., Xie, X., Huang, C.H., 2020. Inactivation of Bacteria by Peracetic Acid Combined with Ultraviolet Irradiation: Mechanism and Optimization. Environ Sci Technol 54, 9652–9661. https://doi.org/10.1021/acs.est.0c02424.

Zhang, Y., Du, N., Wang, L., Zhang, H., Zhao, J., Sun, G., Wang, P., 2015. Physical and chemical indices of cucumber seedling leaves under dibutyl phthalate stress. Environmental Science and Pollution Research 22, 3477–3488. https://doi.org/10.1007/S11356-014-3524-1/FIGURES/6.

ANEXO – Material complementar

Supplementary material

Table .C1 Concentration of temperature, soluble solids, and pH

Natural				
Time	**treatments**	**Temperature**	**Soluble solids**	**pH**
0	**1**	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	2	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	3	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	4	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	5	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	6	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	7	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	8	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
	9	25.000 ± 0.000	18.000 ± 0.000	4.680 ± 0.000
72	**1**	22.25 ± 0.25	14.529 ± 0.171	4.085 ± 0.015
	2	22.000 ± 0.000	14.571 ± 0.214	4.095 ± 0.035
	3	22.250 ± 0.250	14.550 ± 0.150	4.045 ± 0.035
	4	22.000 ± 0.000	14.55 ± 0.107	4.110 ± 0.010
	5	22.000 ± 0.000	14.593 ± 0.150	4.130 ± 0.010
	6	22.000 ± 0.000	14.421 ± 0.064	3.925 ± 0.015
	7	22.000 ± 0.000	14.464 ± 0.107	3.995 ± 0.015
	8	22.250 ± 0.250	13.800 ± 0.129	4.290 ± 0.010
	9	22.000 ± 0.000	15.257 ± 0.043	4.020 ± 0.010
120	**1**	22.250 ± 0.250	14.529 ± 0.286	4.085 ± 0.030
	2	22.000 ± 0.000	14.571 ± 0.357	4.095 ± 0.025
	3	22.250 ± 0.250	14.550 ± 0.250	4.045 ± 0.015
	4	22.000 ± 0.000	14.55 ± 0.179	4.11 ± 0.005
	5	22.000 ± 0.000	14.593 ± 0.250	4.13 ± 0.005
	6	22.000 ± 0.000	14.421 ± 0.107	3.925 ± 0.010
	7	22.000 ± 0.000	14.464 ± 0.179	3.995 ± 0.005
	8	22.250 ± 0.250	11.00 ± 0.214	4.115 ± 0.015
	9	22.000 ± 0.000	15.257 ± 0.071	4.020 ± 0.005
168	**1**	20.750 ± 0.250	9.900 ± 0.400	4.07 ± 0.01
	2	22.000 ± 0.000	10.00 ± 0.500	5.385 ± 1.465
	3	21.500 ± 0.000	9.950 ± 0.350	3.930 ± 0.030
	4	21.500 ± 0.000	9.950 ± 0.250	3.920 ± 0.050
	5	21.000 ± 0.000	10.050 ± 0.350	3.865 ± 0.015
	6	21.500 ± 0.000	9.650 ± 0.150	3.745 ± 0.005
	7	21.500 ± 0.000	9.750 ± 0.250	3.795 ± 0.005
	8	21.500 ± 0.000	8.200 ± 0.300	4.080 ± 0.010
	9	21.500 ± 0.000	11.600 ± 0.100	3.750 ± 0.000

Pulped				
Time	**Strain**	**Temperature**	**Soluble solids (Brix)**	**pH**
0	**1**	20,000±0,000	15,000±0,000	4,600±0,000
	2	20,000±0,000	15,000±0,000	4,600±0,000

	3	20,000±0,000	15,000±0,000	4,600±0,000
	4	20,000±0,000	15,000±0,000	4,600±0,000
	5	20,000±0,000	15,000±0,000	4,600±0,000
	6	20,000±0,000	15,000±0,000	4,600±0,000
	7	20,000±0,000	15,000±0,000	4,600±0,000
	8	20,000±0,000	15,000±0,000	4,600±0,000
	9	20,000±0,000	15,000±0,000	4,600±0,000
72	**1**	22,250±0,250	11,850±0,050	4,060±0,060
	2	22,000±0,000	11,125±0,425	3,880±0,010
	3	22,250±0,250	10,825±0,275	3,945±0,025
	4	22,000±0,000	11,000±0,450	4,075±0,045
	5	22,000±0,000	11,550±0,200	3,940±0,010
	6	22,000±0,000	11,300±0,300	3,815±0,025
	7	22,000±0,000	11,675±0,025	4,000±0,020
	8	22,250±0,250	11,375±0,025	4,000±0,010
	9	22,000±0,000	11,600±0,150	3,970±0,000
120	**1**	22,250±0,250	11,850±0,083	4,060±0,005
	2	22,000±0,000	11,125±0,708	3,880±0,010
	3	22,250±0,250	10,825±0,458	3,945±0,045
	4	22,000±0,000	11,000±0,750	4,075±0,040
	5	22,000±0,000	11,550±0,333	3,940±0,005
	6	22,000±0,000	11,300±0,500	3,815±0,005
	7	22,000±0,000	11,675±0,042	4,000±0,020
	8	22,250±0,250	11,375±0,042	4,000±0,030
	9	22,000±0,000	9,3333±0,25	3,710±0,040
144	**1**	20,750±0,250	8,700±0,100	3,915±0,015
	2	22,000±0,000	7,250±0,850	3,685±0,005
	3	22,250±0,000	8,041±0,550	3,825±0,055
	4	22,000±0,000	8,333±0,900	3,950±0,025
	5	21,000±0,000	8,100±0,400	3,840±0,000
	6	22,000±0,000	8,833±0,600	3,705±0,000
	7	20,500±0,000	8,350±0,050	3,810±0,010
	8	20,500±0,000	7,750±0,050	3,855±0,035
	9	20,500±0,000	8,200±0,300	3,700±0,050

1: *Meyerozyma caribbica* (CCMA 1993); *2: Meyerozyma caribbica* (CCMA1950); *3: Meyerozyma caribbica* (CCMA1992); *4: Meyerozyma caribbica* (CCMA1617); *5: Hanseniaspora uvarum* (CCMA 1944); *6: Pichia Kluyveri* (CCMA1658); *7: Meyerozyma guillermondii* (CCMA1737); *8: Cystofilobasidium ferigula* (CCMA1647); *9: Meyerozyma caribbica* (CCMA1735).

