DÉBORA MARA DE JESUS CASSIMIRO

IMPACTO DA UTILIZAÇÃO DE BACTÉRIAS ÁCIDO-LÁTICAS E LEVEDURAS SOBRE A QUALIDADE DO CAFÉ FERMENTADO POR VIA ÚMIDA

LAVRAS-MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, para a obtenção do título de Doutora.

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IMPACT OF THE USE OF LACTIC ACID BACTERIA AND YEAST ON THE QUALITY OF WET FERMENTED COFFEE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, para a obtenção do título de Doutora.

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LAVRAS-MG
2022

A Jesus por me sustentar, inspirar e direcionar em todas as etapas.

Aos meus pais e irmãos por estarem sempre ao meu lado.

Ao meu esposo Eluane, por me compreender, incentivar e encorajar.

A todos que de forma direta e/ou indireta contribuíram para
que a concretização dessa etapa fosse possível.

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"*Ninguém ignora tudo. Ninguém sabe tudo. Todos nós sabemos alguma coisa e todos nós ignoramos alguma coisa. Por isso, aprendemos sempre."*

(Paulo Freire)

RESUMO

A fermentação úmida é bastante difundida em algumas regiões produtoras de café, como Havaí, América Central e Colômbia, contudo no Brasil a técnica é pouco utilizada. Este trabalho objetivou avaliar a inoculação das bactérias do ácido lático - *Leuconostoc mesenteroides* CCMA1105 e *Lactiplantibacillus plantarum* CCMA1065 – e das leveduras - *Saccharomyces cerevisiae* CCMA 0543 e *Torulaspora delbruecki* CCMA 0684 - como culturas iniciadoras para fermentar café arábica e café conilon, utilizando a metodologia SIAF (fermentação por anaerobiose autoinduzida). Carboidratos presentes nos frutos foram utilizados no metabolismo microbiano, resultando na produção de metabólitos que impactaram na qualidade do produto final. Além disso, compostos voláteis de diferentes classes (ácidos, ésteres, álcoois, cetonas, pirazinas, furanos, dentre outros) foram identificados entre as amostras de café verde e torrado, auxiliando na formação do perfil sensorial da bebida. Nas amostras do café arábica em que as cepas foram inoculadas as notas finais variaram entre 79,0 e 83,2, portanto, os cafés inoculados podem ser classificadaos como "cafés especiais", de acordo com a Specialty Coffee Association (SCA). Com relação ao café conilon, cafés provenientes de quatro tratamentos fermentados podem ser classificados como bebidas finas (80,0-89,0), enquanto o tratamento Controle e três fermentados podem ser classificados como bebidas *premium* (70,0-79,0). Portanto, a fermentação úmida de frutos de café inoculados com bactérias do ácido lático e leveduras utilizando a metodologia SIAF surge como nova possibilidade para a potencialização da qualidade dos cafés arábica e conilon.

Palavras-chave: SIAF. Fruto íntegro. *L. mesenteroides*. Descritores sensoriais.

ABSTRACT

Wet fermentation is widespread in many coffee-producing regions such as Hawaii, Central America, and Colombia. Nevertheless, Brazilian coffee producers rarely use this technique. Thus, this present study aimed to evaluate the inoculated fermentation of coffee beans using the lactic acid bacteria *Leuconostoc mesenteroides* CCMA1105 and *Lactiplantibacillus plantarum* CCMA1067, and the yeast species *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbruecki* CCMA 0684. Those microorganisms were employed as starter cultures to ferment arabica and conilon coffee beans using the self-induced anaerobiosis fermentation (SIAF) method. The carbohydrates of the coffee beans served as the substrate for microbial metabolism, producing metabolites that benefited the quality of the final product. Moreover, volatile compounds that helped form the beverage's sensory profile, such as acids, esters, alcohols, ketones, pyrazines, and furans, were identified in the green and roasted coffee samples. Arabica coffee samples inoculated with the strains achieved final grades ranging from 79.0 to 83.2. Hence, they were classified as “specialty coffees” according to the Specialty Coffee Association. Regarding conilon coffee samples, four of the fermentation treatments provided coffee classified as fine beverage (80.0-89.0), while the Control treatment and three fermented ones were classified as premium beverage (70.0-79.0). Therefore, considering the results obtained, the wet fermentation of arabica and conilon coffee beans, inoculated with lactic acid bacteria and yeasts, using the SIAF method emerges as a new alternative for enhancing these products' quality.

Keywords: SIAF methodology. Whole fruit. *L. mesenteroides*. Sensory descriptors.

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PRIMEIRA PARTE

1 INTRODUÇÃO

Segundo Coltro *et al*. (2006), *Coffea arabica* e *Coffea canephora* (variedades robusta e conilon) são as duas espécies que dominam o mercado mundial. A bebida oriunda do café arábica é caracterizada como mais suave, ácida e frutada, por outro lado, a bebida do robusta é caracterizada por amargor forte e pronunciado (BERTRAND *et al*., 2003).

O processamento do café pode ser realizado de três formas distintas: via seca, via semisseca e via úmida. Na via seca (ou natural) os frutos de café são diretamente conduzidos a terreiros suspensos (ou plataformas de cimento) para fermentarem, e a secagem ocorre simultaneamente ao processo fermentativo. Na via semisseca, o café é despolpado e a fermentação ocorre com os grãos diretamente expostos ao sol. No processamento úmido, o café é descascado e desmucilado, sendo direcionado a tanques contendo água onde a fermentação se processa (EVANGELISTA *et al*., 2014a, 2015; SILVA *et al*., 2008).

Durante a fermentação espontânea do café, diferentes grupos de microrganismos (bactérias, leveduras e fungos filamentosos) metabolizam os substratos presentes na polpa e mucilagem dos frutos, produzindo metabólitos (como ésteres, álcoois, ácidos orgânicos e outros) que contribuem para a formação do perfil sensorial da bebida e podem potencializar sua qualidade (PEREIRA *et al*., 2022). No entanto, o uso de microrganismos selecionados (culturas iniciadoras) é uma vertente que tem sido explorada e pode originar cafés com características peculiares e distintas, quando comparados aos cafés fermentados espontaneamente (EVANGELISTA *et al*., 2014a, 2014b). A utilização dessas culturas permite consistência ao processo, confiabilidade no desempenho fermentativo, além de segurança do alimento (SOUZA, 2017).

Diversos estudos acerca do efeito da inoculação dos microrganismos para fermentação do café foram realizados, utilizando leveduras (isoladamente ou em cocultivo) em processos naturais ou semissecos (BRESSANI *et al*., 2020, 2021a, 2021b; ELHALIS *et al*., 2020a; RIBEIRO *et al*., 2017). Por outro lado, o método úmido ainda é pouco utilizado no Brasil, assim como o uso de bactérias láticas para fermentação do café (RIBEIRO *et al*., 2020; VALE *et al*., 2019). Essa é uma linha de pesquisa que pode ser mais explorada, para que o efeito da utilização desse grupo de microrganismos sobre a qualidade do café seja estudada e elucidada.

2 REFERENCIAL TEÓRICO

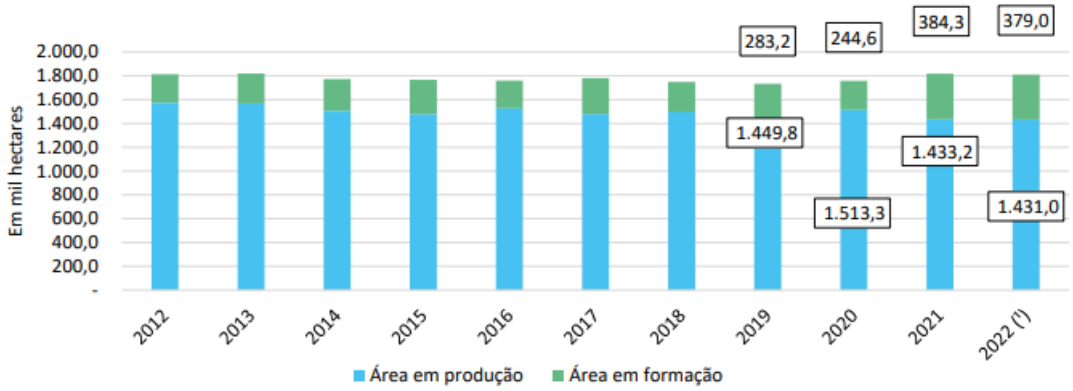
2.1 Café: Origem e mercado

O café é originário da Etiópia, sendo que os holandeses foram responsáveis por trazerem a planta para as Américas do Sul e Central. O café chegou ao Brasil em 1727, quando o português Francisco de Mello Palheta trouxe as primeiras mudas da rubiácea para o estado do Pará. A planta foi trazida da Guiana Francesa, e desde aqueles tempos seu valor comercial era notável. Em 1781 João Alberto de Castello Branco iniciou o plantio no Rio de Janeiro. Em seguida, o cultivo do café teve início em São Paulo, que a partir da década de 1980 passou a ser o principal produtor nacional. A partir do século XVIII o cultivo do café no país concebeu muitos avanços à nação. Os lucros provenientes do cultivo do café, intensificado a partir das décadas de 1930 e 1940 em São Paulo, formam os responsáveis pela implementação das estradas de ferro e avanço da urbanização e, além disso, o deslocamento do centro de poder do Nordeste para o Sudeste também pode ser atribuído ao cultivo do café (ASSOCIAÇÃO BRASILEIRA DA INDÚSTRIA DE CAFÉ - ABIC, 2021).

Dentre as bebidas não alcoólicas, o café é uma das mais consumidas mundialmente (SAKIYAMA; FERRÃO, 2015). Segundo a Companhia Nacional de Abastecimento (CONAB, 2021), foram produzidos 47.716 milhões de sacas (60 kg) entre cafés arábica e conilon. O volume é 24,4% menor, comparado à safra anterior. Os estados com maior produção foram Minas Gerais (22.142,3 milhões de sacas), Espírito Santo (total de 14.166 milhões de sacas – sendo 11.221,00 milhões de sacas de café Conilon e 5.890 de arábica) e São Paulo (4.007,2 mil sacas).

Para o ano de 2022, a expectativa de produção é de 38.783,9 milhões de sacas de arábica e 16.959,2 milhões de sacas de conilon. A estimativa da área destinada ao cultivo do café arábica está em torno 1.809,98 hectares (quase 80% da área total destinada à cafeicultura nacional), e Minas Gerais é o estado com maior área com cultivo da espécie, 1.316,59 hectares - cerca de 70% da área ocupada por cultivo de arábica no país (FIGURA 1). Por outro lado, a estimativa em área total destinada ao cultivo do conilon é de 427 mil hectares, sendo que o estado do Espírito Santo comporta a maior área destinada a essa finalidade no país. Tem-se expectativa de que cerca de 285,4 mil hectares sejam destinados ao cultivo do conilon (CONAB, 2022) (FIGURA 2).

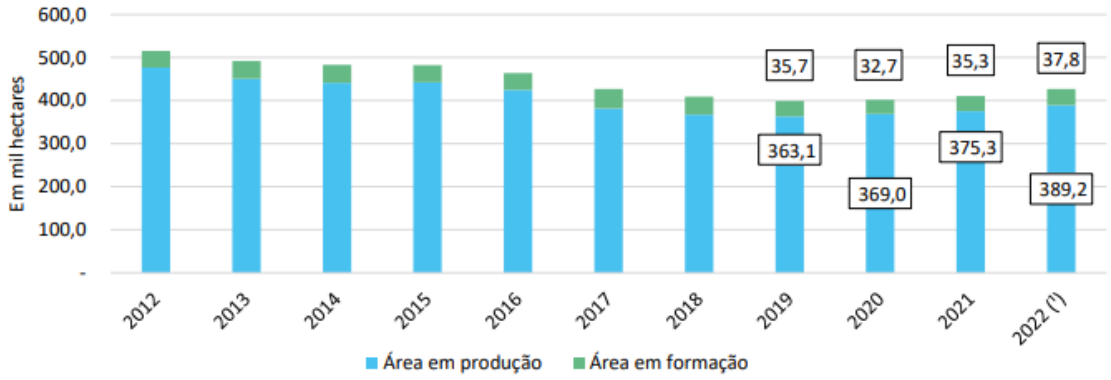
Figura 1 - Áreas em formação e em construção destinadas ao cultivo de café arábica no Brasil.



(1): Estimativa em Janeiro/2022.

Fonte: CONAB (2022).

Figura 2 - Áreas totais em formação e em construção destinadas ao cultivo de café conilon no Brasil.



(1): Estimativa em Dezembro/2021.

Fonte: CONAB (2022).

O café arábica é reconhecido por apresentar maior equilíbrio entre os compostos químicos, levando-o a ser mais valorizado no mercado. Os grãos de café arábica usualmente dão origem a bebidas mais aromáticas, com acidez mais pronunciada e menos encorpadas. No entanto, um estudo revelou que a bebida oriunda do café arábica apresentou menor intensidade de atributos desejáveis de aroma e sabor (como amadeirado, chocolate/chocolate amargo, amadeirado, sabor amargo), em comparação à bebida do *Coffea canephora* (MENDONÇA *et al*., 2007; RIBEIRO *et al*., 2014; SAKIYAMA; FERRÃO, 2015; SENIDE; CHAMBERS; CHAMBERS, 2020). Por outro lado, a variedade conilon (predominante no Brasil) destaca-se pelo valor industrial e alta resistência à seca. Os grãos de conilon são largamente utilizados para produção de cafés solúveis e em *blends* com café arábica, pois conferem corpo e equilibram a acidez, uma vez que possuem maior teor de sólidos solúveis e acidez inferior ao arábica. A bebida originária dos grãos de conilon normalmente aporta como características maior amargor,

sabor amadeirado e baixa acidez, além de ser encorpada. A mistura (*blend*) entre arábica e conilon objetiva aproveitamento do potencial sensorial de cada café, enriquecendo-se assim o perfil de sabor e aroma do produto final (DA SILVA, 2018; PEREIRA *et al*., 2020; RIBEIRO *et al*., 2014). As duas espécies de café mais consumidas no mundo originam bebidas com características distintas. Contudo, além do consumo dos grãos de arábica e conilon isoladamente, existe, como dito, a possibilidade de realizar-se a mistura dos dois cafés, buscando um equilíbrio no perfil sensorial do produto final. Pesquisas têm sido desenvolvidas visando melhoria da qualidade dos cafés, para que novos públicos possam ser atingidos, agregando mais valor ao produto, o que consequentemente impulsiona a demanda e o mercado de cafés.

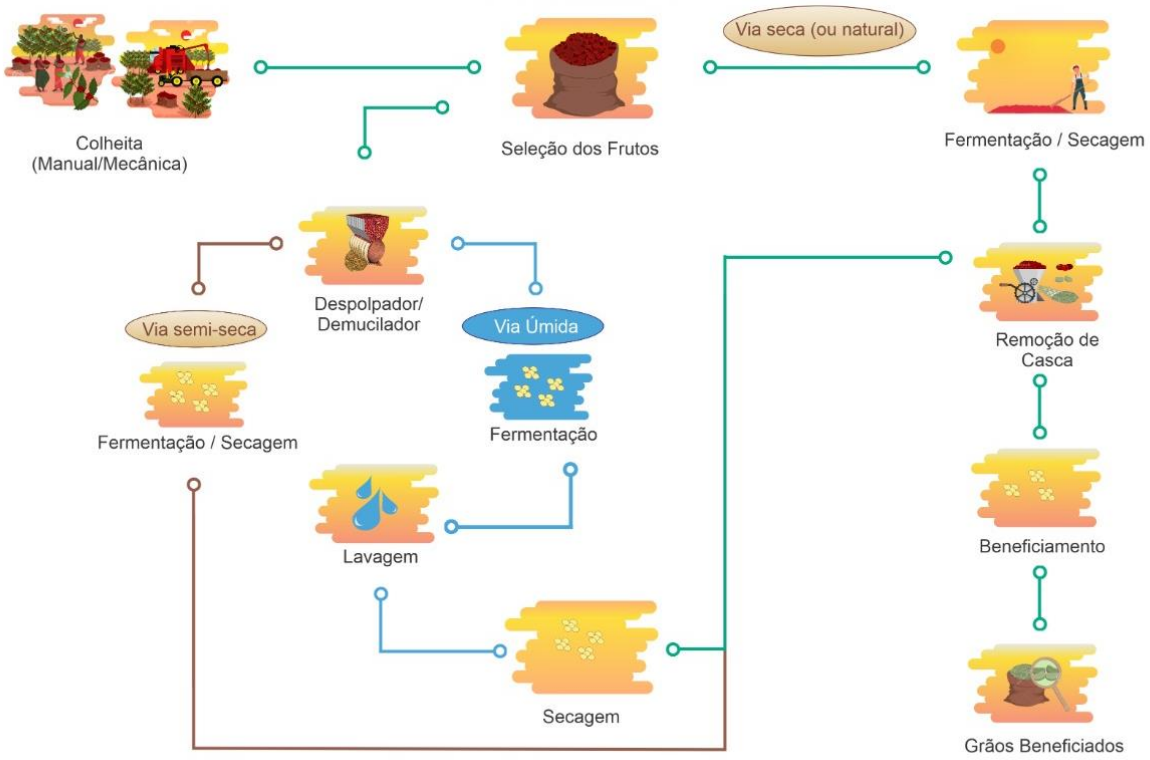
2.2 Processamento do café

No momento da colheita inicia-se o processamento do café, porém, fatores que impactam sobre a qualidade do produto devem ser observados desde o plantio (BORÉM *et al*., 2008). Imediatamente após a colheita, é importante que o processamento do café seja iniciado o mais breve possível, evitando-se que os frutos sejam deteriorados e/ou sofram fermentação indesejável (BORÉM *et al*., 2008; HAMDOUCHE *et al*., 2016).

Diferentes práticas pós-colheita são responsáveis pela melhoria na qualidade e preservação do café (HAMDOUCHE *et al*., 2016). A fermentação é uma etapa do processamento de grande relevância para a qualidade e formação do perfil sensorial do produto final, sendo usualmente utilizados três métodos para fermentar o café (FIGURA 3). O processamento por via seca consiste na utilização do fruto em sua totalidade. Esses são conduzidos diretamente para secagem sob o sol, em terreiros suspensos ou pátios de cimento. Nesse tipo de processamento a fermentação da polpa e da mucilagem pode durar até 20 dias, período que envolve também a secagem dos frutos. No processamento semisseco, intermediário entre os processamentos seco e úmido, o café é descascado e os grãos direcionados para secagem ao sol junto com a maior parte ou toda a mucilagem. Os grãos são secos ao ar livre, o que pode demandar de 10 a 15 dias, dependendo das condições climáticas. Nesse período, os microrganismos envolvidos no processo degradam toda a mucilagem que ainda possa estar aderida aos grãos (BRANDO; BRANDO, 2015; EVANGELISTA 2014b; SILVA, 2000). Nos cafés fermentados por via úmida a polpa é removida mecanicamente e os cafés despolpados são conduzidos para biorreatores contendo água, e a fermentação pode compreender um período entre 6 e 72 horas. Durante a fermentação, a mucilagem remanescente é degradada e então

solubilizada. O processo úmido é utilizado para o café arábica e para uma pequena porcentagem do café robusta, embora a tendência para o processo úmido no *Cofea canephora* esteja aumentando. Após essa etapa, os grãos são retirados dos reatores, lavados e secos ao sol (BRANDO; BRANDO, 2015; EVANGELISTA *et al*., 2015).

Figura 3 - Fluxograma do processamento do café.



Fonte: Da autora (2022).

A etapa de secagem visa reduzir a umidade dos grãos de 60 para 11-12% (independentemente do tipo de processamento utilizado), prevenindo-se assim o desenvolvimento de fungos filamentosos. Esse é o segundo processo pós-colheita mais relevante, pois uma secagem bem conduzida origina cafés com aromas e sabores distintos, superiores (BORÉM *et al*., 2008). Após essa etapa, os grãos podem ser armazenados por alguns meses, sem comprometimento de sua qualidade, antes de serem torrados e envasados (BELITZ *et al*., 2009).

2.3 Qualidade do café

A qualidade do café é caracterizada pelo sabor e aroma, com influência de diferentes fatores pré-colheita (espécie e variedade do café, grau de maturação, efeitos da adubação,

microrganismos) e pós-colheita (fermentação, secagem, torra, beneficiamento, armazenamento), que juntos irão garantir a expressão final da qualidade do produto (CHALFOUN; FERNANDES, 2013; LIVRAMENTO *et al*., 2017).

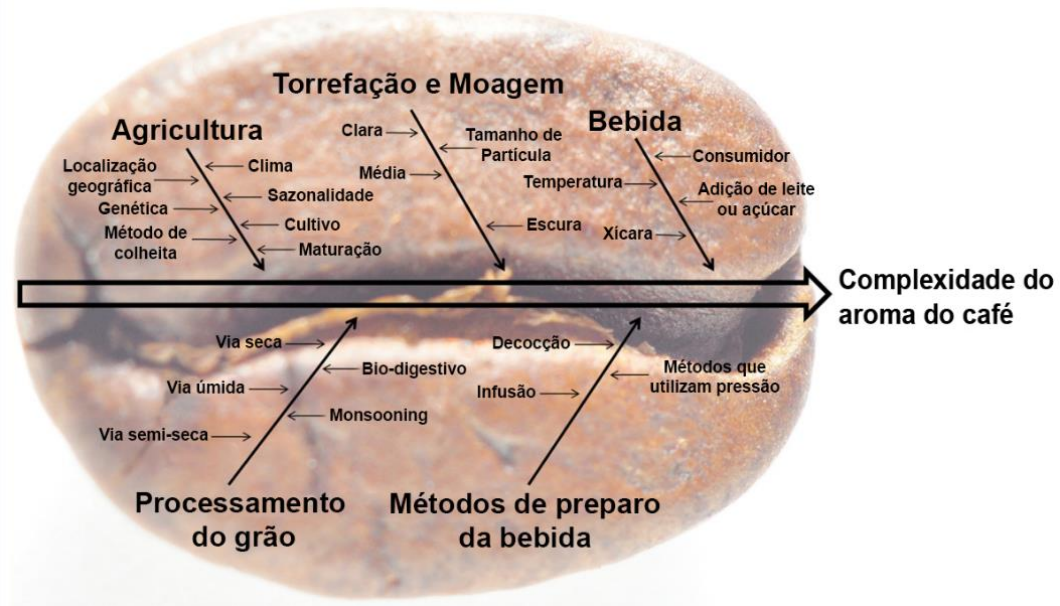
O aroma e sabor do café são características formadas durante o processamento e capazes de impulsionar o aumento no consumo da bebida, uma vez que contribuem para a qualidade dos grãos e consequentemente da bebida do café. A formação de ambos são processos complexos, envolvem a participação conjunta de compostos voláteis e não voláteis, tais como ésteres, cetonas, aldeídos, proteínas, aminoácidos, açúcares, compostos fenólicos, ácidos graxos e, ainda, ação enzimática (CHIN; EYRES; MARRIOTT, 2015; LEE *et al*., 2015).

A fermentação do café consiste na combinação de reações (químicas e biológicas), em que moléculas mais complexas presentes na mucilagem são metabolizadas por microrganismos e, quando bem conduzida, exerce efeito benéfico sobre a qualidade do produto. A presença dos microrganismos nessa etapa acarreta em atributos sensoriais peculiares, originando, por exemplo, cafés com sabor mais suave, menos corpo e pronunciada acidez (via úmida) ou, ainda, bebidas com mais corpo, adstringência e menor acidez (via seca ou natural). Assim sendo, as bebidas oriundas de cafés processados por diferentes métodos apresentarão características distintas. Contudo, quando o processo é bem conduzido, darão origem a bebidas de qualidade igualmente superior (CHALFOUN; FERNANDES, 2013). O uso de culturas iniciadoras nessa etapa do processo pode auxiliar no controle da fermentação. Podem originar produtos finais diferenciados, visto que a produção de ácidos orgânicos e compostos voláteis pode ser otimizada, além disso algumas cepas são capazes de inibir o crescimento de fungos filamentosos produtores de toxinas. Esses fatores impactam na qualidade sensorial da bebida e ao mesmo tempo na segurança do produto (EVANGELISTA *et al*., 2014a; MASSAWE; LIFA, 2010).