Table.C2. Volatile compounds of natural process

	Time	Index	FC	RC	1	2	3	4	5	6	7	8	9
Acids													
Propanoic acid	11476	1665	N/D	N/D	N/D	N/D	N/D	19.409 ± 4.830	N/D	N/D	58.806 ± 0.000	N/D	N/D
Benzeneacetic acid	24701	2445	N/D	1.780 ± 0.049	2.306 ± 0.528	1.708 ± 0.012	1.705 ± 0.094	2.231 ± 0.319	1.977 ± 0.005	2.120 ± 0.692	2.233 ± 0.283	2.318 ± 0.032	2.216 ± 0.610
Butanoic acid, 3-methyl-	13642	1939	6.563 ± 1.211	63.681 ± 26.389	103.256 ± 25.055	134.938 ± 16.886	103.993 ± 30.685	71.453 ± 7.514	93.761 ± 17.748	294.914 ± 29.471	288.435 ± 0.859	298.129 ± 12.179	353.694 ± 27.228
n-Hexadecanoic acid	30622	2679	4.433 ± 1.416	1.717 ± 0.121	1.636 ± 0.240	1.525 ± 0.577	1.858 ± 0.423	1.243 ± 0.297	1.696 ± 0.633	1.334 ± 0.451	0.733 ± 0.139	0.658 ± 0.050	0.540 ± 0.141
n-Decanoic acid	21459	2317	0,321 ± 0,108	N/D	N/D	N/D	3.128 ± 0.000	N/D	N/D	N/D	N/D	N/D	N/D
Benzoic acid	23336	2391	N/D	N/D	1.393 ± 0.000	1.327 ± 0.118	1.350 ± 0.000	1.422 ± 0.131	1.313 ± 0.055	14.212 ± 3.483	6.548 ± 1.696	4.693 ± 0.593	2.905 ± 0.243
2-Butenoic acid, 3-methyl-	15473	2067	N/D	N/D	16.104 ± 3.574	21.134 ± 0.940	16.885 ± 3.849	12.858 ± 1.565	15.68 ± 4.114	62.658 ± 9.485	59.200 ± 0.111	54.014 ± 3.184	63.939 ± 4.155
Alchols													
1,6-Octadien-3-ol, 3,7-dimethyl-	11838	1718	7.572 ± 1.260	2.503 ± 0.947	3.303 ± 0.947	4.282 ± 1.170	4.144 ± 1.568	3.582 ± 0.471	3.955 ± 0.970	14.068 ± 2.964	14.235 ± 0.169	19.101 ± 0.390	19.377 ± 1.545

Phenylethyl Alcohol	17104	2146	N/D	N/D	10.044 ± 2.911	7.951 ± 0.621	8.198 ± 2.087	8.894 ± 1.239	9.683 ± 2.373	20.798 ± 4.485	27.64 ± 0.697	36.867 ± 0.742	44.268 ± 4.369
3-Pentanol	7340	1059	N/D	9.767 ± 3.119	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
(S)-3-Ethyl-4-methylpentanol	11289	1637	8.982 ± 1.670	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Octen-3-ol	10110	1464	7.086 ± 0.752	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Octanol	12132	1761	5.157 ± 0.902	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
n-Pentadecanol	24827	2450	0.316 ± 0.054	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Hexanol	8249	1193	17.703 ± 2.228	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Octen-1-ol, (E)-	12920	1876	3.573 ± 0.423	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Nonanol	13755	1947	1.362 ± 0.123	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Heptanol	10322	1495	0.985 ± 0.070	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Furan													
2-Furancarboxaldehyde, 5-methyl-	12019	1744	N/D	573.981 ± 78.024	577.022 ± 64.870	657.375 ± 69.557	573.378 ± 91.332	459.609 ± 12.661	529.857 ± 33.368	1429.376 ± 55.61	1409.192 ± 0.552	1520.076 ± 16.892	1355.328 ± 60.856
Benzofuran, 2,3-dihydro-	22724	2367	N/D	5.537 ± 0.162	7.227 ± 1.108	7.492 ± 0.111	8.396 ± 0.535	8.782 ± 0.465	8.582 ± 0.621	7.966 ± 0.012	7.411 ± 0.987	11.523 ± 0.207	10.574 ± 3.048
3(2H)-Furanone, dihydro-2-methyl-	5461	783	N/D	35.914 ± 0.413	16.091 ± 0.958	56.132 ± 0.025	21.700 ± 11.829	9.946 ± 2.401	11.256 ± 3.210	46.698 ± 32.604	25.214 ± 1.239	74.249 ± 5.545	57.392 ± 33.673
Furfural	9819	1422	N/D	1082.178 ± 91.970	1023.951 ± 64.102	1224.442 ± 123.992	976.024 ± 123.755	952.653 ± 52.060	1057.038 ± 27.474	2356.658 ± 159.565	2133.457 ± 19.206	2213.303 ± 20.120	1949.974 ± 51.746
Ethanone, 1-(2-furanyl)-	10699	1551	N/D	140.981 ± 24.158	137.466 ± 18.309	165.179 ± 21.581	131.514 ± 24.095	103.531 ± 3.690	130.897 ± 7.985	384.977 ± 4.852	373.671 ± 2.576	399.831 ± 8.488	364.325 ± 14.056
2-Furanmethanol, acetate	11196	1624	N/D	136.015 ± 36.096	132.701 ± 24.660	159.871 ± 29.610	154.911 ± 41.915	100.445 ± 12.156	118.453 ± 9.601	313.961 ± 12.077	322.349 ± 0.474	439.404 ± 5.627	370.112 ± 26.272
2-Furanmethanol	13473	1927	N/D	541.589 ± 81.784	693.800 ± 133.794	727.846 ± 33.214	688.455 ± 91.795	531.369 ± 95.774	589.476 ± 79.386	1228.628 ± 62.489	1226.822 ± 4.765	1264.051 ± 26.066	1147.087 ± 67.884
trans-Furfurylideneaceto	17005	2142	N/D	3.093 ± 0.643	4.595 ± 0.220	4.428 ± 0.129	4.779 ± 0.432	3.930 ± 0.112	4.145 ± 0.461	5.672 ± 2.717	8.808 ± 0.909	11.957 ± 0.201	10.280 ± 2.059

ne													
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	18834	2214	N/D	31.691 ± 2.416	41.435 ± 8.694	40.148 ± 6.075	44.954 ± 3.132	40.598 ± 9.163	43.294 ± 10.127	99.663 ± 21.716	109.909 ± 13.578	95.625 ± 0.466	99.534 ± 10.172
.alpha.-Furfuryliden-.alpha.-furylmethylamine	21135	2305	N/D	20.748 ± 1.636	25.167 ± 1.015	21.170 ± 0.521	25.161 ± 2.232	19.329 ± 0.791	21.395 ± 3.057	24.193 ± 8.839	28.221 ± 3.060	38.017 ± 0.045	38.560 ± 9.688
Furan, 2-[(methyldithio)methyl]-	24459	2435	N/D	N/D	N/D	N/D	N/D	N/D	2.972 ± 0.054	5.652 ± 1.790	3.672 ± 0.281	10.855 ± 0.559	11.559 ± 3.724
2(5H)-Furanone	14711	2014	N/D	32.405 ± 2.161	45.831 ± 11.311	44.754 ± 3.389	46.069 ± 1.406	37.739 ± 13.232	38.521 ± 4.691	N/D	N/D	N/D	N/D
5-Hydroxymethyldihydrofuran-2-one	23906	2414	N/D	7.622 ± 0.843	7.912 ± 2.018	7.116 ± 0.589	7.793 ± 0.054	7.205 ± 0.471	7.449 ± 0.294	12.656 ± 0.044	N/D	N/D	N/D
2-Furanmethanol, 5-methyl-	14401	1992	N/D	N/D	N/D	N/D	N/D	4.563 ± 0.078	N/D	N/D	N/D	N/D	N/D
(Pg-245)2-Furanmethanethiol, 5-methyl-	24982	2456	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	1.266 ± 0.065	1.361 ± 0.000