O valor comercial do café e consequentemente sua qualidade estão intimamente relacionados ao seu sabor e aroma. Tais parâmetros são dependentes da composição química e do tratamento térmico aplicado aos grãos (CAPORASO *et al*., 2018). A presença conjunta de cetonas, ácidos e aldeídos, compostos voláteis e não voláteis é a responsável pelo aroma e sabor do café, bem como compostos fenólicos, enzimas, ácidos graxos, açúcares, proteínas e aminoácidos (CHIN; EYRES; MARRIOT, 2015; LEE *et al*., 2015). É no cafeeiro onde a geração do sabor tem início, pois ali os seus precursores vão se formando, no decorrer do desenvolvimento dos frutos. A complexidade desse atributo desenvolve-se ao longo das várias etapas do processamento dos grãos, até que possa ser percebido na xícara. A Figura 4 representa os fatores que exercem influência sobre o sabor do café, desde o local do cultivo até a xícara

(SUNARHARUM; WILLIAMS; SMYTH, 2014).

Figura 4 - Fatores que influenciam na complexidade do sabor do café, desde a fazenda até a xícara.



Fonte: Adaptado de Sunarharum, Williams e Smyth (2014).

O aroma é um dos parâmetros mais importantes na avaliação sensorial da bebida do café (SUNARHARUM *et al*., 2014). Os precursores do aroma presentes nos grãos verdes são componentes essenciais na formação dos compostos voláteis e do aroma do café torrado. Diferentes concentrações de precursores aromáticos (proteínas, carboidratos, ácido clorogênico e trigonelina) nos grãos verdes acarretam em cafés de diferentes perfis sensoriais (LEE *et al*., 2015).

Alcaloides, proteínas, trigonelina, açúcares livres, ácidos clorogênicos e lipídeos são exemplos de compostos químicos presentes no café verde responsáveis pelos compostos voláteis presentes no café torrado (RIBEIRO *et al*., 2009). A reação de Maillard e outras termicamente catalisadas ocorridas no momento da torra são as responsáveis pela formação do aroma do café, em temperaturas geralmente superiores a 200 ºC (LEE *et al*., 2015). A reação de Maillard é responsável pela geração de compostos voláteis relevantes, como pirimidinas, fenóis e furanos, sendo essa geração a principal responsável pela formação dos compostos que originam o aroma do café. Durante a reação, moléculas contendo nitrogênio e enxofre são produzidas, especialmente compostos heterocíclicos, como pirróis e tiofenos (LEE *et al*., 2015).

Alguns compostos, como a trigonelina e ácido 3,4-dicafeilquínico, correlacionam-se intimamente com a elevada qualidade do café (FARAH *et al*., 2006). A importância da trigonelina está relacionada especialmente à sua degradação durante a torra, originando

compostos voláteis, essencialmente pirimidinas e pirroles (FARAH, 2012). Além disso, a trigonelina e ácidos clorogênicos possuem propriedades importantes, como atividade antioxidante e antimicrobiana (LEE *et al*., 2015). Os ácidos clorogênicos são considerados ácidos primários na composição do café, durante a torra originam as lactonas, que se correlacionam ao sabor amargo da bebida. A cafeína é outro composto relevante para a qualidade do produto, descrita como um composto estável ao calor, com sabor amargo característico e efeito estimulante, e contribui para o amargor da bebida (FARAH, 2012).

Na etapa de torrefação ocorre degradação e/ou formação, liberação de diversos compostos químicos através das reações de Maillard, degradação de Strecker, bem como quebra da trigonelina, pigmentos, lipídeos e ácido quínico, além da interação entre produtos intermediários que irão impactar no sabor da bebida (BUFFO; CARDELLI-FREIRE, 2004). A torra influencia diretamente na qualidade a bebida, pois converte o aroma do café verde e cru em aromas agradáveis e peculiares do café torrado, ocorrendo um aumento drástico de inúmeros compostos aromáticos (CZERNY; GROSCH, 2000; CZERNY; MAYER; GROSCH, 1999).

2.4 Microbiota associada ao café

Açúcares, polissacarídeos e outras substâncias mais simples fazem parte da constituição da mucilagem do café, originando ambiente propício ao desenvolvimento dos microrganismos que realizam a fermentação. O principal objetivo da fermentação é degradar a camada de mucilagem que contém pectina, um dos compostos utilizados como substrato pela microbiota presente para síntese de precursores responsáveis pelo sabor e aroma da bebida (SILVA *et al*., 2008). A pectina é um heteropolissacarídeo complexo, em sua estrutura ligações do tipo α-1,4 unem resíduos de ácido d-galacturônico, e algumas enzimas atuam sobre esse composto presente na mucilagem do café. Os frutos de café aportam enzimas que degradam a camada de mucilagem, contudo, somente a ação de enzimas endógenas não é suficiente para a completa remoção da camada. Nesse sentido, o desenvolvimento de microrganismos que também apresentam habilidade para produzirem essas enzimas é favorecido, a partir do momento em que eles utilizam a mucilagem como substrato durante a fermentação dos frutos. Dentre essas enzimas produzidas encontram-se poligalacturonase - principal enzima envolvida na fermentação do café - que catalisa a hidrólise das ligações glicosídicas do tipo α-1,4; pectina metilesterase – que desesterifica os grupos metoxila da pectina, acarretando em formação de metanol e ácido péctico; e pectinaliase – responsável por catalisar reações de quebra da pectina. A produção da pectinase está intimamente relacionada à ação microbiana (SILVA *et al*., 2013,

2000).

Após a colheita, a fermentação dos frutos de café ocorre naturalmente (fermentação espontânea). Além disso, microrganismos estão presentes em todas as etapas pré e pós-colheita e, ao desenvolverem atividade, impactam sobre a qualidade do produto final. Bactérias, leveduras e fungos filamentosos, de distintos gêneros, constituem a microbiota do café. A diversidade dos microrganismos é dependente da variedade do café, fatores ambientais da região onde o cultivo foi realizado (temperatura, umidade, população do solo, altitude), composição do fruto, bem como do método de processamento utilizado. A "responsabilidade" pelo processo fermentativo é mais comumente atribuída aos dois primeiros grupos. Dentre as bactérias presentes durante o processo fermentativo do café, os gêneros mais comuns são *Lactobacillus*, *Bacillus*, *Weisella*, *Arthrobacter* e *Acinetobacter*, e com relação às leveduras, *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Candida*, *Kluyveromyces* e *Rhodotorula.* Algumas espécies são comuns durante o processo fermentativo em diferentes regiões como também podem ocorrer espécies específicas de cada região (BATISTA *et al*., 2009; ELHALIS *et al*., 2020a; MARTINS *et al*., 2020; SILVA *et al*., 2008; VILELA *et al*., 2010).

2.4.1 Bactérias

Diferentes gêneros de bactérias fazem parte do processamento do café. A heterogeneidade desse grupo de microrganismos relaciona-se diretamente ao tipo de processamento utilizado para fermentar o café (SILVA *et al*., 2008).

O estudo de Silva *et al*. (2000) sobre a diversidade da microbiota associada à maturação e processamento natural do café em diferentes fazendas revelou que, com poucas exceções, o grupo das bactérias era o mais numeroso. Do total de isolados, 43,8% pertenciam a esse grupo. No início da fermentação natural, quando o teor de umidade é mais elevado (cerca de 69%), bactérias estão presentes em maior número. Contudo, no decorrer do processamento o café vai perdendo umidade e a atividade de água é reduzida, favorecendo a ocorrência de leveduras e fungos filamentosos (SILVA *et al*., 2008). Em outro trabalho que avaliou a diversidade microbiana durante a fermentação/secagem do *Coffea canephora* em diferentes altitudes (300 e 600m), as populações de bactérias foram maiores do que as de leveduras bactérias ácido-láticas, apresentaram maiores populações no final da secagem, no café fermentado a 600 m (PEREIRA *et al*., 2021).

Nas fermentações úmidas a presença de bactérias também foi estudada. Em cafés australianos processados por esse método, foram identificadas bactérias mesófilas aeróbias,

ácido-láticas e do ácido acético (ELHALIS *et al*., 2020c). Ribeiro *et al*. (2018) identificaram bactérias mesófilas e ácido-láticas ao trabalhar com fermentação úmida de três diferentes variedades de café arábica. Avallone *et al*. (2001) identificaram bacilos Gram-negativos, pertencentes aos gêneros *Erwinia* e *Klebsiella*, como mais frequentes no processamento úmido e, além desses, relatou que no grupo das BAL, *Leuconostoc mesenteroides* foi a espécie mais frequente.

Com relação ao processamento semisseco, Vilela *et al.* (2010) relataram que nas primeiras 24 horas de fermentação as bactérias destacaram-se em população e as espécies predominantes foram *Bacillus subtilis*, *Escherichia coli*, *Enterobacter agglomerans*, *Bacillus cereus* e *Klebsiella pneumoniae*.

De acordo com Evangelista *et al*. (2014a), a população de bactérias é superior à de leveduras no início do processo, provavelmente porque nessa etapa a umidade e a atividade de água são mais elevadas, favorecendo o desenvolvimento desse grupo de microrganismos. Dentre os gêneros mais comuns presentes durante a fermentação, os autores relataram *Lactobacillus*, *Bacillus*, *Klebsiella*, *Acinetobacter*, *Arthrobacter* e *Weissella*.

2.4.2 Leveduras

Grande parte dos estudos relacionados à fermentação do café tem sido dedicado ao isolamento e identificação de leveduras durante o processamento. Atualmente, essa linha de pesquisa tem avançado notavelmente. Sabe-se, por exemplo, que a produção de metabólitos que impactam diretamente sobre a qualidade do café (ésteres e álcoois) podem ser produzidos por este grupo de microrganismos. O uso de fungos leveduriformes tem auxiliado na melhoria das características químicas e sensoriais do café, originando bebidas com perfil sensorial distinto e muitas vezes peculiar (BRESSANI *et al*., 2020; ELHALIS *et al*., 2020b; EVANGELISTA *et al*., 2014b). Outro papel importante atribuído a esses microrganismos é a capacidade de produzir enzimas pectinolíticas, cuja função foi relatada anteriormente (MASOUD; JESPERSEN, 2006).

Masoud *et al*. (2004) estudaram a comunidade de leveduras do café na África Oriental e relataram que a população aumentou durante o processo fermentativo. *Pichia anomala*, *Pichia kluyveri* e *Hanseniaspora uvarum* foram as três espécies dominantes nas diferentes etapas do processamento. Além dessas, também foram isoladas: *Torulaspora delbrueckii*, *Issatchenkia orientalis*, *Pichia ohmeri*, *Kluyveromyces marxianus*, *Candida pseudointermedia. Hanseniaspora uvarium*, *Candida xylopsoci*, *Candida railenensis*, *Pichia fermentans* e

Wickerhamomyces anomalus. No processamento úmido, foram isoladas e identificadas algumas leveduras que fazem parte da ecologia microbiana do café em diversas partes do mundo. No entanto, *Pichia kudriavzevii* foi isolada e identificada pela primeira vez nesse tipo de processamento (ELHALIS *et al*., 2020a). Das espécies identificadas nesse tipo de processamento, alta atividade pectinolítica foi demonstrada pelas espécies *P. kluyvery* e *P. anomala*. Por outro lado, *H. uvarum* denotou atividade da poligalacturonase (MASOUD; JESPERSEN, 2006).

Silva *et al*. (2000, 2008) relataram que dentre os gêneros mais comumente encontrados associados ao processamento natural (ou via seca) estão *Pichia, Debaromyces, Candida, Saccharomyces e Arxula*. Trabalhos recentes em que cafés despolpado e natural foram fermentados, relataram que espécies pertencentes ao gênero *Candida* foram identificadas, além de *Debaromyces hansenii*, *Pichia kluyveri* e *Torulaspora delbrueckii*. As espécies detectadas como mais abundantes compreenderam *Meyerozima caribbica*, *Rhodotorula mucilaginosa*, *Cystofilobasidium ferigula*, *Saccharomyces cerevisiae* e *H. uvarum* (MARTINS *et al*., 2020; PEREIRA *et al*., 2022). Pereira *et al*. (2021) isolaram 89 leveduras da fermentação do *Coffea canephora* pertencentes aos gêneros *Candida* (39%), *Meyerozima* (35%), *Hanseniaspora* (18%) e *Picchia* (8%). *M. caribbica* e *M. guilliermondii* estavam presentes nas amostras de todos os ambientes estudados, sendo que a segunda espécie foi a mais encontrada durante a fermentação/secagem.

2.5 Culturas iniciadoras

Como forma de controlar a fermentação, culturas iniciadoras podem ser utilizadas, objetivando modular, controlar e favorecer o desenvolvimento de características agradáveis e desejáveis ao paladar dos consumidores, agregando valor e qualidade à bebida do café. Além disso, essas culturas demonstraram capacidade de prevenir o desenvolvimento de fungos produtores de toxinas, como *Aspergillus carbonarius* e *A. ochraceus.* Microrganismos selecionados proporcionam consistência ao processo, confiabilidade no desempenho fermentativo e segurança do alimento. As culturas iniciadoras devem ser capazes de colonizar o produto e dominar outros microrganismos ao longo do processo de fermentação (SILVA *et al*., 2013; SOUZA *et al*., 2017).

Massawe e Lifa (2010) realizaram experimentos com bactérias ácido-láticas (*Leuconostoc, Weisella* e *Lactobacillus*) e leveduras (*Pichia anomala* e *Pichia kluyveri*) como culturas iniciadoras na fermentação úmida do café arábica. A inoculação de *Pichia fermentans*

no café (variedade Catuaí) por Pereira *et al*. (2015) favoreceu aumento na concentração de metabólitos como etanol, acetaldeído, acetado de etila e acetato de isoamila. Os resultados da análise sensorial corroboram a hipótese de que a utilização de culturas selecionadas apresenta potencial de melhoria da qualidade do café, uma vez que a qualidade da bebida foi considerada superior (87 pontos), além da percepção mais intensa dos aromas florais e sabor de baunilha.

Recentemente, Cassimiro *et al*. (2022) utilizaram cepas de bactérias ácido-láticas e leveduras para fermentação úmida do café arábica (var. Catuaí vermelho). A qualidade foi potencializada e todas as fermentações inoculadas receberam pontuação superior a 80. Além disso, houve aumento nos teores dos ácidos lático e acético, que impactaram na acidez do produto. As culturas iniciadoras alteraram o perfil de compostos voláteis, produzindo compostos específicos. A análise sensorial revelou que a doçura foi intensificada, assim como a acidez e o corpo das bebidas. A inoculação intensificou e/ou modificou alguns descritores sensoriais, como vinho e frutas vermelhas (quando *L. plantarum* foi inoculado em cocultivo com *S. cerevisiae*), notas de caramelo foram mais evidenciadas no café fermentado por *L. mesenteorides* + *T. delbrueckii*.

A qualidade do café (Catuaí amarelo) fermentado por via semisseca foi avaliada por Martínez *et al*. (2017), aplicando-se inoculação direta (pulverização da solução de leveduras) e método do balde (em que a solução de leveduras foi adicionada aos grãos), objetivando avaliar qual deles apresentaria melhor efeito sobre a persistência dos inóculos (*S. cerevisisae*, *C. parapsilosis* e *T. delbrueckii*) e metabólitos produzidos. Análise sensorial foi realizada para verificar se a inoculação contribuiu para a qualidade do café. A população de leveduras foi maior quando o método do balde foi utilizado. De forma geral, quando o método do balde foi utilizado maiores teores de ácido lático foram observados. Com relação aos compostos voláteis (grãos verdes), maior quantidade de compostos foi detectada quando o método do balde foi utilizado. Nos grãos torrados, quando a inoculação direta foi aplicada, maior número de compostos foi detectado no tratamento em que *C. parapsilosis* foi inoculada, no método direto (tratamentos em que *S. cerevisiae* e *T. delbrueckii* foram inoculadas) a quantidade de voláteis foi maior que nos demais tratamentos. A análise sensorial revelou que todos obtiveram pontuação superior a 80 (cafés especiais), pelo método direto a bebida do café inoculado com *T. delbrueckii* destacou-se, obtendo pontuação de 81,5, contudo, no método do balde café inoculado com *S. cerevisiae* e o Controle obtiveram maiores notas (81,4). O método de inoculação em balde favorece o desenvolvimento de microrganismos, especialmente no café inoculado com *S. cerevisiae*.

As culturas iniciadoras surgiram e vem sendo cada vez mais utilizadas como alternativas

para fermentação do café, visando sua melhoria e obtenção de perfis diferenciados para as bebidas. Um trabalho recente utilizou *M. caribbica* e *P. kluyveri* como culturas iniciadoras para fermentação do café conilon, provadores não treinados conseguiram perceber as alterações sensoriais nesse café e, além disso, foi possível aumento na pontuação do café inoculado quando comparado à fermentação espontânea (SILVA *et al*., 2021). Características peculiares, perfis distintos, potencialização e/ou modificação do sabor e aroma são características atribuídas à atuação das cepas selecionadas. Esses microrganismos tornaram-se valiosa ferramenta para melhorar a qualidade do café e, além de agregarem valor ao produto, permitem maior controle, modulação e previsibilidade com relação às características finais da bebida.

2.6 Fermentação do café utilizando metodologia SIAF (self-induced anaerobiosis fermentation)

Dentre as etapas envolvidas no processamento do café, a fermentação é caracterizada como a uma das mais importantes para melhoria da qualidade do produto final (PEREIRA *et al*., 2022). Existem diferentes métodos para processamento do café e a concentração de oxigênio varia conforme cada um deles. Recentemente a metodologia SIAF tem sido aplicada à fermentação do café, a utilização de biorreatores fechados permite que a condição de anaerobiose seja alcançada gradativamente, devido ao metabolismo microbiano. Os microrganismos utilizam o O_2 remanescente para suas reações metabólicas, liberando CO_2, compostos voláteis e não voláteis. Trata-se de uma nova abordagem para obtenção de cafés com características peculiares, uma vez que a produção de ácidos orgânicos, álcoois e outros metabólitos é intensificada. Além dessas características, a metodologia impacta positivamente o desempenho de bactérias ácido-láticas e leveduras (CASSIMIRO *et al*., 2022; DA MOTA *et al*., 2022; PEREIRA *et al*., 2022).

Bressani *et al*. (2021b) utilizaram a metodologia SIAF para fermentação de cafés inoculados com três leveduras (*S. cerevisiae*, *T. delbrueckii* e *Candida parapsilosis*) processados pelo método seco. Diversos compostos foram produzidos durante a fermentação nos biorreatores, especialmente pertencentes à classe dos álcoois e ésteres. Cafés em que as leveduras foram inoculadas em concentrações de aproximadamente 10^6 cells/g receberam maiores pontuações e foram caracterizados por diferentes descritores sensoriais, como exemplo, o sabor de especiarias foi evidenciado nos tratamentos em que *C. parapsilosis* foi inoculada. Ainda, nos tratamentos em que *S. cerevisiae* foi utilizada como cultura *starter*, notas de nozes/amêndoas foram mais marcantes.

Pereira *et al*. (2022) estudaram a influência da metodologia SIAF sobre a microbiota e qualidade sensorial do café de diferentes regiões (Monte Carmelo, Três Pontas, Carmo de Minas e Lajinha) e condições ambientais. Elevado número de microrganismos foram isolados (380), compreendendo-se bactérias mesófilas, ácido-láticas e leveduras. Houve predominância dos gêneros *Bacillus, Leuconostoc, Lactobacillus, Staphylococcus, Pediococcus* e *Weisella*. A metodologia (café fermentado em terreiro ou SIAF) e o método de processamento (natural ou despolpado) influenciaram no perfil sensorial do café nas diferentes regiões. Cafés naturais em que a SIAF foi utilizada foram caracterizados por notas frutadas, cana-de-açúcar e açúcar caramelizado (Carmo de Minas); cana-de-açúcar, caramelo, chocolate, herbáceo (Três Pontas). Nos cafés despolpados, notas frutadas, herbáceas e amadeiradas destacaram-se em Carmo de Minas, e frutado, amadeirado e caramelo em Três Pontas. Por outro lado, os produtos originados da fermentação padrão dos cafés naturais apresentaram notas herbáceas e canavieiras dominantes em Carmo de Minas, frutadas em Três Pontas, herbáceas em Monte Carmelo e Lajinha. Nos cafés despolpados, açúcar caramelizado (Carmo de Minas), chocolate (Três Pontas), caramelo (Monte Carmelo) e lenhosas e herbáceas (Lajinha) foram as dominantes. A metodologia SIAF mostrou-se capaz de potencializar o perfil sensorial dos cafés de todas as regiões estudadas, no entanto, nos cafés de Monte Carmelo utilizando grãos despolpados houve variação mais significativa nas notas sensoriais. Alta dominância do atributo frutado foi observada no café dessa fermentação, quando comparado ao café da fermentação natural.

A metodologia SIAF também impactou positivamente na melhoria da qualidade de cafés (var. catuaí vermelho e catuaí amarelo) fermentados pelo método úmido e método natural, respectivamente. Bactérias ácido-láticas e leveduras foram utilizadas como culturas iniciadoras. Na bebida proveniente do catuaí vermelho a metodologia favoreceu o desenvolvimento das culturas iniciadoras, resultando em bebidas com perfis diferenciados e pontuação superior a 80, além disso alguns atributos (doçura, acidez e corpo) e descritores, como notas florais, lácteas, frutas cítricas, caramelo, castanha e amêndoas, foram mais perceptíveis. Com relação ao catuaí amarelo, foram observados impactos sobre a concentração de compostos não voláteis e voláteis, como alta produção de ácidos orgânicos que influenciaram os atributos sensoriais das bebidas. Compostos voláteis específicos, como maltol, responsável por notas frutadas e de caramelo, e 1,2-ciclopentadiona, 3-metil (notas de caramelo) e butirolactona tiveram sua produção potencializada. O café do método SIAF apresentou intensa acidez, doçura e corpo médio (CASSIMIRO *et al*., 2022; DA MOTA *et al*., 2022).

Os resultados dos trabalhos em que a SIAF foi utilizada foram promissores. A metodologia foi capaz de favorecer o desenvolvimento dos microrganismos, potencializar a

qualidade do café, influenciar positivamente a produção de compostos que impactaram diretamente sobre as características químicas e sensoriais das bebidas. Portanto, a metodologia torna-se potencial ferramenta a ser utilizada, visando otimizar o processo fermentativo, bem como a melhoria da qualidade do café.

3 CONSIDERAÇÕES FINAIS

A fermentação do café é uma etapa de grande relevância para que a qualidade do produto final possa ser melhorada, envolve a participação conjunta de microrganismos, especialmente bactérias e leveduras, que utilizam substratos presentes na casca e mucilagem para a produção dos metabólitos responsáveis pelo perfil sensorial do produto final. Quando a fermentação ocorre de forma espontânea, sem controle, pode originar cafés com características que não são agradáveis ao paladar dos consumidores e ter sua qualidade afetada negativamente. Nesse sentido, a utilização dos microrganismos selecionados (culturas iniciadoras) pode contribuir expressivamente para a potencialização da qualidade do café, uma vez que podem inibir o desenvolvimento de microrganismos indesejáveis, reduzindo riscos associados à segurança dos alimentos, reduzir o tempo de fermentação e melhorar o perfil sensorial da bebida, pois compostos específicos pertencentes a diferentes classes (ésteres, álcoois, ácidos, dentre outros) são produzidos, além de permitirem modulação e padronização do processo. A metodologia SIAF surge como nova tecnologia a ser aplicada em conjunto com diferentes tipos de fermentações do café (seco, semisseco e úmido) e tem-se mostrado eficaz no que diz respeito a impactar positivamente o desenvolvimento dos microrganismos e a qualidade final da bebida.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - Coinoculation of lactic acid bacteria and yeasts increases the quality of wet fermentated Arabica coffee

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Abstract

Wet coffee fermentation is widely used in coffee-producing regions such as Colombia and Hawaii, but it is not widespread in Brazil. This study aimed to evaluate inoculating the lactic acid bacteria *Leuconostoc mesenteroides* CCMA1105 and *Lactiplantibacillus plantarum* CCMA1065 and the yeasts *Saccharomyces cerevisiae* CCMA0543 and *Torulaspora delbrueckii* CCMA0684 as starter cultures on wet coffee fermentation using the SIAF method (self-induced anaerobiosis fermentation). The microbial activity resulted in high consumption of the carbohydrates glucose (98.6%), fructose (97.6%), and sucrose (100%), in addition to the production of lactic and acetic acids, impacting the final quality of the beverage. A total of 108 volatile compounds belonging to 17 classes were identified in the green and roasted coffee samples, including 2,3-butanediol produced by lactic acid bacteria, contributing to coffee's aromatic profile. The final scores for the coffees from the different treatments ranged from 79.0 to 83.25. The inoculated fermentations were classified as specialty according to the Specialty Coffee Association. Therefore, whole coffee fruit processed via wet using SIAF method and yeast and lactic acid bacteria starter is an alternative for improving wet fermented coffee quality and obtaining coffee beverages with a different sensory profile.