(Pg-228)2-Propenal, 3-(2-furanyl)-	16304	2114	N/D	N/D	N/D	5.888 ± 0.058	N/D	5.135 ± 0.250	5.569 ± 0.456	N/D	N/D	N/D	N/D
5-Isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	18485	2200	N/D	3.03 ± 4.285	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Bis(2-furfuryl)disulfide	16722	2131	N/D	5.032 ± 7.117	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pyrroles													
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	19597	2244	N/D	47.099 ± 12.507	26.634 ± 1.202	24.235 ± 1.364	28.952 ± 2.580	23.938 ± 2.706	24.137 ± 3.352	38.630 ± 11.985	45.262 ± 3.570	51.941 ± 2.181	46.728 ± 9.680
1H-Pyrrole-2-carboxaldehyde	18407	2197	N/D	60.553 ± 4.354	82.506 ± 11.038	76.909 ± 1.777	88.376 ± 11.485	66.393 ± 8.278	69.443 ± 8.946	178.939 ± 29.956	170.786 ± 0.886	190.273 ± 8.889	186.564 ± 16.778
5-Hydroxymethylfurfural	24010	2418	N/D	13.822 ± 19.547	36.231 ± 0.014	N/D	N/D	42.205 ± 0.001	45.987 ± 0.001	32.134 ± 1.076	38.748 ± 4.219	39.972 ± 0.642	31.342 ± 9.024
Aldehyde													
Benzeneacetaldehyde, .alpha.-	17380	2157	N/D	2.152 ± 0.325	3.061 ± 0.603	2.645 ± 0.025	2.643 ± 0.644	2.530 ± 0.082	3.001 ± 0.772	7.871 ± 1.773	8.359 ± 0.510	10.475 ± 0.247	10.199 ± 1.597

ethylidene-													
5-Acetoxymethyl-2-furaldehyde	20374	2275	N/D	1.117 ± 1.58	N/D	N/D	N/D	3.477 ± 0.001	3.364 ± 0.247	4.073 ± 1.442	5.188 ± 0.625	5.288 ± 0.109	4.812 ± 1.030
Benzaldehyde, 3-hydroxy-4-methoxy-	24749	2447	N/D	N/D	N/D	N/D	N/D	N/D	N/D	1.937 ± 0.001	1.571 ± 0.001	1.943 ± 0.001	2.356 ± 0.364
Benzeneacetaldehyde	13100	1902	42.173 ± 36.899	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2,4-Heptadienal, (E,E)-	10790	1565	11.650 ± 2.094	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2,4-Octadienal, (E,E)-	14280	1984	1.470 ± 0.268	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2,4-Decadienal, (E,E)-	15185	2047	1.931 ± 0.078	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Octenal, (E)-	9651	1398	10.163 ± 1.793	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Octadecanal	18675	2208	8.282 ± 0.132	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Tetradecanal	17349	2155	0.998 ± 0.104	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

2,4-Dodecadienal, (E,E)-	15868	2095	1.687 ± 0.268	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Thiophenecarboxaldehyde	13836	1953	N/D	8.427 ± 2.369	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1,2-Benzenedicarboxaldehyde	11008	1596	30.605 ± 2.825	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4-Hydroxy-2-methoxybenaldehyde	24759	2448	1.160 ± 0.211	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Esteres													
Methyl salicylate	15042	2037	15.205 ± 2.233	10.311 ± 2.028	17.647 ± 2.060	10.808 ± 1.090	20.511 ± 3.945	16.672 ± 1.019	13.957 ± 1.795	39.879 ± 12.644	59.973 ± 3.858	66.167 ± 1.924	51.775 ± 7.704
Benzeneacetic acid, ethyl ester	15254	2052	18.900 ± 1.928	N/D	N/D	N/D	19.096 ± 4.948	13.331 ± 0.544	13.077 ± 2.243	33.839 ± 8.762	41.566 ± 1.903	55.949 ± 4.368	61.904 ± 5.899
Benzoic acid, 2-hydroxy-, ethyl ester	15577	2074	1.422 ± 0.196	N/D	12.803 ± 1.907	N/D	13.216 ± 0.000	12.577 ± 0.611	8.525 ± 0.001	23.367 ± 0.000	31.555 ± 2.873	54.753 ± 0.669	23.503 ± 3.494
1,2-Benzenedicarboxyli	24301	2430	1.704 ± 0.983	N/D	N/D	N/D	15.665 ± 0.000	N/D	N/D	N/D	N/D	N/D	N/D

c acid, bis(2-methylpropyl) ester													
Hexadecanoic acid, ethyl ester	21263	2310	N/D	3.789 ± 5.359	18.850 ± 0.000	10.441 ± 0.000	17.873 ± 1.624	15.257 ± 1.203	16.377 ± 1.751	N/D	N/D	N/D	N/D
Hexadecanoic acid, methyl ester	20821	2292	3.393 ± 0.213	N/D	1.140 ± 0.491	1.014 ± 0.123	1.436 ± 0.140	1.162 ± 0.076	1.133 ± 0.069	N/D	N/D	N/D	N/D
Benzeneacetic acid, methyl ester	14849	2024	7.711 ± 0.601	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Acetic acid, 2-phenylethyl ester	15700	2083	1.024 ± 0.044	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
N-Cbz-glycylglycine p-nitrophenyl ester	16599	2126	17.899 ± 1.722	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
12-Octadecenoic acid, methyl ester	23230	2387	0.277 ± 0.033	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Cyclobutanecarboxylic acid, 4-tridecyl ester	16760	2132	1.403 ± 0.992	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pentadecanoic acid, 14-methyl-, methyl ester	20820	2292	N/D	1.215 ± 0.098	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Tetradecanoic acid, ethyl ester	18899	2220	0.391 ± 0.029	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Ethyl Oleate	23603	2402	0.199 ± 0.040	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Benzoic acid, ethyl ester	13489	1928	2.178 ± 0.345	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
9,12-Octadecadienoic acid, ethyl ester	24079	2421	3.492 ± 1.035	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Ketone													
2-Butanone, 1-(acetyloxy)-	11349	1646	N/D	60.424 ± 14.365	76.671 ± 21.912	81.350 ± 6.320	70.050 ± 10.820	46.592 ± 7.444	59.846 ± 5.939	192.576 ± 32.326	195.975 ± 6.859	236.362 ± 10.516	232.923 ± 9.197
4-Cyclopentene-1,3-dione	11925	1730	N/D	6.861 ± 9.702	9.304 ± 0.854	12.823 ± 1.189	9.759 ± 1.285	9.969 ± 0.694	11.794 ± 0.739	26.848 ± 1.222	24.889 ± 0.967	23.504 ± 0.936	23.830 ± 1.101
1,2-Cyclopentanedione, 3-methyl-	16162	2109	N/D	N/D	22.804 ± 7.118	19.322 ± 0.000	20.139 ± 14.241	N/D	N/D	63.412 ± 12.133	61.694 ± 1.993	N/D	60.456 ± 5.537
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	17060	2144	N/D	6.138 ± 0.538	9.570 ± 2.816	8.330 ± 0.920	9.421 ± 1.845	6.518 ± 1.535	7.701 ± 1.847	27.812 ± 6.736	26.204 ± 1.191	29.228 ± 0.488	27.712 ± 2.904

5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	16352	2116	0.740 ± 0.011	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Pentadecanone, 6,10,14-trimethyl-	19864	2255	0.361 ± 0.056	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenols													
Ethanone, 1-(1H-pyrrol-2-yl)-	17840	2175	N/D	33.954 ± 3.314	51.435 ± 9.379	47.761 ± 2.017	54.434 ± 9.070	41.111 ± 5.993	43.073 ± 7.458	125.105 ± 29.774	120.589 ± 2.929	143.121 ± 4.576	139.713 ± 13.721
(Pg199)Phenol, 4-ethyl-2-methoxy-	18486	2200	N/D	N/D	9.698 ± 1.891	8.088 ± 0.335	9.429 ± 2.257	6.517 ± 0.310	7.209 ± 1.788	19.443 ± 6.226	18.937 ± 0.718	30.395 ± 0.198	25.732 ± 4.722
(Pg199)2-Methoxy-4-vinylphenol	20500	2280	N/D	N/D	154.420±0.484	141.485±4.995	145.176±0.000	154.203±8.607	152.725±24.281	158.798±66.206	184.215±20.909	276.928±7.130	248.737±72.185
Phenol, 2-methoxy-3-(2-propenyl)-	20105	2264	0.187 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenol	18179	2188	0.464 ± 0.054	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Acetophenone	13281	1914	1.900 ± 0.131	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenol, 4-(1,1-dimethylpropyl)-	22834	2372	0.595 ± 0.006	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Hydrazine, (phenylmethyl)-	17104	2146	39.304 ± 4.385	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy-	23338	2392	N/D	0.551 ± 0.780	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pyrans													
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	21392	2315	N/D	N/D	N/D	N/D	N/D	15.208 ± 1.767	16.156 ± 2.883	30.693 ± 1.095	31.681 ± 4.024	19.371 ± 0.001	26.617 ± 4.649
Pyrazines													
Pyrazine, methyl-	6005	863	N/D	127.534 ± 41.465	362.16 ± 243.781	235.968 ± 274.165	350.933 ± 119.195	368.169 ± 92.682	147.182 ± 14.682	574.573 ± 227.654	415.093 ± 29.130	322.897 ± 39.349	1162.437 ± 37.364
Pyrazine, 2-ethyl-6-methyl-	8976	1296	N/D	68.832 ± 17.138	83.576 ± 24.233	78.846 ± 37.185	65.823 ± 6.899	36.905 ± 6.622	47.352 ± 4.398	201.050 ± 60.334	271.290 ± 10.374	300.191 ± 6.719	285.395 ± 32.259
Pyrazine, 2-ethyl-5-methyl-	9137	1322	N/D	38.444 ± 27.379	71.256 ± 0.001	84.631 ± 16.461	46.349 ± 6.982	58.094 ± 5.200	69.529 ± 8.619	270.117 ± 12.025	251.098 ± 8.988	193.982 ± 117.544	198.454 ± 134.136
Pyrazine, 2-ethyl-3-methyl-	9434	1365	N/D	20.781 ± 3.198	23.844 ± 7.450	28.853 ± 1.170	20.847 ± 5.168	14.595 ± 6.164	22.944 ± 3.932	101.116 ± 5.133	71.314 ± 37.415	110.728 ± 10.734	105.638 ± 2.949

Pyrazine, trimethyl-	9625	1393	N/D	N/D	66.338 ± 20.231	72.204 ± 5.348	58.605 ± 0.001	41.527 ± 25.808	66.106 ± 10.162	248.704 ± 23.190	264.166 ± 2.452	235.406 ± 5.772	261.859 ± 11.158
Pyrazine, 3-ethyl-2,5-dimethyl-	10273	1488	N/D	47.643 ± 13.917	71.511 ± 18.256	74.401 ± 8.477	61.265 ± 17.357	55.838 ± 1.547	71.408 ± 10.608	289.676 ± 30.641	292.600 ± 0.568	310.561 ± 1.395	315.823 ± 15.304
5H-5-Methyl-6,7-dihydrocyclopentapyrazine	13251	1912	N/D	8.094 ± 2.003	13.359 ± 0.001	11.500 ± 0.376	10.613 ± 2.718	9.165 ± 1.134	11.075 ± 1.783	39.814 ± 6.734	38.528 ± 0.598	40.594 ± 0.471	45.731 ± 2.829
1-(6-Methyl-2-pyrazinyl)-1-ethanone	14154	1975	N/D	23.837 ± 3.661	33.256 ± 6.116	29.405 ± 0.411	30.059 ± 3.364	28.042 ± 4.591	31.626 ± 4.321	91.993 ± 26.389	110.301 ± 7.195	111.694 ± 3.655	111.834 ± 11.677
2-Acetyl-3-methylpyrazine	13969	1962	N/D	9.591 ± 1.82	12.989 ± 2.867	11.682 ± 0.051	11.188 ± 2.094	10.297 ± 1.603	12.591 ± 1.997	51.357 ± 0.001	48.461 ± 1.986	51.086 ± 0.001	51.967 ± 4.075
Pyrazine, 2,3-dimethyl-	8140	1176	N/D	N/D	35.703 ± 15.303	35.639 ± 11.341	20.410 ± 0.000	34.496 ± 13.485	23.662 ± 6.830	55.282 ± 13.768	60.239 ± 23.522	122.6 ± 0.000	41.156 ± 18.714
Pyrazine, 3,5-diethyl-2-methyl-	11119	1612	N/D	N/D	9.196 ± 0.001	N/D	N/D	10.967 ± 0.001	12.861 ± 0.001	N/D	43.942 ± 0.001	N/D	54.296 ± 0.001
Pyrazine, 2-methyl-6-(1-propenyl)-, (Z)-	13773	1949	N/D	N/D	4.992 ± 0.449	4.747 ± 0.204	4.778 ± 0.620	4.532 ± 0.020	5.127 ± 0.720	N/D	13.154 ± 1.015	16.714 ± 0.051	13.332 ± 0.001
1-Methylpyrrolo[1,2-	15825	2092	N/D	N/D	N/D	N/D	N/D	5.926 ± 0.570	7.566 ± 0.001	N/D	N/D	N/D	N/D