Keywords: SIAF method, whole fruit, coffee fermentation, sensory descriptors

1. Introduction

The post-harvest processing of coffee fermentation has a role in forming flavor, aroma, and other attributes related to the final product's quality (Elhalis et al., 2021a). In addition, several methods for coffee fermentation have been performed on-farm. In addition, the self-induced anaerobic process (SIAF) has been shown a new approach to obtaining coffees with unique sensory characteristics (Pereira et al., 2022).

In the SIAF method, anaerobiosis is gradually formed by the microbial metabolism that uses the remaining O_2 for their metabolic reactions releasing CO_2, volatile and non-volatile compounds. Also, it positively impacts the fermentative performance of lactic acid bacteria and yeasts during natural and pulped coffee processing (Bressani et al., 2021a; Da Mota et al., 2020; Martins et al., 2020). However, this method has not yet been tested for the wet process.

In the wet process, the fruits are selected in flotation tanks are depulped, leaving a thin inner pulp layer and silverskin on the seed. Then they are submerged in water for between 6 and 72 hours and later taken to the drying stage. Usually, fruits processed by this method result in more acidic drinks, medium body and sweetness, and a more striking balance (Borém et al., 2006).

Bacteria and yeasts participate in the wet fermentation of coffee. However, yeasts were recently identified during wet fermentation of Australian coffees and later used as starter cultures (Elhalis et al., 2021a, 2020b). Some microorganisms in this group produce organic acids and extracellular enzymes that help improve the quality of coffee (Bressani et al., 2020). The genera *Leuconostoc* and *Lactobacillus* species were identified in coffees processed by the wet method (Avallone et al., 2001; Elhalis et al., 2020b). Ribeiro et al. (2018) also reported that these two genera were frequently identified in three Arabica coffee varieties (Ouro Amarelo, Mundo Novo, and Catuaí Vermelho) fermented by the wet process.

Quality improvement via the action of selected microorganisms has stimulated research into optimizing coffee production processes (Lee et al., 2016, 2015). Starter cultures provide

consistency of the process, reliability of performance fermentative, and food security (Souza et al., 2017).

Lactic acid bacteria (LAB) are of great economic importance and are widely used to produce fermented products (Holzapfel and Wood, 2014). These bacteria are among the main groups involved in coffee fermentation. In addition, the metabolism of LAB might contribute to the removal of mucilage. LAB uses the sugars in this layer to produce metabolites that could impact coffee quality — especially lactic acid, contributing to the final product's acidity — and enhancing volatiles production during wet fermentation. In addition, yeasts have been used as starter cultures for coffee fermentation (Bressani et al., 2020, 2018; Da Mota et al., 2020; Elhalis et al., 2020a, 2020b). On the other hand, LAB starter (alone or in cocultivation with yeasts) was poorly investigated to improve coffee quality (Ribeiro et al., 2020);
Vale et al., 2019). As far as we know, this is one of the few times that *Leuconostoc mesenteroides* has been used as a starter culture for coffee cherries processed via the wet process.

This study aimed to evaluate the impact of inoculation with lactic acid bacteria and yeasts on the chemical and sensory quality of coffee subjected to wet processing using the SIAF method. Besides, the dynamic behavior of the starter cultures was evaluated by quantitative real-time PCR (qPCR).

2. Materials and Methods

2.1 Coffee

Coffea arabica L. of the Catuaí Vermelho variety from the Boa Vista farm (19°03'02.0"S 46°31'34.9"W; 1200 m above sea level) Campos Altos, Minas Gerais, Brazil, was fermented by wet process.

2.2 Starter cultures and culture conditions

Saccharomyces cerevisiae CCMA0543, *Torulaspora delbrueckii* CCMA0684, *L. mesenteroides* CCMA1105, and *Lactiplantibacillus plantarum* CCMA1065 (former *Lactobacillus plantarum*) were used as starter cultures and obtained from the Culture Collection of Agricultural Microbiology (CCMA, according to its acronym in Portuguese) of Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. The microorganisms were isolated from different coffee varieties and processing methods (Ribeiro et al., 2018; Silva et al., 2000).

The inoculated fermentations were identified as **M**: *L. mesenteroides;* **P**: *L. plantarum;* **MP**: *L. mesenteroides + L. plantarum;* **MT**: *L. mesenteroides + T. delbrueckii;* **MS**: *L. mesenteroides + S. cerevisiae*; **PT**: *L. plantarum + T. delbrueckii,* and **PS**: *L. plantarum+S. cerevisiae.* The spontaneous fermentation (Control) was performed under the same conditions but without inoculation.

Yeast cells were reactivated and grown in YEPG broth [10 g/L yeast extract (Merck); Peptone 20 g/L (HiMedia); 20 g/L dextrose (Merck)] at 30 °C until reaching a final concentration of 10^9 cells/mL. The bacterial strains were reactivated and cultivated at 30 ºC in MRS broth (MRS) (De Man Rogosa Sharpe, Merck, Darmstadt, Germany) until a 10^9 cells/mL concentration. After growth, the cell suspension from both groups (bacteria and yeast) was centrifuged (7000 RCF, 10 min) and resuspended in 500 mL of distilled water. This solution was applied to the coffee, obtaining a final concentration of approximately 10^7 cells/mL.

2.3 Coffee fermentation

Coffee fruits were fermented by the wet process following the SIAF method (self-induced anaerobiosis fermentation). Fermentations were carried out for 72 h in closed high-density polyethylene bioreactors containing 50 L of whole coffee fruit and approximately 20 L of freshwater. The fermentation process was performed with and without inoculation (Control) of lactic acid bacteria and yeast. The starter cultures were inoculated single and co-culture (Fig.

S1). After fermentation, the coffee was washed, mechanically pulped (Ecoflex, Pinhalense, São Paulo, Brazil), and transferred to suspended terraces for sun drying until 11-12% moisture content. Temperature, pH, and soluble solids content were measured during the fermentative process. The samples (150 g) were collected every 36 h and frozen at -20 °C for chemical and microbiological analysis. The high-barrier packages (double kraft paper and internally by an oxygen impermeable plastic film) were used to store coffee beans for further sensory analysis. All fermentations were performed in triplicate.

2.4 High-performance liquid chromatography (HPLC)

Carbohydrates (fructose, glucose, sucrose and mannitol), organic acids (acetic, butyric, citric, lactic, malic, propionic, and succinic acid), and bioactive compounds (chlorogenic acid, caffeine, and trigonelline) were evaluated by high-performance liquid chromatography (HPLC) (Shimadzu, model LC-10Ai, Shimadzu Corp., Japan).

A UV-VIS detection system (SPD-10Ai) was used for the analysis of organic acids (Shim-pack SCR 101-H column, 7.9 mm × 30 cm) and refractive index detector (RID-10Ai) Shim-pack SCR-101C column for bioactive compounds (C18 reversed-phase AG 120 column, 150 x 4.6 mm, 5 μm).

Carbohydrates and acids were evaluated with 0, 36, and 72 h of fermentation. For extraction, 3 g of coffee was homogenized with 20 mL of Milli-Q water was vortexed at room temperature for 10 min, and were centrifuged twice at 12.745 RCF/ 4 ºC for 10 min. Only to acid, the pH solution was adjusted to 2.11 using a perchloric acid solution (16 mM) and, after centrifuged at 12.745 RCF/ 4 °C for 10 min. The supernatant was filtered through a 0.22 μm cellulose acetate membrane. Operating conditions and results were performed as described by Evangelista et al. (2014).

The bioactive compounds were evaluated during the fermentative process (0, 24 and 72 h). Extraction and analysis conditions were according to the methodology of Malta and Chagas

(2009). The coffee fruit (0.5 g) were grounded and homogenized with 50 mL Milli-Q water and boiled for 3 min to extract total compounds. The solution was centrifuged at 12.745 RCF/ 4 ºC for 10 min, and the suspension was filtered through a 0.22 µm cellulose acetate membrane (Merck Millipore). The C18 reversed-phase column (AG 120 column, 150 x 4.6 mm, 5 µm)

The trigonelline, caffeine, and chlorogenic acid were identified UV–vis detection set at 272 nm wavelength and equipped with a Shimadzu C18 (AG120) column (150 × 4.6 mm, 5 µm) was used. The mobile phase consisted of methanol: water: acetic acid (20:79:1) at 0.6 mL/min, 30 °C. The identification and quantitative analysis were performed by injecting the different standard concentrations under the exact condition of the analyses of the samples. All analyses were performed in triplicate.

2.5 Analysis of volatile compounds

2.5.1 Volatile compound extraction by headspace solid-phase microextraction (HS-SPME)

Green and roasted coffee beans samples (2 g) were extracted by headspace solid-phase microextraction (HS-SPME). The samples were heated in a water bath (15 min/60 °C) until the system reached equilibrium. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm SPME fiber (Supelco Co., Bellefonte, PA, USA) was used (30 min/60 °C) for the absorption of volatile compounds.

2.5.2 Volatile compounds analysis by gas chromatography coupled to mass spectrometry (GC/MS)

Volatile compounds were analyzed in a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010) using a Carbowax column (30 m x 0.25 mm x 0.25 mm). The operating conditions were the same as those described by Ribeiro et al. (2017). Alkane standards (C10-C40) were used to identify and calculate each compound's retention index (RI). The mass spectra generated for each compound were compared with those of the

NIST 11 library, and the RI of each compound was also calculated.

2.6 DNA extraction from samples and quantitative real-time PCR (qPCR)

The starter culture populations were monitored during the fermentation and the dry process by qPCR. DNA strain and samples were extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the "DNA Purification from Tissues" protocol. The starter culture was cultivated separately in YEPG broth for yeasts and MRS broth for lactic acid bacteria, incubating at 30 °C for 24 h. The yeasts were counted using a Neubauer chamber, and the standard plate count counted the bacteria in MRS agar (De Man Rogosa Sharpe, Merck, Darmstadt, Germany). Serial dilution (1:10) was performed from 10^3 to 10^8 cells/mL to prepare the standard curve. Each point of the curve was analyzed in triplicate.

The qPCR analysis was performed using a Rotor-Gene Q system (Qiagen, Hombrechtikon, ZH, Switzerland). Each reaction had a final volume of 25 µL: 12.5 µL of Rotor-Gene SYBR Green master mix (Qiagen, Stockach, Konstanz, Germany), 2.5 µL (10 µM) of each primer (Invitrogen, São Paulo, SP, Brazil), 1 µL of DNA, and 6.5 µL of ultrapure water. The mixture was heated to 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 10 s. The cycling temperature was increased by 1 °C every 5 s from 50 °C to 99 °C to obtain the melting curve (Batista et al., 2015). Specific primers were used (Table S1) (Cho et al., 2010; Díaz et al., 2013; Mastrigt et al., 2019; Zott et al., 2010). Specificity was evaluated in GenBank BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.7 Sensory analysis

Coffee samples were prepared according to the Specialty Coffee Association (SCA, 2018). Five cups from the same sample were evaluated (8.25 ± 0.25 g of coffee per 150 mL of water). Six trained tasters with Q Grader coffee certification evaluated the coffee samples. The Q-Graders were asked to select the sensory descriptors of aroma and flavor that they considered

appropriate to describe the coffee. An unstructured scale assessed the intensity of sweetness, acidity, body, and aftertaste.

2.8 Statistical analysis

The experiment was conducted by applying a completely randomized design. The results were subjected to ANOVA, and the means were compared by the Scott-Knott test ($p < 0.05$) using the statistical program RStudio, version 3.3.3. The principal component analysis (PCA) was run to volatile compounds using XLSTAT software (Addinsoft, version 2020.1.3). The Pearson correlation coefficient was used to calculate the correlations between the acidity, body, finish, sweetness, astringency, bitterness, and score using Origin software (version 2020). Heatmap was performed using the XLSTAT 2019 2.1 software for the aroma and flavor descriptors sensory.

3. Results

3.1 Physicochemical characterization

Temperature, pH, and soluble solid are parameters that must be checked during fermentation, as they allow the monitoring of microbial activity. The temperature increased during the fermentative process while soluble solids and pH value were reduced. The coffee mass temperature varied from 20 to 33 ºC. A higher temperature variation was reached for the fermented M and MP (11.5 °C). Freshly harvested and spontaneously fermented coffee had an average concentration of 16 °Brix and a pH of 5.7. After 72 h of fermentation, this concentration showed below 10 °Brix and pH 4.5. Spontaneous fermentation was characterized as the most acidity (pH 4.5) (Table S2).

3.2 Monitoring of starter cultures

The population of *L. mesenteroides, L. plantarum, S. cerevisiae,* and *T. delbrueckii* were monitored by qPCR (Fig. 1). In spontaneous fermentation, higher concentrations of *L. mesenteroides* and *S. cerevisiae* (approximately 6 $\log_{10}$ cells/mL) followed by *T. delbrueckii* (3.49 $\log_{10}$ cells/mL) and *L. plantarum* (2.70 $\log_{10}$ cells/mL) (Fig. 1) were detected. The population dynamics varied over the fermentation period. *L. mesenteroides* had a larger population (9.56 $\log_{10}$ cells/mL) at the beginning of fermentation when compared with co-cultures fermentation (about 8 $\log_{10}$ cells/mL). These results suggest an antagonistic interaction between the microorganisms which reduced its growth.

L. plantarum showed the best growth with 36 h of fermentation, and the higher population achieved was in fermented PS (6.20 $\log_{10}$ cells/mL). After 72 h of fermentation, *S. cerevisiae* achieved viability between 5.59 – 6.25 $\log_{10}$ cells/mL, while *T. delbrueckii* was 3.65 to 7.79 $\log_{10}$ cells/mL. The largest microbial density was reached in both fermentations when inoculated with *L. mesenteroides.*

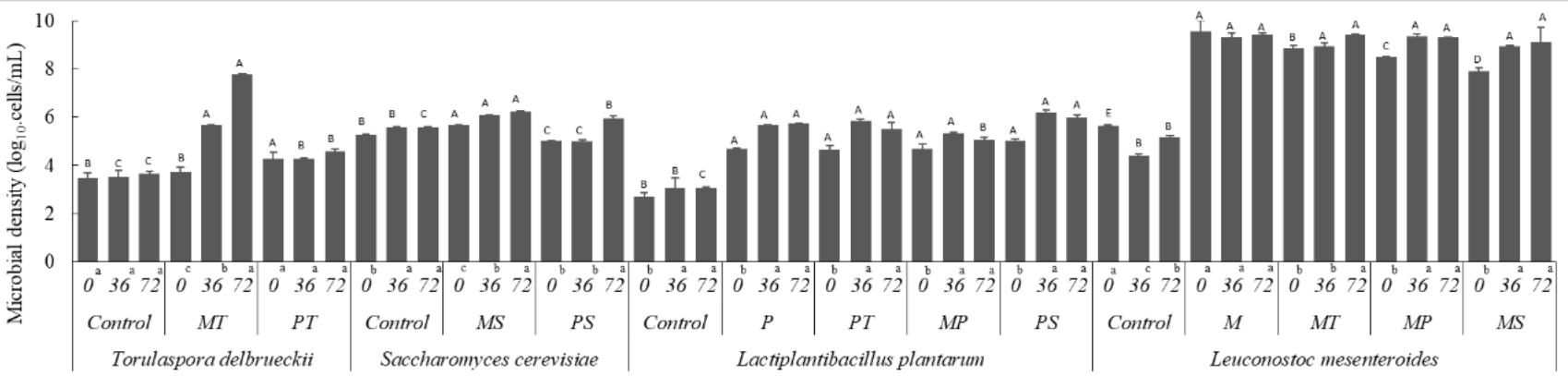

Fig. 1. Microbial density during coffee fermentation. Columns followed by the same capital letter (for each microorganism) did not differ significantly within the same time by the Scott-Knott test ($p>0.05$). The column followed by the same lowercase letter at each fermentation time (0, 36, and 72 hours) did not differ significantly for the Scott-Knott test ($p>0.05$).

3.3 Carbohydrates

The concentrations of fructose, glucose, sucrose and mannitol were evaluated (Table 1). At the beginning of fermentation, high concentrations of sucrose, glucose, and fructose were detected in MS (20.17 g/kg), M (86.09 g/kg), and M (93.14 g/kg), respectively. All fermentations showed carbohydrate consumption after 72 h of fermentation. Sucrose was not detected in the spontaneous fermentation and PS. The reducing sugars fructose and glucose were intensely consumed during processing in the fermented MS (in which 97.6% of fructose and 98.6% of glucose were used) and PS (in which there was consumption of 92.1% of fructose and 96.0% of glucose) after 72 h of fermentation. In spontaneous fermentation, the glucose concentration increases 2 times after 36 h of fermentation. Fermentation with *S. cerevisiae* inoculated showed the lowest concentrations of fermentable sugars. Mannitol was produced in all treatments; however, the MS fermentation (17.50 g/kg) showed the highest content being 7.9 times higher than at the beginning (2.20 g/kg).

Table 1. Carbohydrate concentration during coffee fermentation

Treatment	Time (h)	Carbohydrate (g/kg)			
		Fructose	Glucose	Sucrose	Mannitol
Control	0	31.00 ± 3.22^{b}	12.01 ± 1.73^{d}	9.36 ± 1.35^{c}	0.75 ± 0.04^{a}
M	0	93.14 ± 0.10^{a}	86.09 ± 0.16^{a}	2.74 ± 0.35^{d}	1.30 ± 0.01 ^{a}
P	0	36.44 ± 1.56^{b}	45.28 ± 0.44^{b}	9.32 ± 0.25^{c}	1.72 ± 0.01^{a}
MT	0	22.02 ± 0.05^{b}	19.47 ± 0.00^{d}	3.88 ± 0.00^{d}	0.70 ± 0.00^{a}
PT	0	32.77 ± 0.87^{b}	24.88 ± 0.67^{c}	0.46 ± 0.03^{d}	1.03 ± 0.03^{a}
MP	0	31.01 ± 3.89^{b}	41.68 ± 0.43^{b}	13.13 ± 0.33^{b}	2.20 ± 0.01^{a}
MS	0	44.27 ± 0.11^{b}	35.75 ± 0.09 ^{b}	20.71 ± 0.04^{a}	2.20 ± 0.01^{a}
PS	0	23.18 ± 0.69^{b}	18.12 ± 0.54^{c}	1.45 ± 0.17^{d}	0.48 ± 0.02^{a}
Control	36	24.98 ± 0.04^{a}	25.04 ± 0.00^{a}	0.04 ± 0.00^{a}	5.65 ± 0.02^{a}
M	36	14.92 ± 0.02^{b}	22.63 ± 0.06^{a}	0.16 ± 0.02^{a}	3.70 ± 0.10^{a}
P	36	32.99 ± 0.01^{a}	36.43 ± 0.03^{a}	0.75 ± 0.00^{a}	5.09 ± 0.02^{a}
MT	36	33.94 ± 0.04^{a}	29.89 ± 0.05^{a}	0.11 ± 0.00^{a}	5.81 ± 0.07^{a}
PT	36	26.16 ± 0.01^{a}	22.33 ± 0.10^{a}	nd	4.75 ± 0.03^{a}
MP	36	38.50 ± 0.02^{a}	33.13 ± 0.10^{a}	0.08 ± 0.01^{a}	5.58 ± 0.02^{a}
MS	36	9.36 ± 0.09^{b}	6.41 ± 0.11^{b}	0.14 ± 0.00^{a}	4.08 ± 0.09^{a}
PS	36	13.85 ± 0.04^{b}	10.36 ± 0.04^{b}	nd	4.34 ± 0.10^{a}
Control	72	22.74 ± 0.01^{a}	26.52 ± 0.01^{a}	nd	12.22 ± 0.00^{b}
M	72	20.41 ± 0.42^{a}	17.91 ± 0.21^{a}	0.18 ± 0.00^{a}	9.70 ± 0.02^{b}
P	72	27.36 ± 0.28^{a}	22.57 ± 0.13^{a}	0.78 ± 0.08^{a}	9.06 ± 0.05^{b}
MT	72	19.36 ± 0.34^{a}	17.43 ± 0.46^{a}	0.13 ± 0.09^{a}	7.48 ± 0.01^{b}
PT	72	25.83 ± 0.00^{a}	19.10 ± 0.01^{a}	0.27 ± 0.02^{a}	6.64 ± 0.00^{b}
MP	72	18.65 ± 0.12^{a}	12.84 ± 0.13^{a}	0.28 ± 0.00^{a}	5.91 ± 0.03^{b}
MS	72	1.02 ± 0.01^{b}	0.50 ± 0.02^{b}	0.18 ± 0.00^{a}	17.50 ± 0.05^{a}
PS	72	1.83 ± 0.09^{b}	0.71 ± 0.08^{b}	nd	7.89 ± 0.04^{b}

nd: not detected

Means ± standard deviation in the same column for each fermentation time, followed by the same lowercase letters, indicate that the treatments do not differ by the Scott-Knott test ($p > 0.05$).

Fermented: M: *L. mesenteroides*; **P**: *L. plantarum*; **MT**: *L. mesenteorides* + *T. delbrueckii*; **PT**: *L. plantarum* + T. *delbrueckii*; **MP**: *L. mesenteroides* + *L. plantarum*; **MS**: *L. mesenteroides* + *S. cerevisisae*; **PS**: *L. plantarum* + *S. cerevisiae*.

3.4 Organic acids

The contents of malic, citric, acetic, lactic, and succinic acids were evaluated during the different fermentations (Table 2). The lactic acid concentration increased during fermentation. Higher concentration were detected in control (11.42 g/kg), P (11.99 g/kg) and PS (11.84 g/kg) after 72 h of fermentation. MS fermentation had the lowest lactic acid concentration (5.12 g/kg). The concentration of acetic acid varied with the type of fermentation reaching 2.78 g/kg (PT) to 21.33 g/kg (PS) at the beginning of fermentation and 0.86 (PS) to 23.41 g/kg (Control) at the end of fermentation. At the end of the fermentation, the acetic acid levels increased approximately 4.8 and 1.5 times in the Control (4.92 to 23.41 g/kg) and P (13.46 g/kg to 21.24 g/kg) fermentation. The fermentation with *L. plantarum* monoculture showed a higher concentration of acetic acid than when inoculated in co-culture. Regarding citric, malic, and succinic acids, it can be observed that their concentrations were less evident at the end of coffee fermentation.

Table 2. Organic acids detected during the fermentation (0, 36, and 72 h)

Treatment	Time (h)	Organic Acid (g/kg)				
		Acetic	Citric	Lactic	Malic	Succinic
Control	0	4.92 ± 0.36^{e}	5.13 ± 0.08^{e}	0.23 ± 0.00^{d}	3.48 ± 0.00^{c}	0.96 ± 0.00^{f}
M	0	1.56 ± 0.15^{g}	7.51 ± 0.2^{c}	1.72 ± 0.15^{b}	3.93 ± 0.02^{c}	3.49 ± 0.26^{a}
P	0	13.46 ± 0.20^{b}	5.93 ± 0.1^{d}	0.58 ± 0.01^{c}	3.70 ± 0.07^{c}	0.17 ± 0.02^{g}
MT	0	12.06 ± 0.52^{c}	7.55 ± 0.01^{c}	1.60 ± 0.12^{b}	5.64 ± 0.17^{a}	2.53 ± 0.03^{d}
PT	0	2.78 ± 0.06^{f}	8.64± 0.1^{a}	nd	3.59 ± 0.10^{c}	2.92 ± 0.05^{b}
MP	0	8.15 ± 0.95^{d}	7.96 ± 0.4^{b}	2.93 ± 0.37^{a}	2.32 ± 0.13^{d}	1.44 ± 0.07^{e}
MS	0	8.93 ± 0.06^{d}	5.59 ± 0.6^{d}	0.80 ± 0.00^{c}	4.45 ± 0.52^{b}	2.52 ± 0.04^{d}
PS	0	21.33 ± 0.36^{a}	4.20 ± 0.4^{f}	0.27 ± 0.00^{d}	6.04 ± 0.06^{a}	2.67 ± 0.04^{c}
Control	36	5.62 ± 0.11^{b}	2.26 ± 0.04^{a}	2.79 ± 0.05^{a}	1.11 ± 0.05^{a}	2.61 ± 0.28^{a}
M	36	8.35 ± 0.11^{a}	2.48 ± 0.09^{a}	3.71 ± 0.11^{a}	1.55 ± 0.04^{a}	0.96 ± 0.03^{a}
P	36	2.37 ± 0.10^{b}	2.01 ± 0.02^{a}	5.89 ± 0.07^{a}	0.52 ± 0.05^{a}	0.54 ± 0.00^{a}
MT	36	6.68 ± 0.31^{b}	2.08 ± 0.02^{a}	1.36 ± 0.02^{a}	1.05 ± 0.15^{a}	0.73 ± 0.06^{a}
PT	36	3.55 ± 0.45^{b}	2.85 ± 0.07^{a}	4.80 ± 0.07^{a}	1.35 ± 0.02^{a}	0.38 ± 0.04^{a}
MP	36	2.19 ± 0.05^{b}	2.51 ± 0.07^{a}	3.09 ± 0.14^{a}	1.22 ± 0.02^{a}	1.37 ± 0.06^{a}
MS	36	4.91 ± 0.22^{b}	2.69 ± 0,08^{a}	5.25 ± 0.02^{a}	0.65 ± 0.02^{a}	1.18 ± 0.09^{a}
PS	36	2.80 ± 0.13^{b}	2.33 ± 0.02^{a}	2.90 ± 0.43^{a}	0.56 ± 0.02^{a}	0.48 ± 0.01^{a}
Control	72	23.41 ± 0.30^{a}	2.34 ± 0.04^{e}	11.42 ± 0.95^{a}	0.39 ± 0.03^{a}	0.50 ± 0.00^{d}
M	72	1.56 ± 0.18^{e}	1.33 ± 0.05^{f}	7.25 ± 0.02^{d}	0.11 ± 0.01^{b}	0.85 ± 0.12^{c}
P	72	21.24 ± 0.01^{b}	3.24 ± 0.4^{c}	11.99 ± 0.32^{a}	0.06 ± 0.01^{b}	2.10 ± 0.02^{a}
MT	72	3.31 ± 0.18^{c}	3.94 ± 0.1^{a}	10.33 ± 0.19^{b}	0.66 ± 0.09^{a}	1.19 ± 0.01^{b}
PT	72	2.83 ± 0.02^{d}	3.27 ± 0.1^{c}	9.70 ± 0.01^{c}	0.20 ± 0.09^{a}	0.84 ± 0.01^{c}
MP	72	1.41 ± 0.16^{e}	3.58 ± 0.1^{b}	9.28 ± 0.96^{c}	0.64 ± 0.09^{a}	0.99 ± 0.01^{c}
MS	72	2.52 ± 0.22^{d}	1.08 ± 0.02^{f}	5.12 ± 0.07^{e}	0.09 ± 0.00^{b}	0.26 ±0.03^{e}
PS	72	0.86 ± 0.04^{f}	2.82 ± 0.1^{d}	11.84 ± 0.07^{a}	nd	0.55 ± 0.01^{d}

nd = not detected

Means ± standard deviation in the same column for each fermentation time, followed by the same lowercase letters, indicate that the treatments do not differ from each other by the Scott-Knott test (p >0.05).

Fermented: M: *L. mesenteroides*; **P**: *L. plantarum*; **MT**: *L. mesenteorides* + *T. delbrueckii*; **PT**: *L. plantarum* + T. *delbrueckii*; **MP**: *L. mesenteroides* + *L. plantarum*; **MS**: *L. mesenteroides* + *S. cerevisisae*; **PS**: *L. plantarum* + *S. cerevisiae*.

3.5 Caffeine, chlorogenic acid, and trigonelline

Fermentation time significantly increased the concentration of chlorogenic acid (24 h) and trigonelline (24 and 72 h). At 24 h of fermentation, the chlorogenic acid varied between 10.00 g/kg (MS) and 16.78 g/kg (Control). An increase of up to 28.47% was observed in the concentration of this compound (MT) compared to the beginning of fermentation. Concerning trigonelline, at 24 h of fermentation, the levels varied between 5.03 g/kg (P) and 7.93 g/kg (MT). At 72 h of fermentation, the trigonelline variation was between 5.66 g/kg (MP) and 8.92 g/kg (MT). The trigonelline content in coffee was potentiated up to 20.74% (24 h of fermentation) and 22.27% (72 h of fermentation); these increases were more evident in P fermentation. The caffeine contents did not differ significantly at any of the times evaluated, ranging from 6.07 g/kg (M) to 9.19 g/Kg (PT) (Table 3).

Table 3. Contents of bioactive compounds detected during fermentation with and without culture starter

Fermented	Time (h)	Bioactive Compound (g/kg)		
		Chlorogenic acid	Caffeine	Trigonelline
Control	0	13.90 ± 0.06[a]	7.30 ± 0.02[a]	7.73 ± 0.05[a]
M	0	13.61 ± 0.12[a]	8.28 ± 0.06[a]	7.70 ± 0.21[a]
P	0	14.48 ± 0.00[a]	7.79 ± 0.01[a]	8.17 ± 0.02[a]
MT	0	11.63 ± 0.05[a]	9.02 ± 0.00[a]	8.18 ± 0.12[a]
PT	0	10.74 ± 0.01[a]	8.79 ± 0.00[a]	7.12 ± 0.21[a]
MP	0	11.18 ± 0.00[a]	8.28 ± 0.01[a]	7.00 ± 0.08[a]
MS	0	12.43 ± 0.03[a]	7.64 ± 0.01[a]	6.21 ± 0.20[a]
PS	0	11.19 ± 0.0[a]	6.60 ± 0.02[a]	6.18 ± 0.16[a]
Control	24	16.78 ± 0.10[a]	7.25 ± 0.00[a]	7.50 ± 0.18[a]
M	24	15.59 ± 0.01[a]	7.68 ± 0.01[a]	6.45 ± 0.10[b]
P	24	13.19 ± 0.02[b]	8.56 ± 0.00[a]	5.03 ± 0.04[b]
MT	24	16.26 ± 0.05[a]	8.99 ± 0.00[a]	7.93 ± 0.08[a]
PT	24	11.82 ± 0.05[b]	9.19 ± 0.00[a]	7.56 ± 0.07[a]
MP	24	14.80 ± 0.02[a]	7.70 ± 0.00[a]	7.89 ± 0.04[a]
MS	24	10.00 ± 0.01[b]	8.46 ± 0.05[a]	5.34 ± 0.02[b]
PS	24	11.01 ± 0.00[b]	6.49 ± 0.01[a]	7.05 ± 0.04[a]
Control	72	12.02 ± 0.05[a]	7.26 ± 0.00[a]	7.72 ± 0.19[a]
M	72	12.64 ± 0.04[a]	6.07 ± 0.00[a]	8.07 ± 0.11[a]
P	72	12.23 ± 0.07[a]	8.29 ± 0.04[a]	6.35 ± 0.23[b]
MT	72	12.05 ± 0.04[a]	7.88 ± 0.00[a]	8.92 ± 0.07[a]
PT	72	13.33 ± 0.13[a]	8.29 ± 0.03[a]	7.87 ± 0.10[a]
MP	72	11.75 ± 0.10[a]	8.33 ± 0.01[a]	5.66 ± 0.04[b]
MS	72	11.50 ± 0.02[a]	6.84 ± 0.15[a]	7.01 ± 0.08[a]
PS	72	12.79 ± 0.01[a]	8.10 ± 0.07[a]	7.10 ± 0.09[a]

Mean ± standard deviation followed by the same letter (in the same column) indicate that the fermented product did not show a significant difference

by the Scott-Knott test ($p>0.05$), within the same fermentation time.

Fermented: M: *L. mesenteroides*; **P**: *L. plantarum*; **MT**: *L. mesenteorides* + *T. delbrueckii*; **PT**: *L. plantarum* + T. *delbrueckii*; **MP**: *L. mesenteroides* + *L. plantarum*; **MS**: *L. mesenteroides* + *S. cerevisisae*; **PS**: *L. plantarum* + *S. cerevisiae*.

3.6 Volatile compounds

108 volatile compounds were detected in the green and roasted coffee beans samples (Table S3). These compounds were classified as acids (15), alcohol (10), aldehyde (6), ester (16), ketone (11), phenol (5), furan (17), furanone (2), lactones (1), pyran (2), pyrazines (13), pyridine (1), pyrroles (6), thiophene (1) and others (2) (Fig. 2).

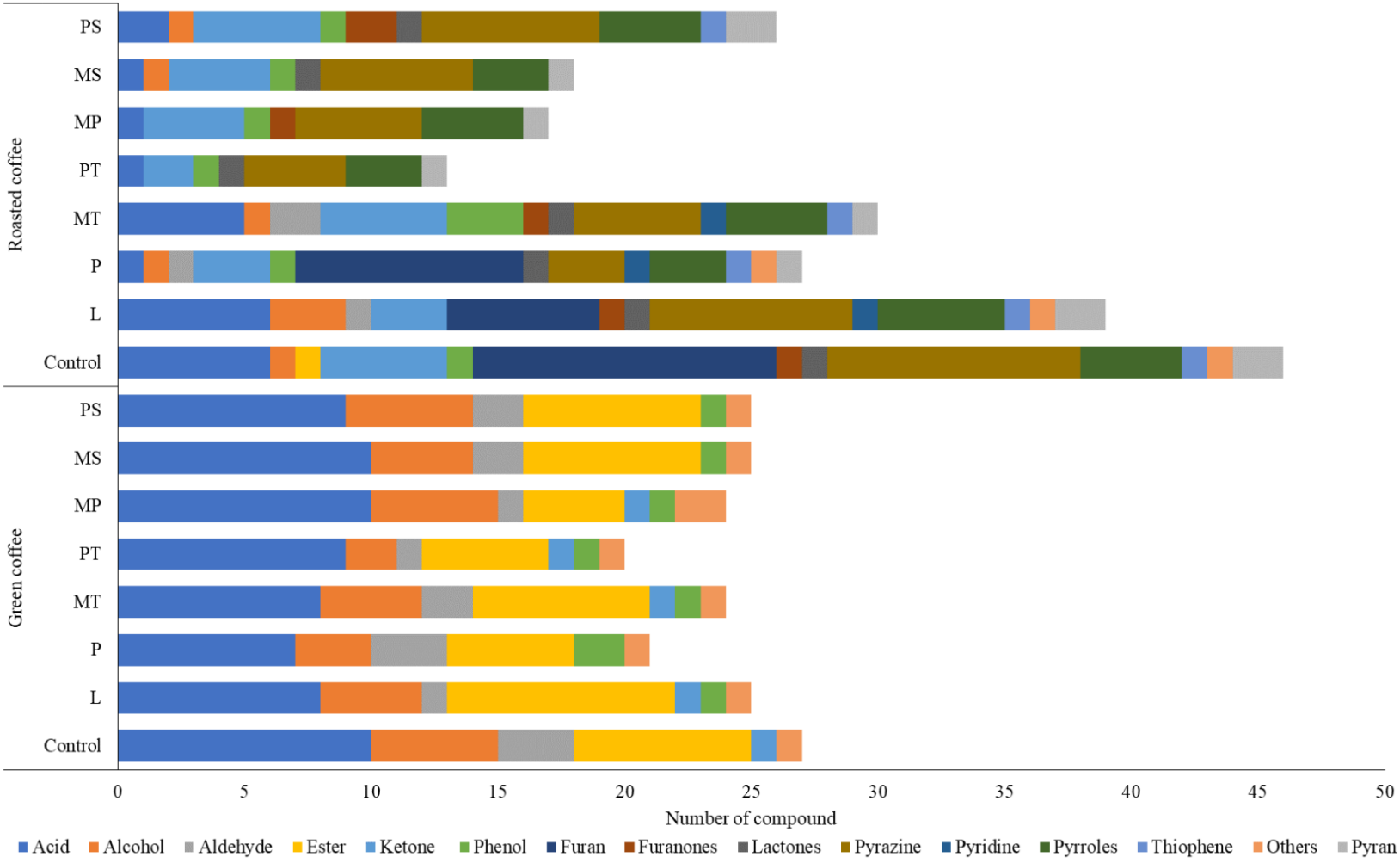

Fig. 2. Volatile group identified by GC-MS in green and roasted coffee

The starter culture changed the volatile compounds profile. 2-cyclopenten-1-one, 3-ethyl-2-hydroxy was produced by the inoculated treatments. The ethanol was produced only in treatments with *S. cerevisiae*. Decanoic acid ethyl ester was produced by the interaction of *S. cerevisiae* with LAB.

Heptadecanol and 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester were detected in the fermented MP. 2-hexanone, 5-methyl-; and phenol, 3-methyl- detected in the fermented MT. 2(3H)-Furanone, dihydro-3-methyl- detected in the fermented PS. 1-dodecanol was produced by all ferments, except for the fermented MT.

The principal component analyses in Fig. 3 display the correlation between the volatile group and inoculation. With 83.77% explained variation, Fig. 3 shows a clear separation of the green coffee and the roasted coffee along PC1 (51.09%). The green coffee beans was correlated with acid, while pyrazine, pyrrole, and ketone were correlated with roasted coffee. In addition, some volatile group was not detected in roasted coffee beans, such as furan, pyridine, and thiophene in fermented PT.

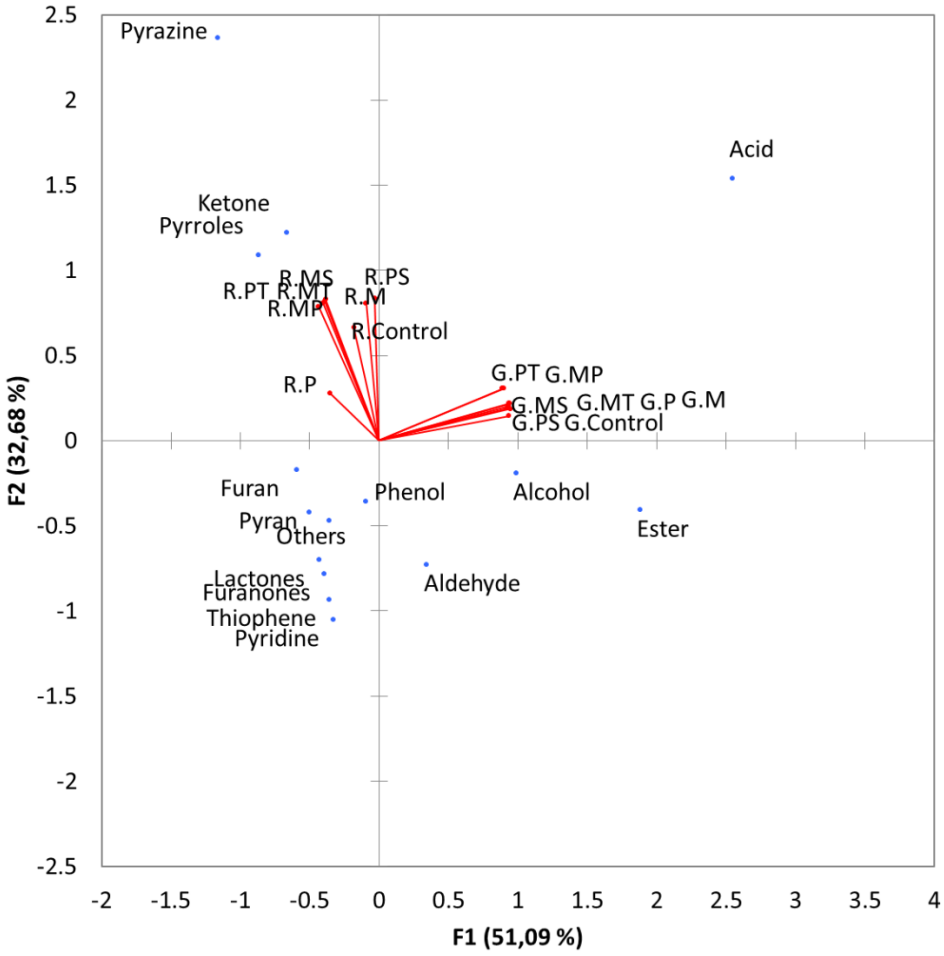

Fig. 3. PCA analysis of volatile group in green and roasted beans. R: roasted coffee; G: green coffee; M: *L. mesenteroides*; P: *L. plantarum*; MT: *L. mesenteorides* + *T. delbrueckii*; PT: *L. plantarum* + T. *delbrueckii*; MP: *L. mesenteroides* + *L. plantarum*; MS: *L. mesenteroides* + *S. cerevisisae*; PS: *L. plantarum* + *S. cerevisiae*.

3.7 Sensory analysis

The spontaneous fermentation coffees were not classified as special (79 points). However, every inoculated fermentation enhanced coffee quality that reached a score above 80, intensifying sweetness, acidity, and body (Fig. 4A). Figure 4B represents the correlation between sweetness, acidity, body, astringency, bitterness, aftertaste, and score. A positive correlation was obtained to score/sweetness and acidity/body. The fermented PT and M produced coffees with higher sweetness (7.25 and 7.5, respectively) and grades (83.25 and 83). PS was the coffee with the highest acidity (6.75) and body (7.5). The spontaneous fermentations coffee was characterized by the aroma notes of nutty and almonds, floral, and dry fruit, and flavor notes of nutty and almonds, honey, milk chocolate, tropical fruit, winey, red fruit, dairy notes, caramelized sugar, floral, dry fruit, caramel, spice, yellow fruit, citric fruit, dark chocolate, orange, woody, and cereal.

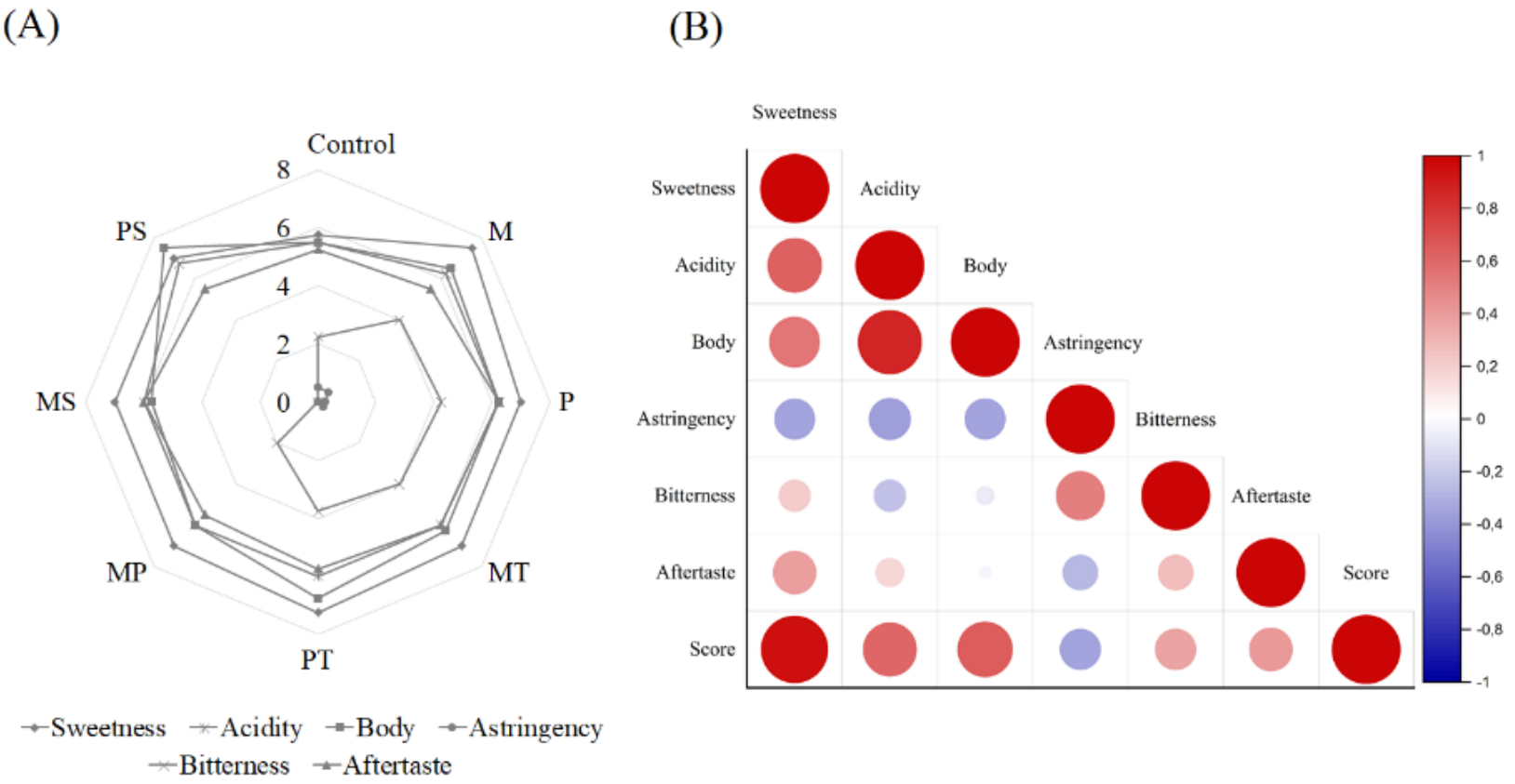

Fig. 4. Sensory attribute intensity (A) and correlation between sensory attribute and coffee beverage score (B)

The microbial inoculation modifies or intensifies some sensory descriptors of the beverage. Some of them stood out in the aroma of the coffee drink, such as wine and red fruits in the fermented PS; milky notes in the fermented P; caramel in the fermented MT and MP, citrus fruits and floral was attributed to the fermented MS; chestnuts and almonds were evidenced in the fermented M, MT, and PT; and milk chocolate in the fermented M, PT, and MP. The flavor of the coffee drink referred to honey in the fermented M, MT, PT, MP, and PS. As for the fermented MS, the flavor of the yellow fruit was more noticeable. Nutty and almond flavors were more eminent (Fig. 5 A-B).

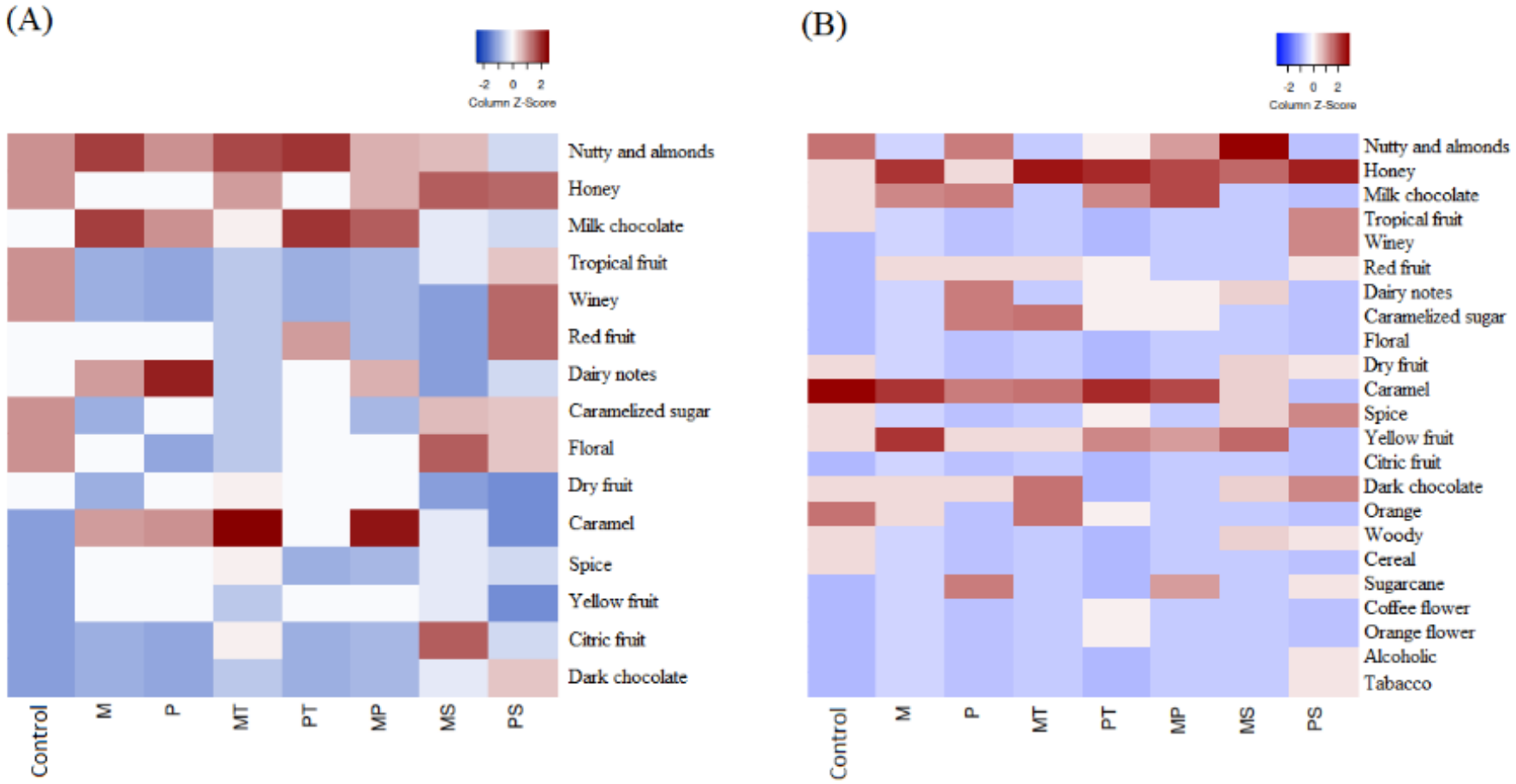

Fig. 5. Heatmap of aroma (A) and flavor (B) descriptors sensory.