a]pyrazine													
4-Methylpyrrolo[1,2-a]pyrazine	18653	2310	N/D	N/D	5.993 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pyridine													
3-Pyridinol, 6-methyl-	12785	1856	N/D	2.129 ± 0.147	53.414 ± 9.932	62.024 ± 8.335	57.053 ± 13.085	39.594 ± 0.648	44.293 ± 4.958	149.991 ± 12.174	146.881 ± 0.613	167.685 ± 1.333	165.742 ± 11.216
2-Pyrrolidinone, 1-butyl-	21652	2325	N/D	8.347 ± 0.001	10.482 ± 4.275	9.227 ± 0.488	N/D	11.081 ± 0.419	10.304 ± 0.729	10.727 ± 1.103	11.204 ± 1.834	10.079 ± 0.842	11.704 ± 2.802
Indolizine	23120	2383	N/D	8.675 ± 0.152	12.386 ± 0.616	12.063 ± 0.056	12.688 ± 0.000	13.832 ± 0.724	13.653 ± 1.777	11.092 ± 4.805	13.271 ± 1.494	20.089 ± 0.884	20.426 ± 5.738
3-Pyridinol	23382	2393	N/D	30.77 ± 0.267	37.087 ± 14.315	33.844 ± 4.783	38.461 ± 0.000	N/D	34.028 ± 1.998	26.419 ± 0.926	39.431 ± 6.273	42.821 ± 0.396	30.145 ± 8.668
Picolinamide	4120	585	N/D	N/D	81.325 ± 9.497	82.095 ± 0.000	57.763 ± 0.078	77.905 ± 37.012	106.673 ± 83.253	84.389 ± 20.525	171.218 ± 13.847	69.788 ± 10.28	162.973 ± 135.144
4(H)-Pyridine, N-acetyl-	14199	1978	N/D	N/D	N/D	N/D	N/D	N/D	N/D	104.586 ± 15.795	N/D	N/D	N/D
Ethyl nicotinate	15974	2101	N/D	N/D	3.567 ± 0.001	N/D	3.539 ± 0.521	2.530 ± 0.174	2.502 ± 0.471	N/D	7.013 ± 0.611	8.913 ± 0.044	7.688 ± 1.547

Pyridine, 2-chloro-6-(2-furanylmethoxy)-4-(trichloromethyl)-	23658	2404	N/D	N/D	N/D	N/D	N/D	N/D	N/D	2.722 ± 0.001	N/D	2.913 ± 0.219	3.777 ± 0.001
2-Pyrrolidinone	19511	2241	N/D	N/D	44.842 ± 3.636	44.900 ± 1.377	51.681 ± 2.359	51.013 ± 5.530	48.468 ± 5.218	81.759 ± 3.272	80.806 ± 7.580	70.765 ± 2.630	82.821 ± 8.733
Pyrimidine													
Pyrimidine, 4,6-dimethyl-	7676	1108	N/D	270.477 ± 138.562	263.073 ± 139.963	324.687 ± 195.244	309.766 ± 0.001	231.332 ± 136.973	311.95 ± 181.508	858.692 ± 436.661	839.528 ± 278.842	542.058 ± 199.534	1221.332 ± 66.299
Pyrrole													
1-Ethyl-2-pyrrolidinone	15211	2049	N/D	2.814 ± 3.980	8.801 ± 2.840	8.685 ± 0.001	12.305 ± 0.078	9.007 ± 2.884	8.030 ± 1.680	23.531 ± 3.284	21.529 ± 3.194	20.817 ± 0.128	22.787 ± 0.822
1H-Pyrrole, 1-(2-furanylmethyl)-	15700	2083	N/D	32.781 ± 10.854	39.487 ± 7.510	43.562 ± 4.229	39.768 ± 11.105	28.392 ± 2.308	36.164 ± 5.272	96.575 ± 18.793	101.346 ± 0.351	138.491 ± 1.009	133.658 ± 13.115
1,5-Dimethyl-2-pyrrolecarbonitrile	12656	1837	N/D	N/D	N/D	N/D	N/D	10.244 ± 0.112	N/D	N/D	N/D	N/D	N/D
Thiophenes													
2-Thiophenemethanol	17430	2159	N/D	2.535 ± 0.378	3.749 ± 0.973	3.899 ± 0.076	4.211 ± 0.785	3.121 ± 0.643	3.218 ± 0.652	9.601 ± 1.959	8.807 ± 0.202	10.515 ± 0.103	10.451 ± 1.086

Other													
Caffeine	24014	2420	4.941 ± 0.000	16.530 ± 1.619	129.644 ± 0.001	132.907 ± 7.502	91.648 ± 0.001	N/D	130.340 ± 0.000	40.993 ± 0.000	14.53 ± 8.256	44.548 ± 0.000	10.772 ± 0.000
Maltol	18004	2181	N/D	63.408 ± 2.395	106.572 ± 14.513	97.153 ± 12.141	124.339 ± 6.410	93.485 ± 16.234	93.361 ± 12.474	193.467 ± 41.271	216.271 ± 19.505	207.389 ± 8.638	187.979 ± 35.171
Heptanediamide, N,N'-di-benzoyloxy-	23336	2391	N/D	N/D	0.696 ± 0.985	1.281 ± 0.183	N/D	1.329 ± 0.001	1.370 ± 0.201	14.212 ± 3.483	7.158 ± 0.833	4.762 ± 0.690	2.905 ± 0.243
4-Ethylbenzoic acid	25380	2472	N/D	N/D	N/D	N/D	N/D	N/D	N/D	1.570 ± 0.307	0.792 ± 0.000	N/D	N/D
Isobutyl ether	11264	1634	N/D	N/D	79.127 ± 20.788	86.866 ± 7.509	72.336 ± 16.194	47.347 ± 7.961	62.392 ± 8.754	183.956 ± 16.048	213.182 ± 7.708	208.933 ± 1.948	203.801 ± 4.502

Nota: 1: *Meyerozyma caribbica* (CCMA 1993); 2: *Meyerozyma caribbica* (CCMA1950); 3: *Meyerozyma caribbica* (CCMA1992); 4: *Meyerozyma caribbica* (CCMA1617); 5: *Hanseniaspora uvarum* (CCMA 1944); 6: *Pichia Kluyveri* (CCMA1658); 7: *Meyerozyma guillermondii* (CCMA1737); 8: *Cystofilobasidium ferigula* (CCMA1647); 9: *Meyerozyma caribbica* (CCMA1735); N/D: Not detected