4. Discussion

The SIAF method performed integral coffees fruits under the water produced beverages with different sensory profiles. The complex formation of coffee flavor involves the chemical composition of the fruit and the microbiota present. The high carbohydrate concentration in whole fruit enhanced the metabolic reactions of the microbiota, contributing to increasing the temperature of the coffee mass reducing the soluble solids and the pH due to the production of organic acids (Elhalis et al., 2020b).

At the beginning of the coffee fermentation, *L. mesenteroides* and *S. cerevisiae* were detected in higher populations. *L. mesenteroides* is one of the species that dominate the wet fermentative processes (Evangelista et al., 2015; Vaughan et al., 2015) due to its adaptability to coffee fermentation stress factors, such as pH and nutrient competition, besides being reported as a producer of pectinase (Avallone et al., 2001). *S. cerevisiae* is a yeast with versatile metabolism and a high capacity to produce enzymes. In coffee fermentation, a high *S. cerevisiae* population contributes to quick degradation of the mucilage layer due to polygalacturonase and pectin lyase production (Buyukpamukcu et al., 2001; Legras et al., 2018; Silva et al., 2013). The microbial consortia involve a complex network with multiple interactions (Braga et al., 2016). Positive interactions between bacteria-bacteria and bacteria-yeast are paramount to enable substrate conversion and improve process performance fermentative (Braga et al., 2016; Canon et al., 2020) as obtained in this co-inoculated fermentation leading to increase population and metabolites microbial. The co-inoculation with yeast and LAB has the potential for forming desirable aroma compounds and producing specialty coffees (Vale et al., 2019).

Carbohydrates present in coffee fruits are among the compounds that play a crucial role in aroma formation. Changes in sucrose, glucose, and fructose concentrations occurred during coffee fermentation. Low sucrose concentrations were detected at the beginning of the

fermentation, possibly because it was hydrolyzed into glucose and fructose. The hydrolases, such as invertase and sucrose hydrolase, are produced from yeast and lactic acid bacteria, respectively (Marques et al., 2016). Furthermore, the dextransucrase has an enzyme associated with the *L. mesenteroides* cleavage sucrose molecule (Lahtinen et al., 2012).

Fructose and glucose were the main sugars detected at the beginning and the end of the fermentation process and were accompanied by an increase in the microbial population and the production of volatile and non-volatile metabolites. The combined inoculation of *L. mesenteroides* and *S. cerevisiae* showed an intense reduction of these carbohydrates and mannitol production. These compounds favor sweet and refreshing tastes contributing positively to coffee's sensory quality (Jung et al., 2012). Heterofermentative LAB can reduce fructose to mannitol through the enzyme mannitol dehydrogenase (Grobben et al., 2001).

The coffee plant produces citric, malic, and succinic acids. These acids are catabolized by microorganisms like lactic acid bacteria in fermentative processes. This conversion involves the conversion of citric acid via oxaloacetate into pyruvate that could be reduced to lactic acid besides liberation of acetate (Wang et al., 2019). Lactic acid fermentation plays a role promising for creating specialty coffees with novel sensory characteristics (Wang et al., 2019). Further, lactic acid is the primary acid produced during the fermentation process.

LAB can produce lactic acid from hexose fermentation via the Embden-Meyerhof pathway and 6-phosphogluconate or malolactic fermentation (Konings et al., 1999). Malolactic fermentation happens when glucose and pH concentration is lower (Konings et al., 1999). In these conditions, lactic acid is produced from malic acid decarboxylation, leading to decreased malic acid.

Moreover, the versatile metabolism of LAB, including *L. mesenteroides* and *L. plantarum*, contribute to acetic and lactic acids formation from the degradation of citric acid under the catalysis of citrate lyase (Evangelista et al., 2015; Papadimitriou et al., 2016; Ribeiro et al., 2018). The acidity is one of the attributes that interferes with beverage quality and is

evaluated for the specialty coffees classification (SCA, 2018). Furthermore, the remaining citric acid in coffee is desirable and may have contributed to the perception of citric acidity in coffee beverages.

Bioactive compounds, such as caffeine, trigonelline, and chlorogenic acid, give functionality to the coffee beverage. However, its concentration depends on various factors such as coffee species, planting conditions, processing and storage conditions, and preparation types (del Castillo et al., 2002; Wu et al., 2021). Caffeine is one of the major bioactive compounds and contributes to the bitter taste in coffee beverages (Farah, 2012). The caffeine level expected to detect varies from 5.0 to 20.0 g/kg in arabica coffee (Malta and Chagas, 2009). Chlorogenic acid is the most abundant phenolic compound in coffee and contributes to bitterness, pigmentation, and astringency in the beverage. These compounds contribute to coffee's flavor. However, its concentration is reduced during the roast (Buffo and Cardelli-Freire, 2004; Kleinwächter et al., 2015).

The trigonelline is the second most abundant alkaloid in coffee seeds, produced from the enzymatic methylation of nicotinic acid. The trigonelline is the precursor for several volatile compounds, such as pyrroles and pyridines, formed during roasting contributing to the bitterness of the coffee beverage (Farah, 2012; Jeszka-Skowron et al., 2016).

Flavor complexity develops throughout coffee beverages' coffee processing and preparation techniques, being influenced by coffee beans' volatile and non-volatile components (Sunarharum et al., 2014). The coffee beans were correlated with acids, while roasted coffee was correlated with pyrroles, pyrazine, and ketone. The increase in temperature during the roast and the presence of sugars intensify their production (Flament, 2001). Ketones positively impact coffee flavor and food safety because their volatile compounds can prevent the development of filamentous fungi (Li et al., 2015). Ester was predominant in green coffees and can be synthesized by esterification, alcoholysis, acidolysis, and transesterification. Therefore,

yeast and lactic acid bacteria can produce it (Liu, 2002; Matthews et al., 2004). The ester formation is associated with the growth of these microorganisms in the initial phase of fermentation (Liu, 2015). Hexadecanoic acid, methyl ester, hexadecanoic acid, ethyl ester are compounds detected in coffee (Bressani et al., 2021b; Hameed et al., 2018; Wei Lee et al., 2017).

The furan is a heterocyclic compound with high volatility and is part of the volatile aroma components generated during the roasting of coffee. Sucrose, glucose, linoleic, and linolenic acids are potential precursors of furan formation in roasted coffee. 2-furanmethanol has antioxidant activity can contribute to sweet, caramel, and astringent flavors in spontaneous fermentation (Osada and Shibamoto, 2006).

The pyrazine is formatted through condensation between amines and carbonyls and was detected in all treatments (Caporaso et al., 2018). 2-Acetyl-3-methylpyrazine may have characterized the caramel flavor detected in fermentations. As expected, the alcohols produced during fermentation decreased after roasting (Elhalis et al., 2021b). Phenylethyl alcohol is usually detected in coffees and its desirable sweet, chocolate, fruity and floral flavor (Elhalis et al., 2021b). In addition, 2,3-butanediol and benzyl alcohol may be produced by microorganisms and involve esterification reactions between alcohols and fatty acids for the coffee ester-forming aroma. It can also be produced by LAB using alternative ways of degrading pyruvate, contributing to a buttery, creamy taste and fruity aromas in the coffee beverage. Other alcohol may contribute to sweet, honey, fruity, and floral aroma in all treatments (Flament, 2001; Ribeiro et al., 2018).

The inoculated fermentation showed that specific volatile compounds, such as the 2-cyclopenten-1-one and 3-ethyl-2-hydroxy can contribute to caramel-like flavor. For example, *S. cerevisiae* is a yeast often used for alcoholic fermentation due to high alcohol production, which contributes to volatiles' beverage viscosity and solubility (Elhalis et al., 2021b; Walker

and Stewart, 2016). In coffee fermentation inoculated with *S. cerevisiae* produced ethanol and decanoic acid ethyl ester is a volatile provide of yeast metabolites, and it was detected in co-culture fermentation of *S. cerevisiae* and lactic acid bacteria. Also known as ethyl decanoate, this compound is a fatty acid ester formed from capric acid and ethanol. Furthermore, decanoic acid ethyl ester can contribute to floral and fruity flavors (Linsenmeier et al., 2010; Walker and Stewart, 2016). Phenol, 3-methyl- detected in MP fermentations may have contributed to the high bitterness of the drink (Ribeiro et al., 2012).

According to the cupping protocol of the Specialty Coffee Association, every inoculated fermentation was classified with specialty based on sensory profile, acidity, body, flavor, and aroma (SCA, 2018). The modulation of the chemical composition of coffee by starter culture resulted in the beverage with a sensory profile specific for each fermentation, such as winey and red fruit in fermented PS; dairy notes in fermented P; caramel in fermented MT and MP, citric fruit, and floral in fermented MS; chestnut and almonds in fermented M, MT, and PT; and milk chocolate in fermented M, PT, and MP. Specific aromas can be related to lactic acid (dairy fruit); cyclopentanedione (caramel); 1-1H-pyrrol-2-yl- (chestnut and almonds); decanoic acid, ethyl ester (floral) and phenylethyl alcohol (chocolate). All coffees showed citric acidity that possibly was influenced by the citric acid detected at the end of fermentation. Acidity is essential for the specialty coffees classification, and its concentration impacts the coffee body (SCA, 2018).The wet process carried out with whole fruits using the SIAF method and initial culture resulted in coffees with high sweetness. The increase in sweetness in wet processing, with stater culture, had not been reported showing that anaerobic conditions intensified this attribute (Elhalis et al., 2021b, 2020a; Wang et al., 2020). Sweetness is another attribute evaluated for specialty coffees and substantially impacted the sensory score. The sweetness coffee involves sugar concentration in coffee beans and the sweet aromatics produced during roasting (Bhumiratana et al., 2011). Metabolism of each starter culture influenced the formation

of the volatile and non-volatile compound and sensory profile. SIAF method and starter cultures have been powerful tools to increase quality and obtain coffees with different sensory profiles.

5. Conclusions

The use of LAB and yeast as starter cultures enhanced the coffee quality due to the self-induced anaerobic conditions that favored the growth of strains and produced higher sweetness coffee. *L. plantarum* CCMA1065 and *S. cerevisiae* CCMA0543 strains in co-cultive showed a high score. However, the single and co-cultivated fermentation intensified specific descriptors. SIAF method with yeast and LAB starters showed a potential method to improve the coffee quality of wet process using whole fruit.

Declaration of competing interest

The authors do not have any conflict of interest.

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Appendix A. Supporting Information - Supplementary Material

Fig. S1. Flowchart of the experiment

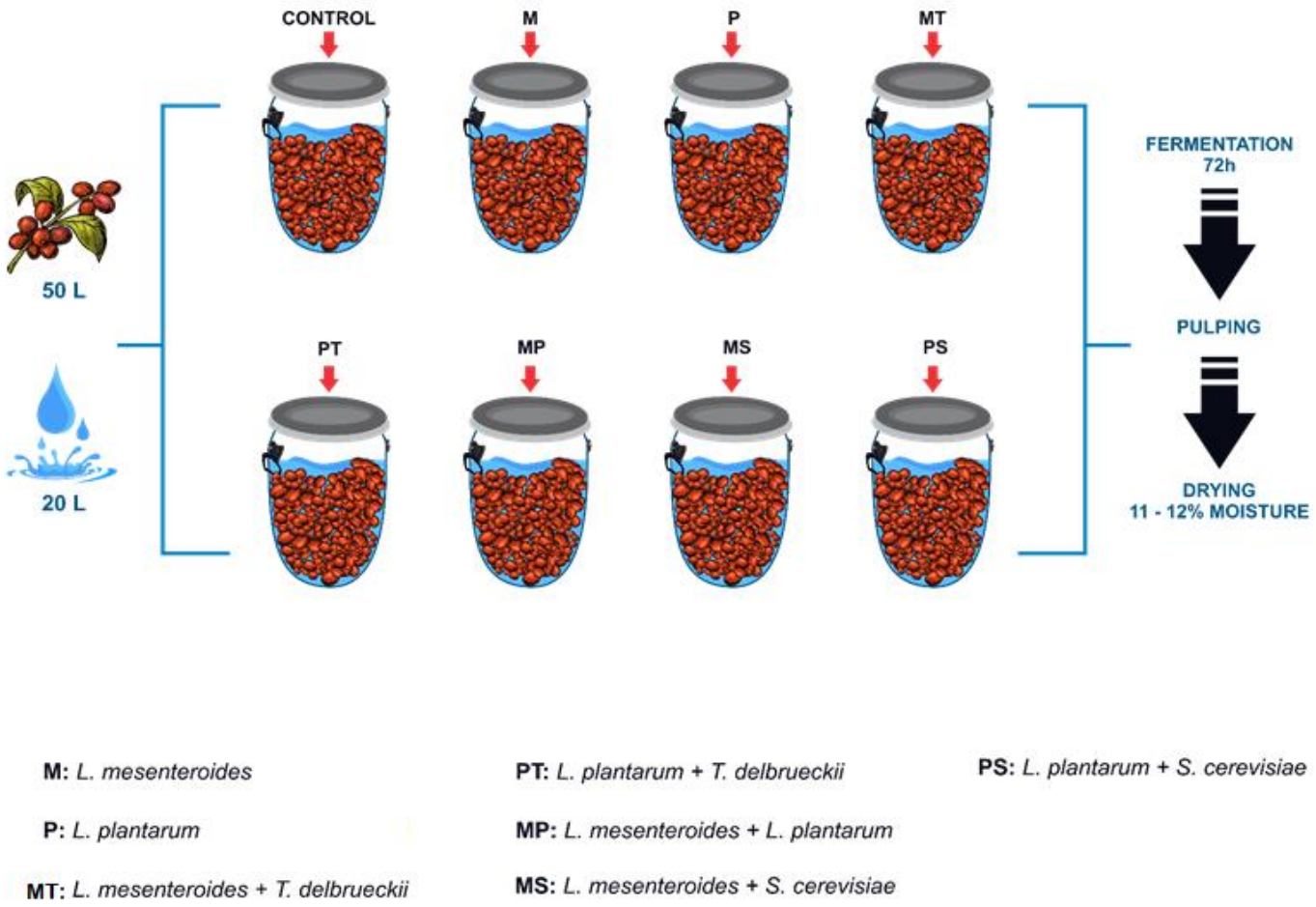

Table S1. Specific primers used for qPCR análisis

Species	Primers		
	Code	Sequence (5'> 3')	References
L. plantarum	plnEF fw	CTATTTCAGGTGGCGTTTTC	Cho et al., (2010)
	plnEF rv	GTGGATGAATCCTCGGACAG	
L. mesenteroides	Fr	AAGTTTCGTGCGATTGCTG	Mastrigt et al., (2019)
	Rv	ATGCCTACTGGATTGGGATG	
S. cerevisiae	SC-5fw	AGGAGTGCGGTTCTTTGTAAAG	Díaz et al., (2013)
	SC-3bw	TGAAATGCGAGATTCCCCT	
T. delbrueckii	Primer 1Tods L2	CAAAGTCATCCAAGCCAGC	Zott et al., (2010)
	Primer 2 Tods R2	TTCTCAAACAATCATGTTTGGTAG	

Table S2. Physicochemical parameters

Samples	Time (hour)	Temperature (°C)	Soluble solid (°Brix)	pH
Control	0	20.00	16.00	5.70
	36	24.00	9.40	4.72
	72	32.00	10.00	4.53
M	0	21.50	14.10	5.71
	36	30.00	10.50	4.45
	72	33.00	10.00	4.37
P	0	22.50	18.00	5.10
	36	28.00	11.60	4.48
	72	32.00	8.00	4.28
MT	0	22.00	11.60	5.30
	36	22.50	16.30	4.43
	72	30.00	11.00	4.40
PT	0	23.00	13.00	5.00
	36	27.00	14.30	4.35
	72	31.00	9.60	4.28
MP	0	21.50	12.30	5.00
	36	29.00	11.60	4.42
	72	33.00	10.30	4.29
MS	0	23.00	11.00	5.00
	36	27.00	11.00	4.38
	72	30.00	9.60	4.26
PS	0	24.00	9.00	5.30
	36	27.00	12.50	4.41
	72	30.00	9.60	4.22

Table S3. Volatile compounds detected of green and roasted Coffee beans fermented in closed biorreactor by the wet fermentation

			Treatment							
Chemical Class/Compound	**LRI**	**Sensory Perception***	Control	M	P	MT	PT	MP	MS	PS
Acid										
Acetic acid	826	Pungent, stinging sour	GB	GB/RB	GB	GB	GB	GB	GB	GB
Hexanoic acid	940	Sour, fatty, sweaty, cheese-like	GB	GB	GB	GB	GB	GB	GB	GB
Heptanoic acid	941	Fatty, nuity, fruity	--	--	--	--	--	GB	GB	GB
Octanoic acid	957	Fatty, waxy, cheese-like	GB	GB	GB	--	GB	GB	GB	GB
Nonanoic acid	971	Waxy, cheese-like	GB	--	--	--	GB	--	GB	--
n-Decanoic acid	987	-	GB			GB	GB	GB	GB	GB
Dodecanoic acid	1013	Rancid flavor	GB/RB	GB		GB	GB	GB	GB	GB
Tetradecanoic acid	1037	-	GB	GB	GB	GB	GB	GB	GB	GB
Pentadecanoic acid	1048	Fatty, weak flavor	GB	GB/RB	GB	GB/RB	GB	GB	GB	GB
n-Hexadecanoic acid	1062	Rancid and acid flavor	GB/RB	GB/RB	GB	GB/RB	GB	GB	GB	GB
Octadecanoic acid	1095	--	GB	GB	GB	GB	--	GB	--	--
Formic acid	837	Unpleasant flavor	RB	--	--	--	--	--	--	--
Propanoic acid	852	Butter, cheese odor	RB	RB	--	RB	--	--	--	RB

Butanoic acid, 3-methyl-	887	Fruity flavor (apple and cherry)	RB	RB	RB	RB	RB	RB	RB	RB
Pentanoic acid, 4-oxo-	995	Sweet, caramel and acid flavor	RB	RB	--	RB	--	--	--	--
Alcohols										
Ethanol	--	--	--	--	--	--	--	--	GB	GB
2,3-Butanediol	--	--	--	RB	--	--	--	--	--	--
Benzyl alcohol	928	--	GB	GB	GB/RB	GB/RB	GB	GB	RB	
Phenylethyl alcohol	938	Sweet, chocolate, fruity and floral flavor	GB	GB	GB	GB	GB	GB	GB	GB
1-Dodecanol	944	--	GB	GB	GB	GB	--	GB	GB	GB
1-Tetradecanol	973	--	GB	--	--	--	--	--	GB	GB
1-Hexadecanol	1000	Faint waxy, nearly odorless	GB	GB	--	GB	--	GB	--	GB
Glicerol	1002	--	--	RB	--	--	--	--	--	--
Heptadecanol	1023	--	--	--	--	--	--	GB	--	--
1,6-Octadien-3-ol, 3,7-dimethyl-	856	Floral	RB	RB	-	-	-	-	-	RB
Aldehydes										
Undecanal	935	--	GB	--	GB	--	--	GB	--	--
Tetradecanal	952	--	GB	GB	GB	GB	--	--	GB	GB
9-Octadecenal, (Z)-	1022	--	--	--	--	--	GB	--	--	--
Vanilin	1025	Vanilla-like	GB		GB	GB	--	--	GB	GB
Pentanal	963	--	--	RB	--	RB	--	--	--	--
Benzeneacetaldehyde,alpha-ethylidene-	936	Floral and fruity flavor	--	--	RB	RB	--	--	--	--

Esters										
Methyl salicylate	909	--	GB	GB	--	GB	GB	--	GB	GB
Decanoic acid, ethyl ester	882	floral and fruity flavour	--	--	--	--	--	--	GB	GB
Benzeneacetic acid, ethyl ester	910	--	GB	GB	--	GB	GB	GB	GB	GB
Dodecanoic acid, ethyl ester	922	--	--	--	--	--	--	--	--	--
Tetradecanoic acid, ethyl ester	955	--	GB	GB	--	--	--	--	GB	GB
Pentadecanoic acid, ethyl ester	969	--	--	--	--	--	--	--	--	--
Hexadecanoic acid, methyl ester	978	--	GB	GB	GB	GB	GB	GB	GB	GB
Hexadecanoic acid, ethyl ester	983	fruity, creamy, balsamic	GB	GB	GB	GB	GB	GB	GB	GB
9-Octadecenoic acid, methyl ester, (E)-	1007	--	GB	--	GB	--	--	--	--	GB
Homosalate	1009	--	--	GB	GB	GB	--	--	--	--
9,12-Octadecadienoic acid, methyl ester	1012	--	GB	--	GB	GB	--	--	GB	--
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1019	--	--	--	--	--	--	GB	--	--
Benzoic acid, tridecyl ester	1042	-	--	GB	--	GB	GB	--	--	--
Benzoic acid, tetradecyl ester	1058	-	--	GB	--	--	--	--	--	--
Benzoic acid, octadecyl ester	1075	-	--	GB	--	--	--	--	--	--
2-Butenoic acid, 3-methyl, ethyl ester	903	Fruit, aple skin, pineaple flavor	RB	--	--	--	--	--	--	--
Ketones										

3,4-Dimethyl-2-pentanone	900	--	--	--	RB	--	--	--	--	--
2-Butanone, 1-(acetyloxy)-	849	Coffee flavor	--	--	--	--	--	--	--	RB
2-Propanone, 1-(acetyloxy)-	825	A peculiar fruitty-buttery odor	--	RB	--	--	--	RB	RB	RB
1,2-Cyclopentanedione	907	Maple flavor, caramel like	RB	--	--	--	--	--	--	--
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-5-methyl	915	Sweet and malty odor	RB	--	--	--	--	--	--	--
1,2-Cyclopentanedione, 3-methyl-	921	Caramel	--	--	--	RB	--	RB	RB	RB
2-Pentadecanone, 6,10,14-trimethyl-	965	--	GB/RB	GB		GB/RB	GB	GB	--	--
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	933	--	--	RB	RB	RB	RB	RB	RB	RB
Ethanone, 1-(1H-pyrrol-2-yl)-	942	Nuts, floral and fruity flavor	RB	RB	RB	RB	RB	RB	RB	RB
Ethanone, 1-(4-hydroxyphenyl)	934	Heavy-floral, herbaceous odor	RB	--	--	--	--	--	--	--
2-Hexanone, 5-methyl-	968	Fruty, red-fruit flavor	--	--	--	RB	--	--	--	--
Phenols										
Phenol, 2-methyl-	960	--			GB	GB	GB	GB	GB	GB
Phenol, 4-ethyl-	972	--		GB	GB					
Phenol, 3-methyl-	959	Flavor unpleasant	--	--	--	RB	--	--	--	--
Cresol	981	--	--	--	--	RB	--	--	--	--
4-Hydroxy-2-methylacetophenone	974	--	RB		RB	RB	RB	RB	RB	RB
Furan										