Table.C3. Volatile compounds of pulped process

	FC	RC	R1	R2	R3	R4	R5	R6	R7	R8	R9
Acids											
Benzeneacetic acid	N/D	2.318 ± 0.656	4.012 ± 0.438	2.347 ± 0.340	2.267 ± 0.006	1.724 ± 0.564	1.810 ± 0.098	2.554 ± 0.501	2.887 ± 0.258	3.558 ± 0.387	5.473 ± 0.869
Butanoic acid. 3-bromo-3-methyl-	N/D	N/D	68.658 ± 7.147	59.427 ± 0.001	54.122 ± 2.272	39.112 ± 2.460	36.539 ± 0.532	59.677 ± 2.251	45.013 ± 17.646	61.208 ± 5.309	53.374 ± 14.067
Butanoic acid. 3-methyl-	4.494 ± 2.143	360.787 ± 4.964	339.709 ± 38.646	292.279 ± 36.188	298.016 ± 14.107	237.226 ± 2.010	212.947 ± 13.567	343.838 ± 6.12	196.781 ± 145.038	323.421 ± 18.38	280.352 ± 72.526
n-Hexadecanoic acid	4.714 ± 1.241	0.568 ± 0.132	0.888 ± 0.012	0.815 ± 0.119	1.044 ± 0.103	0.663 ± 0.069	0.975 ± 0.105	1.318 ± 0.363	1.857 ± 0.640	1.564 ± 0.390	2.902 ± 0.001
Propanoic acid	N/D	68.631 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Butenoic acid. 2-methyl-. (Z)-	N/D	70.477 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Pyrroles											
1H-Pyrrole-2-carboxaldehyde	N/D	205.099 ± 22.800	271.968 ± 9.733	225.655 ± 17.397	247.306 ± 10.718	171.014 ± 23.113	167.506 ± 12.498	227.703 ± 17.185	213.858 ± 35.035	280.390 ± 19.902	255.546 ± 50.321
1H-Pyrrole-2-carboxaldehyde. 1-methyl-	N/D	184.776 ± 1.106	11.100 ± 0.001	N/D	17.128 ± 0.000	N/D	N/D	N/D	16.927 ± 0.000	N/D	N/D

1H-Pyrrole-2-carboxaldehyde. 5-methyl-	N/D	N/D	83.376 ± 1.863	66.762 ± 0.392	79.383 ± 3.759	44.941 ± 12.361	49.254 ± 6.120	60.469 ± 9.126	68.706 ± 5.166	100.460 ± 9.186	88.982 ± 16.867
1H-Pyrrole. 1-(2-furanylmethyl)-	N/D	N/D	150.179 ± 6.359	166.968 ± 11.48	169.792 ± 2.868	191.453 ± 18.079	253.537 ± 8.527	174.938 ± 1.440	133.305 ± 52.372	176.714 ± 7.536	167.660 ± 24.185
5-Hydroxymethylfurfural	N/D	N/D	43.784 ± 5.318	20.129 ± 2.131	17.124 ± 0.024	17.700 ± 6.940	13.614 ± 0.454	19.772 ± 3.931	24.531 ± 2.411	27.763 ± 0.491	30.404 ± 3.159
Aldehyde											
5-Acetoxymethyl-2-furaldehyde	N/D	5.559 ± 1.160	8.234 ± 0.356	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Benzeneacetaldehyde. .alpha.-ethylidene-	N/D	7.608 ± 2.036	8.773 ± 0.098	6.794 ± 0.420	7.457 ± 0.062	6.58 ± 1.792	7.760 ± 0.125	7.99 ± 0.692	7.527 ± 1.132	8.810 ± 0.715	9.250 ± 1.591
Benzaldehyde. 3-hydroxy-4-methoxy-	N/D	2.216 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Benzeneacetaldehyde	24.459 ± 12.098	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2.4-Octadienal. (E.E)-	0.356 ± 0.000	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Octenal. (E)-	1.631 ± 0.000	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Octadecanal	1.241 ± 0.281	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Tetradecanal	0.224 ± 0.028	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2.4-Dodecadienal. (E.E)-	0.702 ± 0.219	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1.2-Benzenedicarboxaldehyde	31.189 ± 3.572	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4-Hydroxy-2-methoxybenaldehyde	1.009 ± 0.561	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Furan											
2-Furancarboxaldehyde. 5-methyl-	N/D	1525.764 ± 9.984	1500.944 ± 85.696	1444.430 ± 78.813	1328.405 ± 25.28	1562.641 ± 26.392	1485.202 ± 29.165	1613.293 ± 3.220	889.981 ± 809.197	1392.292 ± 0.001	1381.425 ± 164.679
Benzofuran. 2.3-dihydro-	N/D	9.219 ± 2.439	15.692 ± 2.216	9.026 ± 0.643	10.477 ± 0.000	8.453 ± 2.722	8.239 ± 0.266	10.719 ± 0.000	N/D	16.940 ± 2.021	16.338 ± 3.579
2-Furanmethanol	N/D	1375.555 ± 15.01	1510.844 ± 123.111	1455.151 ± 135.527	1492.401 ± 59.285	1042.175 ± 20.823	948.908 ± 39.276	1534.298 ± 9.116	863.398 ± 923.580	1644.718 ± 45.496	1529.456 ± 297.897
2.5-Dimethyl-4-hydroxy-3(2H)-furanone	N/D	98.951 ± 21.204	173.552 ± 7.805	99.726 ± 3.434	105.162 ± 7.706	57.637 ± 14.452	53.369 ± 10.232	88.628 ± 9.376	96.397 ± 25.223	169.237 ± 15.894	157.052 ± 45.204

3(2H)-Furanone. dihydro-2-methyl-	N/D	95.144 ± 6.672	70.786 ± 61.947	81.314 ± 44.025	102.405 ± 5.612	27.226 ± 4.706	49.763 ± 37.309	79.091 ± 71.121	104.612 ± 0.000	98.750 ± 0.690	70.997 ± 64.465
2-Furanmethanol. acetate	N/D	409.694 ± 3.100	393.907 ± 35.814	463.009 ± 34.654	529.743 ± 23.971	650.597 ± 27.869	714.177 ± 10.547	523.667 ± 12.925	233.027 ± 283.468	468.759 ± 7.600	425.545 ± 49.341
Bis(2-furfuryl)disulfide	N/D	N/D	N/D	N/D	N/D	55.888 ± 0.000	72.815 ± 0.000	N/D	N/D	N/D	N/D
Ethanone. 1-(2-furanyl)-	N/D	474.632 ± 18.046	430.384 ± 38.463	435.312 ± 31.441	424.728 ± 17.491	497.471 ± 26.188	465.205 ± 16.216	502.706 ± 2.743	445.026 ± 0.000	410.568 ± 1.273	379.102 ± 58.683
Ethanethioic acid. S-(2-furanylmethyl) ester	N/D	N/D	20.120 ± 2.822	9.829 ± 4.176	8.591 ± 0.655	12.715 ± 4.146	15.360 ± 1.422	12.974 ± 5.873	12.776 ± 0.878	18.255 ± 3.055	16.714 ± 4.688
2-Furanmethanol. Propanoate	N/D	N/D	N/D	N/D	N/D	24.991 ± 0.633	33.606 ± 0.764	N/D	N/D	N/D	N/D
trans-Furfurylideneacetone	N/D	11.964 ± 2.428	16.627 ± 0.489	14.173 ± 0.061	16.800 ± 0.047	12.922 ± 3.209	14.660 ± 0.049	14.668 ± 1.365	16.104 ± 0.000	18.724 ± 1.611	17.313 ± 2.955
Furfural	N/D	2133.825 ± 29.457	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
.alpha.-Furfuryliden-.alpha.-furylmethylamine	N/D	39.66 ± 11.908	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Furan. 2-[(methyldithio)methyl]-	N/D	8.728 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Esters											
Methyl salicylate	7.190 ± 0.526	40.07 ± 6.799	52.007 ± 0.969	N/D	N/D	47.58 ± 6.378	39.046 ± 0.422	40.823 ± 0.979	16.413 ± 0.000	N/D	38.505 ± 0.000
Ketones											
2-Butanone. 1-(acetyloxy)-	N/D	313.253 ± 11.457	330.463 ± 28.611	335.318 ± 41.393	332.712 ± 19.646	232.557 ± 3.822	206.322 ± 1.031	352.078 ± 5.209	352.664 ± 0.000	354.555 ± 8.134	319.301 ± 62.832
2-Cyclopenten-1-one. 3-ethyl-2-hydroxy-	N/D	35.613 ± 6.620	32.424 ± 3.021	32.264 ± 3.500	33.956 ± 1.340	29.991 ± 0.539	30.613 ± 0.001	34.397 ± 0.809	34.536 ± 0.001	35.482 ± 1.872	32.002 ± 7.018
1.2-Cyclopentanedione. 3-methyl-	N/D	87.377 ± 0.001	121.838 ± 7.344	94.507 ± 10.359	106.491 ± 6.171	48.185 ± 0.001	N/D	85.553 ± 5.081	93.510 ± 17.800	137.620 ± 11.879	117.789 ± 36.687
1-Hydroxy-2-butanone	N/D	N/D	89.389 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2.3-Pentanedione	N/D	266.121 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4-Cyclopentene-1.3-dione	N/D	28.972 ± 1.020	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5.9-Undecadien-2-one. 6.10-dimethyl-. (E)-	0.531 ± 0.104	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Pentadecanone. 6.10.14-trimethyl-	0.256 ± 0.081	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Pyrazines											
2-Acetyl-3-methylpyrazine	N/D	51.900 ± 6.371	56.948 ± 1.957	52.407 ± 2.968	49.182 ± 0.001	N/D	N/D	52.913 ± 1.328	N/D	59.811 ± 3.172	50.068 ± 0.001
Pyrazine. methyl-	N/D	280.438 ± 191.167	286.501 ± 18.879	386.453 ± 52.521	801.597 ± 340.996	306.227 ± 54.142	479.726 ± 448.778	851.466 ± 586.256	432.283 ± 0.001	285.868 ± 27.496	666.124 ± 442.665
Pyrazine. 2-ethyl-5-methyl-	N/D	303.278 ± 1.870	283.341 ± 29.088	259.600 ± 20.469	271.229 ± 12.063	242.301 ± 109.067	N/D	328.706 ± 0.766	318.526 ± 0.001	290.349 ± 0.001	233.403 ± 24.338
Pyrazine. 3-ethyl-2.5-dimethyl-	N/D	259.619 ± 11.847	257.336 ± 19.153	253.670 ± 23.573	218.586 ± 6.738	244.468 ± 0.001	243.835 ± 0.925	269.337 ± 3.064	175.85 ± 189.775	252.421 ± 9.327	232.143 ± 38.273
Pyrazine. 2-methyl-5-(1-propenyl)-. (E)-	N/D	20.089 ± 0.001	21.704 ± 0.274	20.235 ± 1.302	21.299 ± 0.541	N/D	N/D	21.002 ± 1.158	21.797 ± 0.001	24.056 ± 2.654	12.793 ± 18.093
Pyrazine. 2-ethyl-3-methyl-	N/D	91.649 ± 9.040	N/D	95.159 ± 0.001	N/D	70.707 ± 3.869	N/D	103.952 ± 0.001	N/D	N/D	N/D
Pyrazine. trimethyl-	N/D	220.076 ± 0.001	214.613 ± 18.418	224.077 ± 18.676	200.91 ± 7.072	145.848 ± 0.301	148.922 ± 5.730	224.14 ± 3.721	123.484 ± 174.632	227.991 ± 0.001	127.190 ± 179.874
1-(6-Methyl-2-pyrazinyl)-1-ethanone	N/D	114.295 ± 15.064	132.867 ± 1.222	118.390 ± 3.929	117.480 ± 3.514	76.741 ± 13.381	72.437 ± 8.878	111.851 ± 3.975	82.316 ± 68.338	137.388 ± 7.661	132.173 ± 21.003
5H-5-Methyl-6.7-dihydrocyclopentapyrazine	N/D	40.601 ± 4.098	43.588 ± 2.581	20.365 ± 28.801	41.297 ± 1.365	32.685 ± 3.994	34.776 ± 0.782	42.928 ± 0.827	44.005 ± 0.000	42.658 ± 0.001	44.921 ± 8.465

Pyrazine. 2-ethenyl-6-methyl-	N/D	N/D	N/D	53.152 ± 3.714	48.991 ± 1.718	57.798 ± 0.014	60.217 ± 0.650	61.188 ± 0.355	59.334 ± 0.001	57.539 ± 0.247	24.615 ± 34.811
Pyrazine. 2-ethyl-6-methyl-	N/D	294.34 ± 8.599	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Quinoxaline	N/D	N/D	N/D	8.546 ± 0.001	N/D	N/D	N/D	N/D	9.652 ± 0.001	13.272 ± 0.001	5.466 ± 7.731
Pyrazine. 2-methoxy-3-(2-methylpropyl)-	4.965 ± 1.865										
Phenols											
Ethanone. 1-(1H-pyrrol-2-yl)-	N/D	148.053 ± 22.319	203.692 ± 9.205	180.320 ± 17.012	207.158 ± 8.276	125.286 ± 24.420	137.921 ± 11.951	168.627 ± 14.605	169.458 ± 27.287	236.193 ± 18.672	206.326 ± 48.158
Cyclobutane-1.1-dicarboxamide. N.N'-di-benzoyloxy-	N/D	2.438 ± 0.214	2.817 ± 0.223	N/D	N/D	1.130 ± 0.000	N/D	1.668 ± 0.141	1.907 ± 0.250	2.313 ± 0.275	2.228 ± 0.501
Phenol. 4-ethyl-2-methoxy-	N/D	N/D	35.617 ± 0.061	37.688 ± 2.927	46.541 ± 0.267	29.424 ± 9.294	45.223 ± 0.855	32.809 ± 4.583	37.047 ± 4.463	57.899 ± 6.482	47.424 ± 10.084
Phenol. 2-methoxy-	N/D	N/D	N/D	55.250 ± 0.001	56.325 ± 1.774	40.436 ± 0.001	58.861 ± 0.001	48.428 ± 0.001	38.615 ± 0.001	58.444 ± 0.001	N/D
4-Hydroxy-3-methylacetophenone	N/D	229.644 ± 66.19	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D