Furfural	829	Caramelic, sweet, bread-like,toasted odor	RB	--	RB	--	--	--	--	--
2-Furanmethanol, acetate	845	Soft and floral flavor	--	--	RB	--	--	--	--	--
Ethanone, 1-(2-furanyl)-	846	Pungent, sweet-caramellic odor and sweet taste	RB	--	RB	--	--	--	--	--
2-Furancarboxaldehyde, 5-methyl-	859	Spicy and slightly caramelized flavor	RB	--	RB	--	--	--	--	--
2-Furanmethanol	876	Mild and slightly caramelized flavor	RB	--	--	--	--	--	--	--
2-Furanone, 2,5-dihydro-3,5-dimethyl	946	--		--	RB	--	--	--	--	--
2-Furanmethanol, 5-methyl-	888	--	RB	--	RB	--	--	--	--	--
5-Ethyl-2-furaldehyde	911	--	RB	--	--	--	--	--	--	--
3(2H)-Furanone, 2,5-dimethtl	913	Green, ketonic, weak flavor	RB	--	--	--	--	--	--	--
Furan, 2-[(methyldithio)methyl]-	914	Coffee-like odor	RB	RB	--	--	--	--	--	--
Furan, 2,2'-[oxybis(methylene)]bis-	924	--	RB	--	--	--	--	--	--	--
3(2H)-Furanone, 4-hydroxy-2,5-dimethyl	925	Sweet odor	RB	--	--	--	--	--	--	--

2,5-Dimethyl-4-hydroxy-3(2H)-furanone	926	--	--	RB	RB	--	--	--	--	--
4-Methyl-5H-furan-2-one	930	Pungent, caramel and sweet flavor	--	RB	--	--	--	--	--	--
Benzofuran, 2,3-dihydro-	943	--	RB	RB	--	--	--	--	--	--
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	926	--	--	RB	RB	--	--	--	--	--
5-Hydroxymethylfurfural	1016	Sweet flavor	RB	RB	RB	--	--	--	--	--
Furanones										
2(5H)-Furanone	895	-	RB	RB		RB		RB		RB
2(3H)-Furanone, dihydro-3-methy-	866	Hydrocarbons, as solvent flavor	--	--	--	--	--	--	--	RB
Lactones										
Butyrolactone	873	Sweet flavor	RB	RB	RB	RB	RB	--	RB	RB
Pyranes										
4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	991	Caramel-like odor	RB	RB	--	--	--	--	--	RB
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	986	--	RB	RB	RB	RB	RB	RB	RB	RB
Pyrazines										
2,6-dimethylpyrazine	801	Sweet	--	RB	RB	--	--	--	--	--
2-ethyl-6-methyl-pyrazine	812	Roasted hazelnut-like taste	RB	RB	--	--	RB	RB	RB	RB
2-ethyl-5-methyl-pyrazine	817	A coffee-like taste	RB	--	--	--	--	RB	RB	RB

trimethylpyrazine	818	--	--	RB	--	RB	RB		RB	RB
2-ethyl-3-methyl-pyrazine	822	Nutty and roasted	RB	RB	--	--	--	--	--	--
2-ethyl-3,5-dimethyl-pyrazine	835	Coffee flavor	--	RB	--	--	--	--	RB	RB
3-ethyl-2,5-dimethyl-pyrazine	840	--	RB	--	--	--	--	--	--	--
2-methyl-6-(1-propenyl)-, (E)-pyrazine	855	--	RB	--	--	RB	--	--	--	--
2-Acetyl-3-methylpyrazine	884	Caramel, bready and roasted flavor	RB	RB	RB	RB	RB	RB	RB	RB
1-(6-Methyl-2-pyrazinyl)-1-ethanone	894	Popcorn flavor	RB	RB	--	RB	RB	RB	RB	RB
2-methyl-5-(1-propenyl)-, (E)-pyrazine	836	--	RB	RB	RB	RB	--	RB	--	RB
Quinoxaline	912	--	RB	--	--	--	--	--	--	--
2-Butyl-3-methylpyrazine	997	Sugar syrup flavor	RB	--	--	--	--	--	--	--
Pyridine										
4(H)-Pyridine, N-acetyl-	896	--	--	RB	RB	RB	--	--	--	--
Pyrroles										
1H-Pyrrole, 1-(2-furanylmethyl)-	915	--	RB	RB	RB	RB	RB	RB	RB	RB
1H-Pyrrole-2-carboxaldehyde	950	Pungent flavor	RB	RB	RB	RB	RB	RB	RB	RB
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	964	Mild flavor	RB	RB	RB	RB	RB	RB	--	RB
1H-Pyrrole, 3-ethyl-2,4-dimethyl-	996	--	--	RB	--	--	--	--	--	--

Indole	1006	Floral notes	RB	RB	--	RB	--	RB	RB	RB
1H-Indole, 3-methyl-	1011	Sweet odor	--	--	--	--	--	--	--	--
Thiophene										
2-Thiophenemethanol	937	--	RB	RB	RB	RB	--	--	--	RB
Others										
Maltol	945	Fruit and caramel	RB	RB	RB	--	--	GB	--	--
Caffeine	1134	--	GB	GB	GB	GB	GB	GB	GB	GB

LRI: Experimental Linear Retention Index; GB: Green Bean; RB: Roasted Bean
--: compound not detected
* Sensory attributes and compounds are taken from: Flament, & Bessière-Thomas, (2002); Lee et al., (2015; 2017); Bressani et al., (2018); Martínez et al, (2017); Ribeiro et al., (2018); da Mota et al., (2020).

ARTIGO 2 - Wet fermentation of Coffea canephora by lactic acid bacteria and yeasts using SIAF method enhances coffee quality

Versão preliminar redigida de acordo com as norms do periódico *Food Microbiology*
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Highlights:

- LAB single and co-inoculated with yeast was studied for the first time in *Coffea canephora*
- *L. mesenteroides* showed better fermentative performance
- The score of inoculated coffees increase of up to 5 points
- Inoculation of LAB and yeasts is feasible alternative for obtaining conilon coffee with superior quality

Abstract

This work aimed to evaluate the impact of simple inoculation and co-cultivation of LAB and yeasts during the wet processed conilon by the SIAF method. Chemical (HPLC and GC-MS), microbial (qPCR) and sensory characterization of the coffees were performed. *L. mesenteroides* was detected in high concentrations in coffee fruits (8.37 log10 cells/mL). The growth of *L. plantarum* was favored in the first 36 h of fermentation. Microbial metabolism resulted in high carbohydrate consumption glucose (96.58%), fructose (93.59%) and sucrose (100%). Lactic and acetic acids were the main acids produced during the fermentation process and contributed positively to the characteristics of the beverage. In addition, 139 volatile compounds (belonging to 16 classes) were identified among green and roasted coffee samples. Specific compounds were detected in the inoculated treatments only: 9,12-octadecadienoic acid, methyl ester, octadecyl trifluoroacetate and benzyl benzoate were detected in the fermentation between *L. mesenteroides* and *L. plantarum*. Tridecanal was detected in co-cultures of BAL with *T. delbrueckii*.The coffee score ranged from 75.33 to 81.33. Coffees from four inoculated treatments were classified as fine (80.0 - 89.0), while coffees from the Control and three inoculated treatments were classified as premium (70.0 - 79.0). *L. mesenteroides* inoculated alone or in co-culture with *S. cerevisiae*; *T. delbrueckii* or *L. plantarum* produced superior quality coffees. Thus, the co-cultivation between lactic acid bacteria and yeasts becomes a new way to improve the quality of conilon coffee and obtain beverages with differentiated and better

sensory profiles.

KEYWORDS: Conilon coffee, *L. mesenteorides*, whole fruits, sensory characteristics

1. Introduction

Conilon coffee is a variety that stands out for its resistance to drought and high commercial value. In Brazil, the average production for the year 2022 is estimated at 16.96 million bags. The state of Espírito Santo is the largest producer, accounting for more than 60% of the national crop (CONAB, 2022). Coffee is a product consumed and known worldwide, and different processing steps are applied, resulting in beverages with different sensory profiles (Shankar et al., 2022).

The different process employed in postharvest coffee mainly aim to remove the mucilage of the cherries and reduction of coffee moisture of beans to 10 to 12% (De Bruyn et al 2016). In wet coffee processing, flotation tanks are used to select the fruits, which are pulped, leaving a thin layer of pulp around the seed. Then, beans are submerged in water between 24 and 72 h, where are fermented, and taken to the drying stage (Schwan and Fleet, 2015). Microbial activity is responsible for the production of flavor and aroma precursor metabolites, yeast and lactic acid bacteria are especially involved in the fermentation step (Shankar et al., (2022).

Bacteria and yeasts are ubiquitous during throughout coffee processing (Elhalis et al., 2020d; Evangelista et al., 2015; Ribeiro et al., 2018). Activity of specific microbial groups are related their intra and interspecies interaction, resulting in the production of extracellular enzymes, volatile and non-volatile metabolites, and pH change that contribute to formation of sensory profile of coffee beverage (da Silva et al., 2021; Elhalis et al., 2020d, 2021).

The beverage *Coffea canephora* was not considered attractive due to the poor quality of the cup. However, the view on the quality of the beverage from this species has changed due to post-harvest management practices, as well as biotechnological advances applied to coffee processing. The use of starter culture has been shown to contribute to the balance of volatiles and non-volatiles, improving the sensory attributes of roasted coffee. Yeasts used as inoculum

reduced the population of filamentous fungi, in addition to potentiating the concentration of organic acids and volatile compounds, improving the final score of the drink (Prakash et al. 2022; da Silva et al., 2022). The fermentative performance of lactic acid bacteria and yeasts as a starter culture in Conilon coffee has been little investigated. There are few published works on this topic, which led us to investigate the effects of inoculation of the two groups in the fermentation of Conilon coffee.

Leuconostoc and *Lactiplantibacillus* are present during the coffee fermentation process (Prakash et al., 2022). Under anaerobic conditions, lactic acid production is intensified, contributing to the perception of acidity and body of coffee beverages (Pereira et al 2016). The amount of oxygen (O_2) varies according to the method used and impacts the quality of the beverage.

Self-induced anaerobic fermentation (SIAF) is a method in which the anaerobic condition is gradually formed by microbial metabolism that uses the remaining O_2 for its metabolic reactions, releasing CO_2, volatile and non-volatile compounds (Pereira et al., 2022). In addition, it positively impacts the fermentative performance of lactic acid bacteria and yeasts during the processing of natural and pulped coffee (da Mota et al., 2020; Martins et al., 2020). However, this method has not yet been tested for the wet process of Conilon coffee.This study aimed to evaluate the impact of inoculation single and co-cultivation of LAB and yeasts during wet process using SIAF method, on the chemical and sensory quality of coffee. Moreover, the dynamic behavior of the starter cultures was evaluated by real-time PCR.

2. Material and Method

2.1 Coffee harvesting and processing

Ripe coffee fruits (cherry coffee), variety Conilon LB1, were harvested mechanically at Fazenda Venturim, located in the municipality of São Domingos do Norte (19°06′22″S

40°35′32″W), Espírito Santo, Brazil, where the experiment was carried out. The whole coffee fruit were processed via wet using SIAF method. Fermentations were performed without (control) and with starter culture (*Leuconostoc mesenteroides* CCMA 1105; *Lactiplantibacillus plantarum* CCMA 1065, *Torulaspora delbrueckii* CCMA 0684 and *Saccharomyces cerevisiae* CCMA 0543) for 72 h in closed high-density polyethylene bioreactors (50 L capacity) containing approximately 32 L of coffee fruits and 16 L of fresh water.

After fermentation, the coffee was washed, mechanically pulped (Ecoflex, Pinhalense, São Paulo, Brazil) and taken to suspended terraces for the drying stage (until the moisture content was 11-12%). The pH (Phmeter - KASVI, model K39-0014PA), temperature and soluble solids (Refractomemter - DFV, model RM-M3) content were measured during the fermentation. Samples (150 g) were collected every 36 h and stored at -20 °C for analysis (chemical and microbiological). High barrier packaging (double kraft paper and internally coated with a plastic film impermeable to oxygen) were used to store the coffee beans for further sensory analysis. All fermentations were performed in triplicate.

2.2 Culture conditions and inoculum preparation

Saccharomyces cerevisiae CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, *Leuconostoc mesenteroides* CCMA 1105 and *Lactiplantibacillus plantarum* CCMA 1065 were obtained from the Agricultural Microbiology Culture Collection of the Federal University of Lavras. The starter culture were isolated from different processing methods and coffee varieties. (Ribeiro et al., 2018; Silva et al., 2000).

The inoculated fermentations were identified as: **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus plantarum*; **MP**: *Leuconostoc mesenteroides + Lactiplantibacillus plantarum;* **MD**: *Leuconostoc mesenteroides + Torulaspora delbrueckii;* **MC**: *Leuconostoc mesenteroides + Saccharomyces cerevisiae;* **PD**: *Lactiplantibacillus plantarum + Torulaspora*

delbrueckii and **PC**: *Lactiplantibacillus plantarum* + *Saccharomyces cerevisiae* (Supplementary material Fig. S1).

Yeast cells were reactivated and grown in YEPG broth [10 g/L yeast extract (Merck); Peptone 20 g/L (HiMedia); 20 g/L dextrose (Merck)] at 30 °C until reaching a final concentration of 10^9 cells/mL. The lactic acid bacteria strains were reactivated and cultivated at 35 ºC in MRS broth (MRS) (De Man Rogosa Sharpe, Merck, Darmstadt, Germany) until a 10^9 cells/mL concentration. After growth, the cell suspension from both groups (bacteria and yeast) was centrifuged (7000 RCF, 10 min) and resuspended in 500 mL of distilled water. This solution was applied to the coffee, obtaining a final concentration of approximately 10^7 cells/mL.

2.3 Real-time PCR (qPCR)

The population of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Leuconostoc mesenteroides* and *Lactiplantibacillus plantarum* was monitored during fermentation. Strain and coffee samples DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden Germany) according to the "DNA Purification from Tissues" protocol. Yeasts and bacteria were grown separately in YEPG broth and MRS both, respectively. The microrganisms were incubation temperatures were 30 ºC (yeasts) and 35 ºC (LAB) for 24 h. Yeasts populations were obtained by Neubauer chamber count, and lactic acid bacteria population were obtained by plating on MRS agar (Merck, Darmstadt, Germany). Serial dilution (1:10) was performed from 10^8 to 10^3 cells/mL to prepare the standard curve. Each point on the curve was analyzed in triplicate, as well as the coffee sample.

A Rotor-Gene Q system (Qiagen, Hombrechtikon, ZH, Switzerland) was used for qPCR analysis. Each reaction had a final volume of 25 µL: 12.5 µL of Rotor-Gene SYBR Green master mix (Qiagen, Stockach, Konstanz, Germany), 2.5 µL (10 µM) of each primer

(Invitrogen, São Paulo, SP, Brazil), 1 μL of DNA and 6.5 μL of ultrapure water. The mixture was heated at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 15 s. The cycling temperature was increased by 1 °C every 5 s from 50 °C to 99 °C to obtain the melting curve (Batista et al., 2015). Specific primers were used (Supplementary material: Table S1) (Cho et al., 2010; Díaz et al., 2013; Mastrigt et al., 2019; Zott et al., 2010). Specificity was evaluated on GenBank BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.4 Determination of organic acids, bioactive compounds, and carbohydrates

Organic acids (acetic, butyric, citric, lactic, malic, and succinic), bioactive compounds (chlorogenic acid, caffeine, and trigonelline), and carbohydrates (sucrose, glucose, fructose, and mannitol) were evaluated by high-performance liquid chromatography (LC-10Ai, Shimadzu Corp., Japan) during coffee fermentation. A UV–VIS detection system (SPD-10Ai) was used for organic acids (Shim-pack SCR 101-H; 7.9 mm × 30 cm column) and bioactive compounds (C18 reversed-phase column AG-120; 150 × 4.6 mm, 5 μm). A refractive index detector (RID-10Ai) was used for carbohydrates (Shim-pack SCR-101C column).

The carbohydrates and organic acids were extracted according to (Ribeiro et al., 2017). The operating conditions for carbohydrate and organic acids were performed as described by da Mota et al. (2020).

The bioactive compounds were extracted and analyzed according to Malta and Chagas (2009). The concentration of each sample was determined through an external calibration method, injecting different concentrations of the standard. The analyses were performed in triplicate.

2.5 Determination of volatile compounds

2.5.1 Volatile compounds

Samples of green and roasted coffee beans (2 g) were ground for headspace solid phase microextraction (HS-SPME) of the volatiles. The samples were heated in a water bath (15 min/60 °C) until the system reached equilibrium. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm SPME fiber (Supelco Co., Bellefonte, PA, USA) was used (30 min/60 °C) for the absorption of volatile compounds. Volatile compounds were analyzed in a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010) using a Carbowax column (30 m x 0.25 mm x 0.25 mm). The operating conditions were the same as those described by Ribeiro et al. (2017). Alkane standards (C10-C40) were used to identify and calculate each compound's linear retention index (LRI). The mass spectra generated for each compound were compared with those of the NIST 11 library.

2.5 Sensory Analysis - Cup Test

Six trained panelists (Q-grader) evaluated the samples, according to Fine Robusta Cupping protocol (ICO, 2010). Each sample was evaluated five times by the same taster. The attributes evaluated were fragrance/aroma, flavor, aftertaste, salinity/acidity, bitterness/sweetness, and mouthfeel using a scale from 6 to 10 points. Descriptive analysis of the samples also was performed.

2.6 Statistical Analysis

The experiment was performed by applying a completely randomized design. The results were subjected to ANOVA, and the means were compared by the Scott-Knott test ($p \leq 0.05$) using the statistical program RStudio, version 3.3.3.

3. Results

3.1 Physicochemical characterization

The initial temperature of the coffee mass ranged from 22 to 24 °C, increasing by up 2 °C during fermentation (Table 1). The soluble solid concentration in coffee fruit ranged from 7 to 16 °Brix, decreasing during fermentation. The PD fermentation showed an intense reduction in the concentration of soluble solids (16 to 8 °Brix). For all fermentation, the initial pH was approximately 4.6 - 5.5 and decreased gradually during the fermentation of 72 h reaching approximately 3.5 - 3.9. The MC fermentation was characterized by higher acidity (3.5).

Table 1. Physicochemical parameters during of fermentation

Fermented	Time (h)	Temperature (°C)	Soluble solid (°Brix)	pH
Control	0	24.0	14.0	5.5
	36	22.0	11.0	4.1
	72	23.0	8.5	3.9
M	0	24.5	13.0	4.8
	36	22.0	11.0	4.0
	72	24.0	7.0	3.7
P	0	24.0	13.5	4.9
	36	22.0	9.0	3.9
	72	24.0	8.5	3.8
MD	0	23.0	13.5	5.3
	36	22.0	11.5	4.0
	72	23.0	11.0	3.8
PD	0	22.0	16.0	5.4
	36	24.0	11.0	4.1
	72	24.0	8.0	3.9
MP	0	24.0	13.9	4.6
	36	22.0	11.2	3.9
	72	23.0	7.8	3.7
MC	0	26.0	16.0	4.8
	36	22.0	11.0	4.1

	72	23.0	9.0	3.5
PC	0	22.0	15.5	5.0
	36	23.0	12.0	4.1
	72	24.0	8.5	3.6

Control: Spontaneous fermentation; **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus plantarum*; **MP**: *Leuconostoc mesenteroides + Lactiplantibacillus plantarum;* **MD**: *Leuconostoc mesenteroides + Torulaspora delbrueckii;* **MC**: *Leuconostoc mesenteroides + Saccharomyces cerevisiae;* **PD**: *Lactiplantibacillus plantarum + Torulaspora delbrueckii*; **PC**: *Lactiplantibacillus plantarum + Saccharomyces cerevisiae*.

3.2 Microbial density of starter cultures

Monitoring of microbial density of *L. mesenteroides*, *L. plantarum*, *S. cerevisiae,* and *T. delbrueckii* was performed by qPCR analysis. After 72 h, *L. mesenteroides* was detected in high concentrations in all the fruits of fermented coffee (8.37 $\log_{10}$ cells/mL). Even though the *L. mesenteroides* population did not show statistical differences between fermentations, the MP fermentation showed the highest microbial density 9.63 $\log_{10}$ cells/mL among fermentations (Fig. 1A).

With 36 h of fermentation, the development of L. plantarum was favored, which showed a population increase of 2.32 log10 cells/mL (P), and 2.48 log10 cells/mL (PD), and 2.44 log10 cells/mL. mL (PC) when compared to the beginning of fermentation. In the PD fermentation, the lowest population growth of L. plantarum occurred with 72 h of fermentation (1.56 log10 cells/mL) (Fig 1B).

In the co-cultivation fermentation, the growth of starter yeast was influenced by the inoculated lactic acid bacteria. Thus, the growth of *T. delbrueckii* was favored in co-inoculated with *L. mesenteroides* (increase of 2.9 $\log_{10}$ cells/mL) and *S. cerevisiae* was favored in co-inoculated with *L. plantarum*, increasing 0.74 $\log_{10}$ cells/mL (Fig. 1C and 1D).

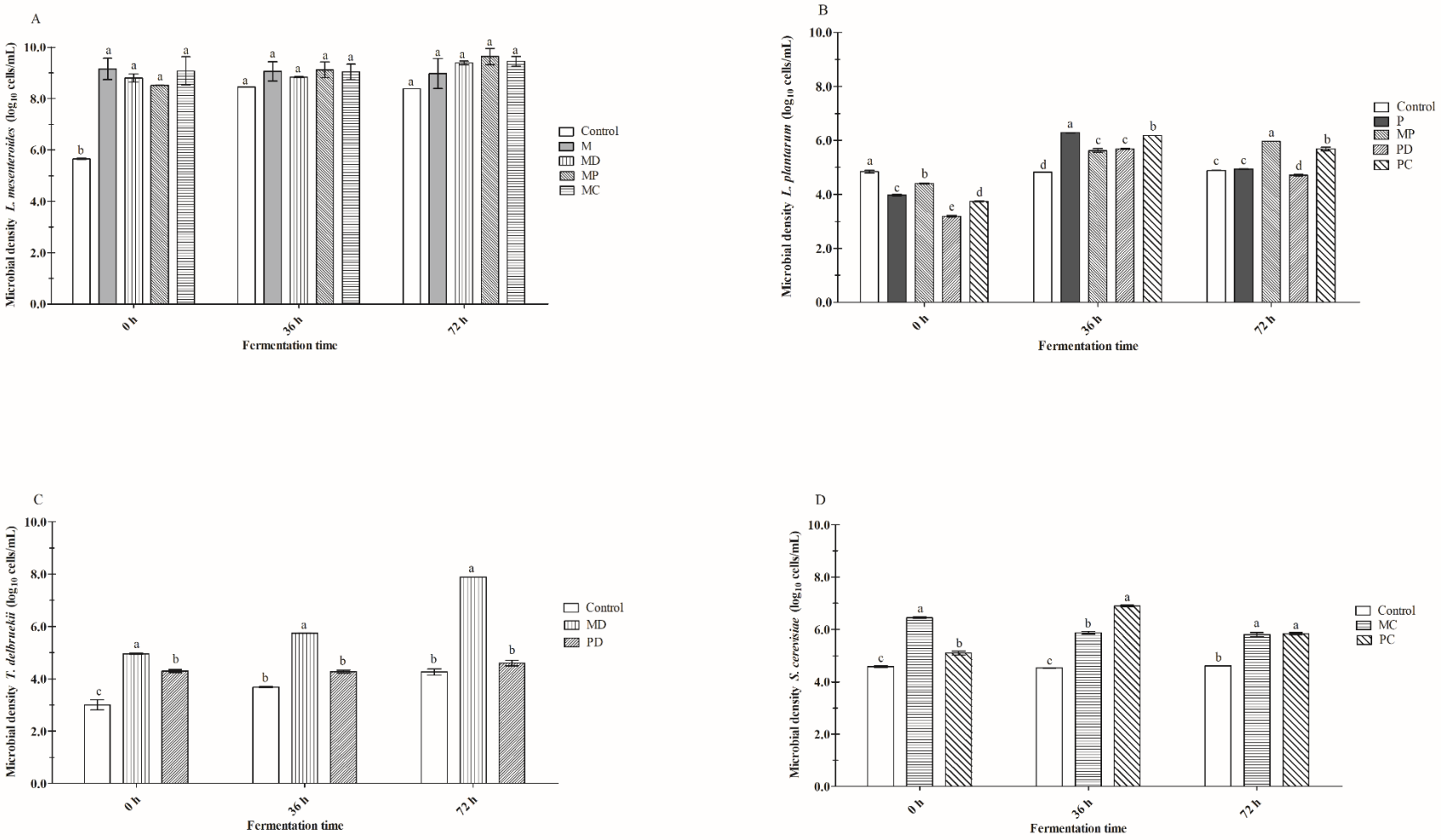

Fig. 1. Microbial density in spontaneous and inoculated coffee fermentations: (A) *L. mesenteroides* CCMA 1105, (B) *L. plantarum* CCMA 1065, (C) *T. delbrueckii* CCMA 0684, (D) *S. cerevisiae* CCMA 0543. **Control**: Spontaneous fermentation; **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus*

plantarum; **MP**: *Leuconostoc mesenteroides + Lactiplantibacillus plantarum;* **MD**: *Leuconostoc mesenteroides + Torulaspora delbrueckii;* **MC**: *Leuconostoc mesenteroides + Saccharomyces cerevisiae;* **PD**: *Lactiplantibacillus plantarum + Torulaspora delbrueckii*; **PC**: *Lactiplantibacillus plantarum + Saccharomyces cerevisiae*.

Mean values followed by the same letter do not differ significantly by the Scott-Knott test ($p > 0.05$) within the same fermentation time.

3.3 Carbohydrates

Sucrose, glucose, fructose, and mannitol were monitored during fermentation. After 72 h of fermentation, P fermentation had the highest concentration of fructose (1.16 g/kg), while M, MD and MP fermentations had higher concentrations of glucose, respectively 0.51, 0.64, and 0.79 g/kg (Fig. 2A and 2B). On the other hand, the co-cultivation treatments showed a total fructose consumption that ranged from 73.48 to 87.88% after 72 h of fermentation. At the end of the process, the co-cultivation of *L. plantarum* with *T. delbrueckii* (PD fermentation) showed glucose consumption above 96.0% (Fig. 2B). Mannitol was produced during fermentations (Fig. 2D). The inoculation of *S. cerevisiae* CCMA 0543 (MC and PC fermentation) showed the highest concentrations of mannitol with 72 h of fermentation.

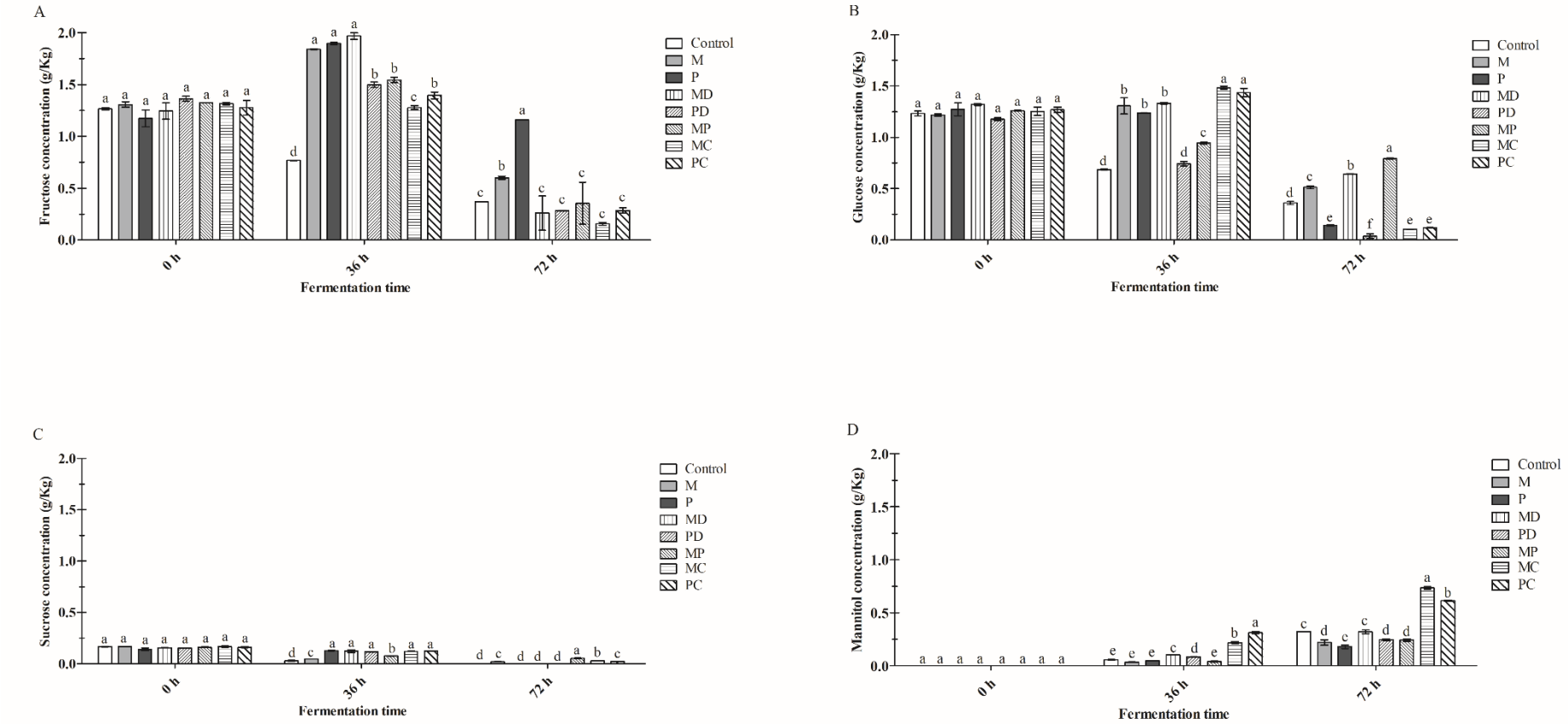

Fig. 2. Carboydrates detected during processing of conilon coffee: (A) Fructose, (B) Glucose, (C) Sucrose, (D) Mannitol. **Control**: Spontaneous fermentation; **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus plantarum*; **MP**: *Leuconostoc mesenteroides* + *Lactiplantibacillus plantarum;* **MD**: *Leuconostoc mesenteroides* + *Torulaspora delbrueckii;* **MC**: *Leuconostoc mesenteroides* + *Saccharomyces cerevisiae;* **PD**: *Lactiplantibacillus plantarum* + *Torulaspora delbrueckii*; **PC**: *Lactiplantibacillus plantarum* + *Saccharomyces cerevisiae*. Mean values followed by the same letter do not differ significantly

by the Scott-Knott test ($p > 0.05$) within the same fermentation time.

3.4 Organic acids

Acetic, citric, lactic, malic and succinic acids were identified in all treatments (Table 2). Lactic acid was detected in the fermented M, P, PD, and PC at the beginning of the fermentation, while succinic acid was detected in the control, MD, PD, MC and PC fermentations. In contrast, acetic acid was detected only in the fermented M, while malic and citric acids were present in all fermentations.

After 36 h of fermentation, the malic acid consumption in the fermentations with *S. cerevisiae* ranged from 63.4% (PC) and 75.7% (MC). The PD fermentation was responsible for the acetic acid production (0.16 g/kg) after 36 h of fermentation. However, after 72 h of fermentation, it was consumed, unlike the other treatments, which showed higher concentrations of this acid at the end of the process.. In relation the fruit composition, the citric acid concentration reduced during fermentation, except to MP. On the

other hand, lactic acid was produced and after 72 h, it reached maximum concentration in the MD and MP fermentations (1.35 and 1.43 g/kg, respectively). The succinic acid concentration increased in most fermentation being more evident in the fermented P and MP fermentations (0.12 g/kg).

Table 2. Organic acids detected during the fermentation (0, 36, and 72 h)

Fermented	Time (h)	Organic acids (g/kg)				
		Acetic	Citric	Lactic	Malic	Succinic
Control	0	n.d	0.89 ± 0.00^{e}	n.d	2.51 ± 0.13^{d}	0.06 ± 0.0^{b}
M	0	0.15 ± 0.00^{a}	1.60 ± 0.01^{b}	0.17 ± 0.00^{a}	3.62 ± 0.24^{c}	n.d
P	0	n.d	0.88 ± 0.01^{e}	0.17 ± 0.00^{a}	3.39 ± 0.13^{c}	n.d
MD	0	n.d	1.30 ± 0.05^{c}	n.d	3.45 ± 0.19^{c}	0.10 ± 0.00^{a}
PD	0	n.d	1.28 ± 0.00^{c}	0.24 ± 0.00^{a}	3.48 ± 0.19^{c}	0.05 ± 0.00^{c}
MP	0	n.d	1.11 ± 0.01^{d}	n.d	2.38 ± 0.16^{d}	n.d
MC	0	n.d	1.79 ± 0.01^{a}	n.d	3.82 ± 0.26^{c}	0.05 ± 0.00^{c}
PC	0	n.d	2.03 ± 0.00^{a}	0.16 ± 0.00^{a}	5.05 ± 0.30^{b}	0.06 ± 0.00^{b}
Control	36	0.08 ± 0.01^{b}	0.97 ± 0.00^{d}	0.17 ± 0.00^{a}	2.33 ± 0.14^{b}	0.07 ± 0.00^{b}
M	36	0.16 ± 0.00^{a}	1.10 ± 0.00^{c}	0.29 ± 0.00^{a}	3.19 ± 0.16^{a}	0.07 ± 0.00^{b}
P	36	n.d	1.42 ± 0.00^{b}	0.28 ± 0.00^{a}	2.39 ± 0.21^{b}	0.07 ± 0.00^{b}
MD	36	n.d	1.77 ± 0.00^{a}	0.31 ± 0.00^{a}	2.52 ± 0.26^{b}	0.08 ± 0.00^{a}
PD	36	0.16 ± 0.00^{a}	1.10 ± 0.00^{c}	0.31 ± 0.00^{a}	2.53 ± 0.16^{b}	0.06 ± 0.00^{c}
MP	36	n.d	1.10 ± 0.00^{c}	0.27 ± 0.00^{a}	2.20 ± 0.15^{b}	0.05 ± 0.00^{d}
MC	36	n.d	0.91 ± 0.01^{d}	0.27 ± 0.00^{a}	0.93 ± 0.13^{d}	0.06 ± 0.0^{c}
PC	36	0.17 ± 0.00^{a}	1.01 ± 0.00^{d}	0.41 ± 0.00^{a}	1.85 ± 0.15^{c}	0.07 ± 0.0^{b}
Control	72	0.18 ± 0.00^{a}	0.78 ± 0.02^{c}	1.05 ± 0.00^{b}	0.81 ± 0.11^{b}	0.07 ± 0.00^{d}
M	72	0.19 ± 0.00^{a}	1.01 ± 0.00^{b}	1.11 ± 0.00^{b}	1.41 ± 0.15^{a}	0.09 ± 0.00^{c}
P	72	0.25 ± 0.00^{a}	0.94 ± 0.00^{b}	1.11 ± 0.00^{b}	1.58 ± 0.14^{a}	0.12 ± 0.00^{a}

MD	72	0.17 ± 0.00[a]	0.55 ± 0,00[c]	1.35 ± 0.05[a]	0.59 ± 0.08[b]	0.08 ± 0.00[c]
PD	72	n.d	0.90 ± 0.00[b]	0.87 ± 0.00[c]	0.34 ± 0.13[c]	0.07 ± 0.00[d]
MP	72	0.20 ± 0.00[a]	1.41 ± 0.00[a]	1.43 ± 0.00[a]	0.88 ± 0.11[b]	0.12 ± 0.00[a]
MC	72	0.17 ± 0.00[a]	0.94 ± 0.00[b]	0.68 ± 0.00[c]	0.67 ± 0.10[b]	0.10 ± 0.00[b]
PC	72	0.18 ± 0.00[a]	1.24 ± 0.00[a]	0.50 ± 0.00[d]	0.76 ± 0.11[b]	0.10 ± 0.00[b]

nd = not detected

Means ± standard deviation in the same column for each fermentation time, followed by the same lowercase letters, indicate that the treatments do not differ from each other by the Scott-Knott test (p >0.05).

Fermented: M: *L. mesenteroides*; **P**: *L. plantarum*; **MT**: *L. mesenteorides + T. delbrueckii*; **PT**: *L. plantarum* + T. *delbrueckii*; **MP**: *L. mesenteroides* + *L. plantarum*; **MS**: *L. mesenteroides + S. cerevisisae*; **PS**: *L. plantarum + S. cerevisiae*.

3.5 Caffeine, trigonelline, and chlorogenic acid

Chlorogenic acid, trigonelline and caffeine showed different concentrations between green and roasted coffee samples (Fig. 3). There were changes in chlorogenic acid and trigonelline levels in all roasted coffee samples. In green coffee, chlorogenic acid levels were more evident in Control (12.68 g/Kg) and in roasted coffee the reduction of this compound reached up to 8.61-fold (MC). The reduction of trigonelline reached 10-fold in the fermented PC. On the other hand, the caffeine content was potentiated in the samples of all fermented coffees. In green coffee, the concentration was more expressive in the fermented M (8.09 g/kg). After roasting, it was observed that the concentration of caffeine reached higher levels, being more evident in the fermented MC (15.18 g/kg), with levels 3.29 higher than in the green beans.

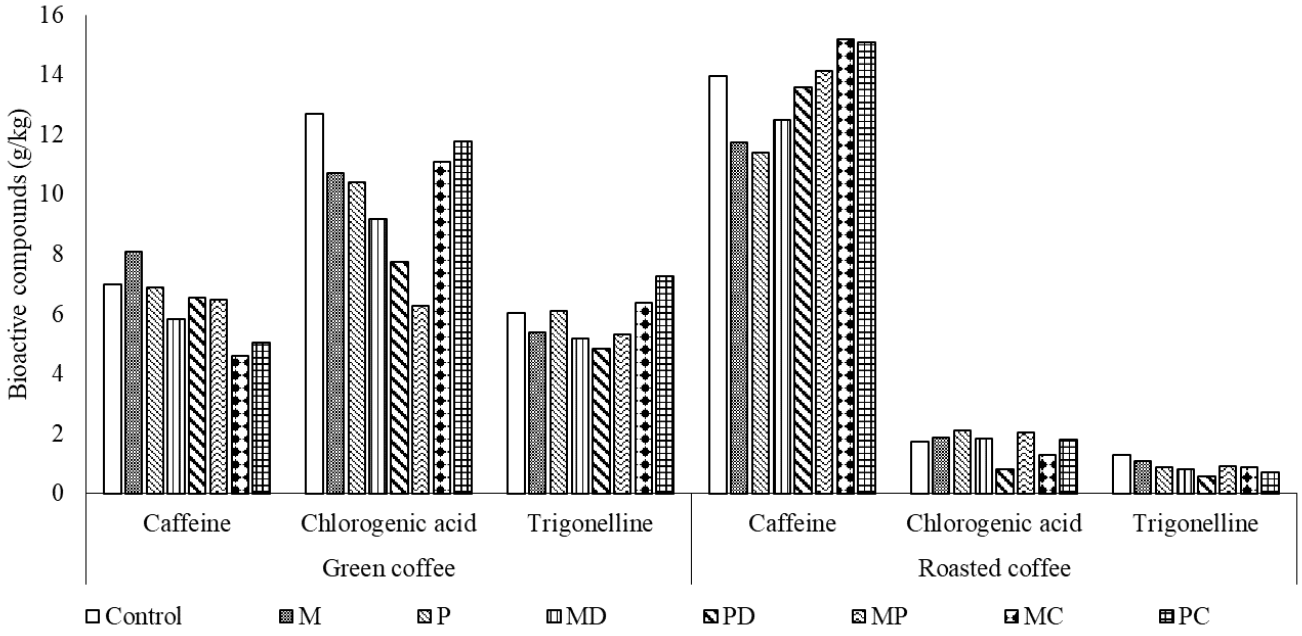

Fig. 3. Caffeine, chlorogenic acid and trigonelline contents in (A) green coffee and (B) roasted coffee

3.6 Profile of volatile compounds

In the samples of green and roasted coffee were identified 137 volatile compounds belonging to 16 chemical classes (Supplementary material Table S2), such as, acids (21), esters (18), pyrazines (15), ketones (12), alcohols (10), furans (14), hydrocarbons (7), phenols (8), aldehydes (6), pyrroles (6), lactone (3), formate (2), furanones (2), pyridines (2), thiophenes (2) and thiazole (1) and others (8). The peak areas corresponding to each compound in the different fermented products can also be observed in Supplementary material (Table S2).

The culture starter modified the profile of volatile compounds. Octadecanoic acid; benzeneacetic acid, ethyl ester; and benzoic acid, 2-hydroxy-, ethyl ester were detected in the inoculated fermentations. Tridecanal was detected in co-cultures of LAB with *T. delbrueckii*. Specific compounds were produced by P (decanol; 2-undecanone; Phenol, 2-methyl-), PC (glycerol; benzoic acid, ethyl ester), PD (hexadecanal), MP (geraniol; 9,12-octadecadienoic acid, methyl ester; benzyl benzoate; tetradecane) and MC (ethyl 9-hexadecenoate) fermentations.

In the roasted coffee, 2-butanone,1-(acetyloxy) was detected in the co-cultivation between *L. mesenteroides* with *L. plantarum* (MP) and *L. mesenteroides* with *S. cerevisiae* (MC). 2-furancarboxaldehyde, 5-methyl-; 1H-pyrrole-2-carboxaldehyde, 1-methyl-; 2-ethyl-6-methyl-pyrazine; and 2,5-dimethyl-4-hydroxy-3(2H)-furanone were detected in all fermentations inoculated. Pantolactone and 2-thiophenemethanol detected in the co-culture of lactic acid bacteria and *S. cerevisiae*. 1-Nonadecanol; 3-hexen-2-one, 5-methyl-; 4-hydroxy-2-methylacetophenone; furan, 2,2'-methylenebis-; 4-methyl-5H-furan-2-one; 2-hydroxy-gamma-butyrolactone was detected in PC fermentation. Furfuryl formate and 2-furanmethanol, propanoate were detected in P fermentation. 1,2-Cyclopentanedione,3-methyl; 2-cyclopenten-1-one, 3-ethyl-2-hydroxy- and 2(5H)-furanone were detected in MC fermentation. 2,6-dimethylpyrazine and 1H-indole, 3-methyl- were detected in MD fermentations. Phenol, 4-ethyl-; 1H-pyrrole, 1-butyl- and 1-hexanone, 1-(2-thienyl)- were detected in PD fermentation.

4-Methylpyrrolo[1,2-a]pyrazine was detected in MP fermentation.

2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-4-methyl (roasted coffee) and benzothiazole (green coffee) was detected in fermentations with lactic acid bacteria (M and P) (Supplementary material: Table S2).

3.7 Analysis of sensory characteristics

The sensory profile was influenced by inoculation. The attributes fragrance/aroma, flavor, aftertaste, salinity/acidity, bitterness/sweetness, and mouthfeel were evaluated using a numerical scale ranging from 6 to 10 points (Supplementary material Table S3). Coffees M, MD, MP, and MC were classified as fine beverage (80.0 - 89.0) while control, P, PD and PC were classified as premium beverage (70.0 - 79.0) (Fig. 4).

The inoculation of *L. mesenteroides* single and in co-cultivation with *S. cerevisiae* and *L. plantarum* achieved the best sensory scores (M: 81, MC: 81.33 and MP: 80.33 pts). Caramel, fruity and spices were the sensory characteristics detected in these coffees. P, PD and PC had similar sensory score (79 pts) but with different sensory profiles. Milk chocolate, caramel, almond, nuts, and spices describe P coffee. Milk chocolate, dark chocolate, nuts, fruity and spices characterized PD coffee. Dark chocolate, caramel, nuts, and spices characterized PC coffee. M (7.75 pts) and MC (7.83 pts) stood out to fragrance attribute. Salinity/acidity showed no significant difference between the fermented coffees. The control was the fermentation that presented the lowest score for all attributes. Furthermore, control and P coffee showed no significant difference for bitterness/sweetness and mouthfeel.

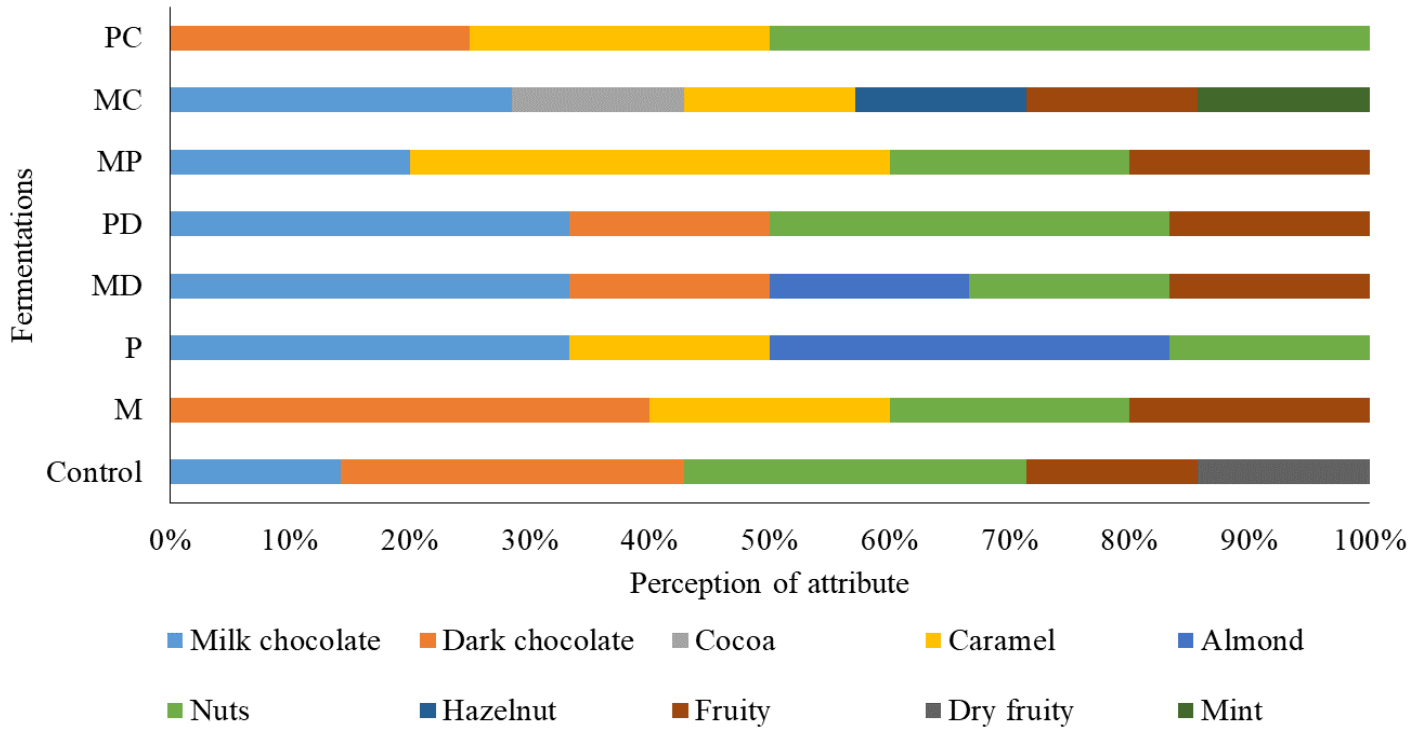

Fig. 4. Sensory descriptors reported by Q-grader during coffee analysis

4. Discussion

The coffee fruits fermented under self-induced anaerobic (SIAF) by microbial activities favored the development of *L. mesenteroides* CCMA 1105 and *L. plantarum* CCMA 1067. Species belonging to the LAB group have the ability to develop in environments with low oxygen concentrations and to ferment available carbohydrates in the absence of oxygen. Furthermore, the additional growth of these strains may be associated with adaptability to the medium and stress factors such as competition with other microorganisms, pH variation and carbohydrate availability (Lahtinen et al., 2012; Pereira et al., 2020; Puerta-Quintero, 2010). Moreover, *L. mesenteroides* is one of epiphytic microbiota of coffee that it seems dominate the entire coffee fermentative process from wet processing (Cruz-O'Byrne et al., 2021; Elhalis et al., 2020; Ribeiro et al., 2018; Pereira et al., 2021).