Phenol. 2-methoxy-3-(2-propenyl)-	0.320 ± 0.150	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenol	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Acetophenone	1.234 ± 0.041	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Phenol. 4-(1.1-dimethylpropyl)-	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Hydrazine. (phenylmethyl)-	29.95 ± 4.055	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Lactones											
2(3H)-Furanone. dihydro-5-propyl-	N/D	47.498 ± 0.001	89.234 ± 8.970	63.256 ± 5.785	84.234 ± 5.086	43.552 ± 15.548	42.192 ± 1.529	56.219 ± 8.406	62.914 ± 5.942	90.481 ± 2.900	91.719 ± 17.361
(S)-(+)-2'.3'-Dideoxyribonolactone	N/D	N/D	N/D	13.025 ± 0.001	N/D	N/D	N/D	11.306 ± 0.001	N/D	N/D	N/D
2-Hydroxy-gamma-butyrolactone	N/D	N/D	N/D	N/D	N/D	9.456 ± 0.001	N/D	N/D	N/D	N/D	N/D
2(3H)-Furanone. dihydro-5-pentyl-	0.903 ± 0.179										
Pyrimidine											
Pyrimidine. 4.6-dimethyl-	N/D	1029.523 ±	1275.974 ±	623.650 ±	1263.827 ±	1030.109 ±	533.680 ±	1077.957 ±	748.883 ±	1392.238 ±	962.476 ±

		528.378	171.665	23.819	77.341	65.771	638.604	619.729	1011.090	7.905	322.892
Pyridine											
Pyridine	N/D	N/D	54.184 ± 0.001	166.000 ± 0.001	169.677 ± 0.001	N/D	27.312 ± 0.001	N/D	N/D	150.609 ± 0.001	689.048 ± 147.327
3-Pyridinol	N/D	32.185 ± 14.313	64.177 ± 8.653	54.911 ± 7.704	75.196 ± 5.724	52.071 ± 20.391	64.927 ± 10.936	57.218 ± 6.372	65.315 ± 7.875	83.323 ± 4.478	73.775 ± 4.422
3-Pyridinol. 6-methyl-	N/D	2.190 ± 1.067	1.748 ± 0.001	3.703 ± 0.001	4.671 ± 0.069	2.953 ± 1.743	4.120 ± 0.628	3.526 ± 0.484	4.104 ± 1.536	5.571 ± 0.594	4.642 ± 0.455
1.2-Ethanediol. 1.2-di-4-pyridinyl-. (R*.R*)-(.+/-.)-	N/D	N/D	188.757 ± 15.177	206.849 ± 17.846	208.778 ± 5.438	213.976 ± 3.103	226.8 ± 6.588	227.266 ± 0.249	201.248 ± 0.001	202.967 ± 2.968	195.271 ± 31.387
2-Pyridinecarboxylic acid	N/D	291.816 ± 261.597	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Pyrrolidinone. 1-butyl-	N/D	8.808 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Indolizine	N/D	16.975 ± 3.568	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Alcohols											
1.6-Octadien-3-ol. 3.7-dimethyl-	3.41 ± 1.113	7.707 ± 0.702	6.831 ± 0.556	4.774 ± 0.499	6.039 ± 0.142	9.723 ± 0.540	9.370 ± 0.895	7.333 ± 0.110	6.387 ± 0.001	4.942 ± 0.001	5.506 ± 0.911

3-Pentanol	N/D	44.287 ± 3.484	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
(S)-3-Ethyl-4-methylpentanol	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Octen-3-ol	3.799 ± 0.745	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Octanol	1.304 ± 0.370	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
n-Pentadecanol	0.268 ± 0.037	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Hexanol	6.009 ± 6.113	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2-Octen-1-ol. (E)-	0.489 ± 0.018	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Nonanol	0.525 ± 0.138	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
1-Heptanol	0.449 ± 0.001	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Thiophenes											
2-Thiophenemethanol	N/D	11.887 ± 1.199	15.076 ± 1.450	12.450 ± 1.726	13.336 ± 0.390	8.339 ± 1.297	8.180 ± 0.581	12.487 ± 0.879	11.071 ± 2.839	15.668 ± 1.177	13.940 ± 3.529

Other											
Maltol	N/D	223.895 ± 56.439	446.516 ± 40.777	318.163 ± 5.606	445.317 ± 30.944	206.666 ± 56.525	234.829 ± 33.846	300.951 ± 53.581	363.581 ± 17.278	545.791 ± 63.513	462.694 ± 116.809
1.3-Dioxan-5-ol. 4.4.5-trimethyl-	N/D		17.748 ± 0.001	32.262 ± 0.001	35.308 ± 0.000	N/D	N/D	N/D	N/D	N/D	46.683 ± 11.264
Caffeine	41.324 ± 0.001	36.997 ± 0.001	45.486 ± 4.297	40.937 ± 0.001	25.058 ± 7.156	20.496 ± 19.192	51.332 ± 6.898	34.056 ± 35.163	37.926 ± 43.086	94.674 ± 0.001	74.354 ± 7.453
m-Aminophenylacetylene	N/D	N/D	25.189 ± 1.849	17.571 ± 0.820	20.241 ± 0.844	16.595 ± 4.950	18.126 ± 0.545	17.079 ± 3.597	19.484 ± 1.038	28.793 ± 3.871	28.365 ± 5.515
1.2-Ethanediol. dipropanoate	N/D	N/D	281.514 ± 29.072	308.385 ± 2.269	293.119 ± 6.956	213.863 ± 4.768	195.888 ± 2.157	313.258 ± 12.283	305.268 ± 0.001	316.478 ± 8.239	282.123 ± 41.379
1H-Imidazole-4-ethanamine. .beta.-methyl-	N/D	N/D	1877.624 ± 127.333	1616.734 ± 74.166	1355.144 ± 29.811	1973.432 ± 75.825	1646.538 ± 32.85	1953.089 ± 0.806	874.127 ± 1077.745	1457.263 ± 4.516	1488.449 ± 165.567

Nota: 1: *Meyerozyma caribbica* (CCMA 1993); 2: *Meyerozyma caribbica* (CCMA1950); 3: *Meyerozyma caribbica* (CCMA1992); 4: *Meyerozyma caribbica* (CCMA1617); 5: *Hanseniaspora uvarum* (CCMA 1944); 6: *Pichia Kluyveri* (CCMA1658); 7: *Meyerozyma guillermondii* (CCMA1737); 8: *Cystofilobasidium ferigula* (CCMA1647); 9: *Meyerozyma caribbica* (CCMA1735); N/D: Not detected.