Yeasts play an important role in coffee fermentation, significantly impacting on coffee flavor and aroma (Elhalis et al., 2020). LAB and yeasts are known to co-exist and cooperate in several fermented foods and beverages such as coffee, cocoa, kefir, kombucha, sourdough and wine (Andreson et al., 2022; Chen et al., 2021; Minnaar et al., 2019; Viesser et al., 2021; Yang et al., 2021). Thus, the co-cultivation between bacteria and yeast is a strategy to improve the microbiological and sensory qualities of the final product. For example, yeasts supply essential amino acids and vitamins to LAB, through proteases production or cell autolysis. The organic acid, such as lactic acid, produced by LAB turn lower the pH contributing to yeasts growth, which reached their maximum population after 72 h of fermentation. These extrinsic characteristics of food fermented provides an unfavorable environment for growth of pathogenic bacteria and filamentous fungi. Besides, the pathogenic control also is supplemented by the production of bacteriocins, which are multifunctional peptides that have antimicrobial activity (Chikindas et al., 2018).

The impact of co-inoculation of LAB and yeasts on the sensory profile is probably due to the interaction symbiotic relationship between them and with the entire microbial community (Adesulu-Dahunsi et al., 2020; Vale et al., 2019). Fructose, glucose, and sucrose are main sugars accumulated during the maturation of coffee fruits, imparting a sweet flavor to the fruit. Sucrose decline during fermentation is likely due to the endogenous and microbial enzymes converting sucrose to glucose and fructose. In bacteria, this hydrolysis occur through hydrolase or sucrose phosphorylase enzymes, and in yeast through action of invertase (Basso, 2019; Kawai et al., 2020a, 2020b; Lahtinen et al., 2012).

In yeast, the hexose, such as glucose are uptake by specific transporters, entering the glycolytic pathway to be metabolized to pyruvate. Therefore, the pyruvate molecule can be processed through the respiratory or the fermentative pathway. In the fermentative pathway ethanol and CO_2 are produced, besides several other by-products, including cell biomass, glycerol, some organic acids, and volatile compound (Faria-Oliveira et al. 2015).

The MC fermentation showed by high reducing sugars consumption, and microbial population and succinic acid production increase. Succinic acid is produced by yeasts, in *S. cerevisiae* this acid can be formed by four main pathways: glyoxylate cycle by isocitrate oxidation, reductive pathway of the TCA cycle, the oxidative pathway of the TCA cycle and from amino acid catabolism (Radler et al., 1993).

Homofermentative and heterofermentative LAB use different metabolic pathways to produce ATP, such as, glycolytic and pentose phosphate pathway, respectively. Lactic acid is the main metabolite produced by LAB, however, heterofermentative ones can produce ethanol, CO_2, and acetate (Faria-Oliveira et al. 2015). Heterofermentative LAB, such as *L. mesenteroides,* are able to produce mannitol from fructose metabolism and under anaerobic conditions the mannitol can be fermented by homofermentative LAB, as *L. plantarum*. Mannitol is formed from fructose in a reaction catalyzed by mannitol dehydrogenase through

the pentose-phosphate pathway (Wisselink et al., 2002). Mannitol confers a sweet and refreshing flavor to fermented products and contributes positively to the quality of coffee (Jung et al., 2012; Wisselink et al., 2002).

The coffee quality also is influenced by acid composition that are generally recognized as flavor precursors for quality descriptors of coffee (Borém et al., 2016). Malic, succinic, and citric acids are part of the fruit composition and contribute to the beverage's acidity (Elhalis et al., 2020; Evangelista et al., 2014). Lactic, acetic, and succinic acids increase during fermentation. Lactic acid bacteria are recognized for producing lactic and acetic acids (Avallone et al., 2002). Species belonging to the genus *Leuconostoc* can perform malolactic fermentation, which malic acid is converted into lactic acid by decarboxylation with the participation of the malolactic enzyme, increasing bacterial survival under environmental stress conditions, such as low pH (Konings et al., 1997). During coffee fermentation, the reduction in malic acid contents and increase in lactic acid contents was especially obtained in the fermented coffees by *L. mesenteroides* CCMA 1105. The increase in acetic acid concentration and reduction in citric acid content may be related to citrate metabolism by LAB (Gemelas et al. 2014).

Chlorogenic acids are among the most relevant phenolic compounds found in coffee, contributing to astringency and pigmentation, furthermore to having a potential antioxidant activity and radical scavenging properties (Duarte et al., 2010). These compounds were present in lower concentrations in the roasted coffee than in the green coffee. Besides, chlorogenic acids are degraded during roasting, contribute to the flavor and aroma of the coffee. They are precursors of compounds responsible for bitterness and contribute to the formation of volatile and non-volatile compounds in the sensory profile of coffee (Duarte et al., 2010; Frank et al., 2006; Martínez et al., 2017). Trigonelline contributes to the flavor of the beverage, and the levels of this compound are greatly affected by the roasting of the grains (Bressani et al., 2018; (Selmar et al., 2015). During roasting, trigonelline degradation gives rise to pyrroles and

pyridines (Flament and Bessière-Thomas, 2001). Caffeine contributes to the bitterness of coffee, especially in the Conilon coffee, where its levels are higher when compared to arabica coffee. This compound is little affected by coffee roasting (Selmar et al. 2015). However, in this study, caffeine levels in roasted coffee were more evident than in green coffee (Fig. 3). During roasting, the beans are subjected to high temperatures, resulting in loss of water, carbon dioxide and volatile compounds, increasing levels of caffeine in roasted coffee (Girma et al., 2020).

Volatile compounds influence the aromatic profile of coffee, directly impacting the quality of the beverage. Most of these compounds are derived from precursors formatted in green coffee. During roasting, thermally catalyzed reactions occur, giving rise to these compounds, which can also be originated from chemical and biochemical reactions during processing (Buffo and Cardelli-Freire, 2004).

Volatile compounds in green coffee can be formed from the metabolism of microorganisms or from chemical reactions during coffee processing. Alcohols class, such as, decanol (only in P), 1-dodecanol, 1-hexadecanol, octadecanol (fermented P and MD), and benzyl alcohol were identified in green coffee, coming primarily from the reduction of aldehydes and methyl ketones corresponding, from glucose degradation or amino acid catabolism by yeasts and bacteria during coffee fermentation (Arora et al., 1995; Cheng, 2010). In this study, volatile acids were present, especially in green coffee. They may be derived from the metabolism of fatty acids by yeasts and bacteria present during the coffee fermentation process. The different volatile compounds, such as, hexanoic, heptanoic, octanoic, nonanoic, and decanoic acids (in both green and roasted coffee) contributed to the sensory profile with notes of nuts, fruit, and acidic odor (Swiegers et al., 2005).

Esters are of great relevance in the composition of coffee and are mainly responsible for the fruity and floral aroma of the beverage. They can be formed by LAB through the

esterification of short-chain free fatty acids with an alcohol molecule and by the metabolism of lipids and acetyl-CoA, by yeasts (Nogueira et al., 2005; Swiegers et al., 2005). Moreover, some esters were detected only in the inoculated treatments, such as benzene acetic acid, ethyl ester; benzoic acid, 2-hydroxy, ethyl ester-; 2-ethyl hexyl salicylate, and isopropyl myristate inferring that they were produced by the LAB and yeasts used as starter cultures in this study. These esters can be correlated, for example, with the fruity notes reported by trained tasters. 9,12-Octadecadienoic acid, methyl ester, octadecyl trifluoroacetate, and benzyl benzoate were detected in MP fermentation. Benzyl benzoate may have influenced fruity notes detected in this coffee beverage.

Relevant volatile compounds were formed during roasting, mainly from the Maillard reaction, contributing to the complex composition of coffee. Pyrazines (for example, 2,6-dimethylpyrazine, 2-ethyl-3-methyl-pyrazine, and 2-ethyl-2,5-dimethyl-pyrazine) can be correlated with the sweet, hazelnut, nut, caramel, roasted, and chocolate notes detected in the different coffees. The formation of pyrazines involves condensing carbonyl and amine groups, producing a Schiff base (Caporaso et al., 2018)

Heterocyclic compounds such as, pyrroles and furans classes, found in roasted coffee are formed during roasting from non-volatile precursors (such as polysaccharides, lipids, proteins and free aminoacids) in green coffee (Lee and Shibamoto, 2002). 1H-pyrrole-2-carboxaldehyde-1-methyl; 1*H*-pyrrole, 1-butyl (PD fermentation); and indole and 1H-indole, 3-methyl (MD fermentation) contributed characteristic notes sweet, green, woody, and floral in the sensory profile this beverage. Furan class, the furfural can contribute to a sweet, caramel odor, arises from the rearrangement of the compounds of Armadori. The formation of 5-hydroxymethylfurfural probably results from the thermal degradation of glucose or other hexoses and contributes to the sweet notes of coffee (control, M, P, MC and PC) (da Silva et al., 2008).

The formation of the coffee's aroma is a complex process; however, volatile compounds play a significant role in the sensory characteristics of the final product. The different functional classes detected was possible to correlate them with the different sensory notes reported by the tasters during cup testing, giving rise to beverages with specific and unique characteristics.

Consumers' search for coffees with differentiated quality is a growing reality, boosting the specialty coffee market (Martinez et al., 2021). Thus, sensory analysis has become a valuable tool for determining the characteristics related to coffee quality.

Leuconostoc mesenteroides inoculation showed the best sensory notes (MP, M, and MC). Caramel and fruity were the sensory notes detected in these coffees. Fruity notes may be associated with esters, acids, ketones, and aldehydes composition. The ability of the starter culture used to produce metabolites that positively influenced the quality of the coffee reinforces the importance of the role of starter cultures in the development of the sensory profile of the beverage, contributing to the improvement of the quality of the coffee.

5. Conclusions

The fermentation using starter cultures of Conilon coffee fruits by the SIAF methodology improved coffee quality. The strains used were benefited by the conditions in the bioreactors (self-induced anaerobiosis), resulting in coffee beverage with differentiated sensory characteristics. *L. mesenteroides* inoculated alone in co-culture with *S. cerevisiae* and *L. plantarum* produced superior quality coffees (fine beverages). Therefore, the wet fermentation of coffee with inoculation of BAL and yeasts by the SIAF methodology becomes a viable alternative for obtaining Conilon coffee with superior quality.

Declaration of competing interest

The authors do not have any conflict of interest.

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Appendix A. Supporting Information - Supplementary Material

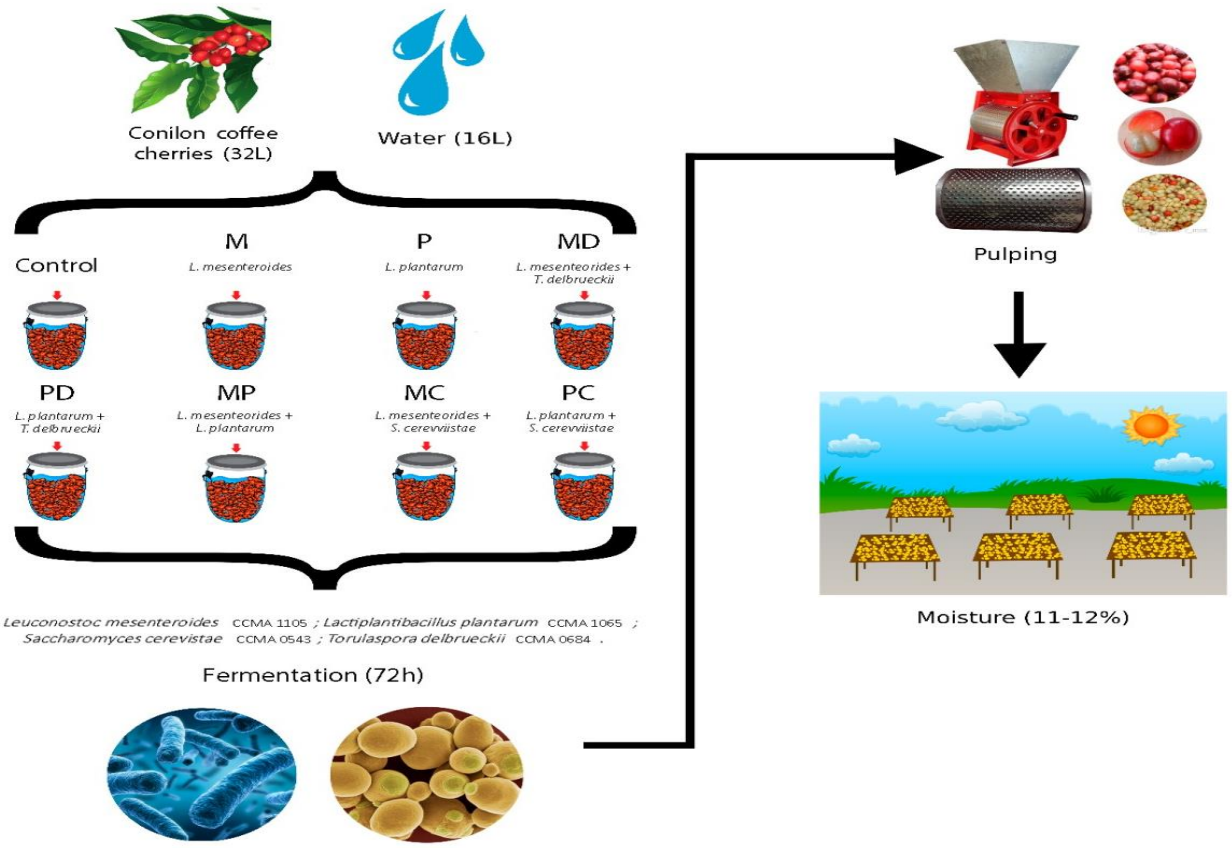

Fig S1. Flowchart of the experiment.

Control: Spontaneous fermentation; **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus plantarum*; **MP**: *Leuconostoc mesenteroides* +

Lactiplantibacillus plantarum; **MD**: *Leuconostoc mesenteroides + Torulaspora delbrueckii*; **MC**: *Leuconostoc mesenteroides + Saccharomyces cerevisiae*; **PD**: *Lactiplantibacillus plantarum + Torulaspora delbrueckii*; **PC***: Lactiplantibacillus plantarum + Saccharomyces cerevisiae***.**

Tables

Table S1 - Specific primers used for qPCR análisis

Specie	Primers		
	Name	Sequence (5'-> 3')	Source
L. plantarum	plnEF fw	CTATTTCAGGTGGCGTTTTC	Cho et al., (2010)
	plnEF rv	GTGGATGAATCCTCGGACAG	
L. mesenteroides	Fr	AAGTTTCGTGCGATTGCTG	Mastrigt et al., (2019)
	Rv	ATGCCTACTGGATTGGGATG	
S. cerevisiae	SC-5fw	AGGAGTGCGGTTCTTTGTAAAG	Díaz et al., (2013)
	SC-3bw	TGAAATGCGAGATTCCCCT	
T. delbrueckii	Primer 1Tods L2	CAAAGTCATCCAAGCCAGC	Zott et al., (2010)
	Primer 2 Tods R2	TTCTCAAACAATCATGTTTGGTAG	

Table S2 - Volatile compounds detected in samples of green and roasted coffee beans.

Class/ Compounds	LRI	Sensory Perception*	Green coffee (10^4 area)								Roasted coffee (10^4 area)							
			Control	M	P	MD	PD	MP	MC	PC	Control	M	P	MD	PD	MP	MC	PC
Acid																		
Acetic acid	826	Pungent, stinging sour	230	940	9.74	40	3.3	20	22	6.2	--	--	--	--	--	720	1200	--
Hexanoic acid	940	Sour, fatty, sweaty, cheese-like odor	2.6	5.4	4.4	3.1	3.6	3.1	--	--	340	--	--	--	--	--	--	100
Heptanoic acid	941	Fatty, nutty, fruit flavor	1.1	4.5	--	2.8	6.3	--	--	2.8	--	--	--	--	--	--	--	--
Octanoic acid	957	Fatty, waxy, cheese-like	6.5	8.5	6	3.9	7	9	7	--	--	--	--	--	--	--	--	--
Nonanoic acid	971	Waxy, cheese-like	23	15	13	5.5	--	20	13	1.2	--	--	--	--	--	--	--	290
Decanoic acid	986	--	10	10	7	--	--	--	--	10	--	--	--	--	--	--	--	--
Dodecanoic acid	1013	Rancid flavor	14	18	9.3	--	14.2	19	37	22	--	--	--	--	--	--	--	4.3
Tetradecanoic acid	1037	-	137	120	100	35	98	77	60	86	370	120	--	--	--	--	--	27

Pentadecanoic acid	1048	Fatty, weak flavor	55	82	65	28	44	59	22	52	--	--	--	--	--	--	--	--
Eicosanoic acid	1044	--	29	--	--	--	30	--	--	--	--	--	--	--	--	--	--	--
n-Hexadecanoic acid	1060	Rancid and acid flavor	480	930	800	120	1.1	250	170	890	130	--	--	--	--	--	--	52
Octadecanoic acid	1076	--	--	130	--	--	--	130	4.6	130	--	130	--	--	--	--	--	--
Benzenacetic acid	1024	Honey flavor	--	--	--	--	--	18	7.5	--	--	--	--	--	--	--	--	--
Propanoic acid	849	Butter, cheese odor	--	--	--	--	--	--	--	--	--	--	--	890	--	--	--	240
Butanoic acid, 3-methyl-	887	Fruity flavor (apple and cherry)	--	3.1	--	3.8	3.6	4.4	2.2	--	1800	2500	3400	2500	2000	1700	2000	1700
Pentanoic acid, 4-oxo-	995	Sweet, caramel and acid flavor	--	--	--	2.3	--	--	6.2	--	--	--	--	--	--	--	--	24
1,2-Benzenedicarboxylic acid	1036	--	--	--	--	--	--	--	--	--	110	100	--	--	--	--	--	20
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)	1018	--	--	--	--	--	--	--	--	--	280	130	--	--	--	--	--	--
Oleic acid	1066	--	--	80	53	--	28	--	--	--	--	--	--	--	--	--	--	--
Pentanoic acid	716	--	--	--	--	2.3	--	--	6.2	--	--	--	--	--	--	--	--	--

2-Butenoic acid, 3-methyl	913	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1300	700
Acetate																		
2-Propanone, 1-(acetyloxy)-	825	--	--	--	--	--	--	--	--	--	1600	3700	4800	4900	2400	2600	6100	5900
2-Butanone, 1-(acetyloxy)-	844	--	--	--	--	--	--	--	--	--	--	--	--	--	--	790	1200	--
Alcohol																		
Benzyl alcohol	928	--	3.5	4.6	5.2	3.9	5.1	5.8	4.3	5	--	--	--	--	--	--	--	--
Phenylethyl alcohol	938	Sweet, chocolate, fruity and floral flavor	4.1	4.5	3.8	7.1	6.7	7.5	8.2	130	--	--	--	--	--	--	--	--
3-Octanol	836	Fatty, cheesy, ketonic, mushroom flavor	--	--	--	--	--	--	--	--	--	--	--	--	--	15000	15000	--
Decanol	943	--	--	--	1.9	--	--	--	--	--	--	--	--	--	--	--	--	--
1-Dodecanol	963	--	9	5.7	2.6	--	1.8	5	--	2	--	--	--	--	--	--	--	--

1-Hexadecanol	973	Faint waxy, nearly odorless	41	26	19	7.2	130	29	22	21	--	--	--	--	--	--	--	--
Octadecanol	980	Green, fatty, cocconut flavor	--	--	4.7	13	--	--	--	--	--	--	--	--	--	--	--	--
1-Nonadecanol	999		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	43
Glycerol	1002	--	--	--	--	--	--	--	--	10	--	--	--	--	--	--	--	--
Geraniol	924	--	--	--	--	--	--	4.2	--	--	--	--	--	--	--	--	--	--
Aldehyde																		
Tridecanal	951	--	--	--	--	8.4	8.7	--	--	--	--	--	--	--	--	--	--	--
Tetradecanal	952	--	1.8	--	1.2	1.4	--	2.9	2.4	6.6	--	--	--	--	--	--	--	--
Pentadecanal	958	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Hexadecanal	982	--	--	--	--	--	1.9	--	--	--	--	--	--	--	--	--	--	--
Heptadecanal	1022	--	--	15	11	2.7	11	--		12	--	--	--	--	--	--	--	--
Vanilin	1025	Vanilla-like	--	--	7.9	11	15	--	7.6	--	--	--	--	--	--	--	--	--
Ester																		
Benzeneacetic acid, ethyl ester	910	--	--	--	--	--	--	--	--	1.25	--	--	--	--	--	--	--	--
Decanoic acid, ethyl ester	922	--	--	--	--	--	--	--	74	--	--	--	--	--	--	--	--	--

Tetradecanoic acid, ethyl ester	955	--	5.3	--	2.1	--	3.2	8.2	7	6.9	--	--	--	--	--	--	--	--
Hexadecanoic acid, methyl ester	978	--	7.8	6	7.1	4.7	1.5	7.2	6.1	5.2	--	--	--	--	--	--	--	--
Hexadecanoic acid, ethyl ester	983	--	74	47	50	85	54	67	100	63	--	--	--	--	--	--	--	--
Homosalate	1009	--	3.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
9,12-Octadecadienoic acid, methyl ester	1012	--	--	--	--	--	--	4.2	--	--	--	--	--	--	--	--	--	--
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1019	--	13	15	11	18	10	15	10	9.8	--	--	--	--	--	--	--	--
Benzoic acid, 2-hydroxy-, ethyl esther	914	--	--	--	18	41	45	36	2.3	51	--	1500	--	1200	--	1100	--	--
Benzoic acid, ethyl ester	885	--	--	14	6.3	24	21	19	65	43	--	--	--	--	--	--	--	--
Linoleic acid, ethyl esther	1016	--	41	36	40	41	10	35	78	51	--	--	--	--	--	--	--	--

Ethyl 9-hexadecenoate	924	--	--	--	--	--	--	--	4.6	--	--	--	--	--	--	--	--	--
Methyl salicilate	908	--	54.8	55.2		56.3	57.5	95.1	83.3	59.4	--	--	--	--	--	--	--	--
2-Ethylhexil salicylate	988	--	--	2.8	2.4	--	2.3	4.7	--	2.7	--	--	--	--	--	--	--	--
Isopropyl myristate	952	--	--	--	3.1	4.3	--	4.9	--	3.6	--	--	--	--	--	--	--	--
Octadecyl trifluoroacetate	943	--	--	--	--	--	--	2.1	--	--	--	--	--	--	--	--	--	--
Benzyl benzoate	1030	--	--	--	--	--	--	2.8	--	--	--	--	--	--	--	--	--	--
Furfuryl formate	830	--	--	--	--	--	--	--	--	--	--	--	--	39000	--	--	--	--
Ketone																		
2-Undecanone	878	--	--	--	4.8	--	--	--	--	--	--	--	--	--	--	--	--	--
5,9-Undecadien-2-one,6,10-dimethyl	923	Mettalic Flavor	3	4.6	3	--	--	3.9	4.1	3.7	--	--	--	--	--	--	--	--
2-Pentadecanone, 6,10,14-trimethyl-	966	--	36	31	37	48	42	64	29	30	--	--	--	--	--	--	--	--

2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-5-methyl	915	Sweet and malty odor	--	--	--	--	--	--	--	--	470	--	--	950	1000	1100	--	470
Ethanone, 1-(1H-pyrrol-2-yl)-	942	--	--	--	--	--	--	--	--	--	2300	2300	2000	2300	2000	2800	2100	1100
4-Hydroxy-3-methylacetophenone	948	--	--	--	--	--	--	--	--	--	190	--	450	490	--	--	--	120
Ethanone, 1-(2-furanyl)-	834	Sweet, grape flavor	--	--	--	--	--	--	--	--	--	1900	1600	--	--	--	29	--
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-4-methyl	933	Sweet, maple-like flavor	--	--	--	--	--	--	--	--	470	--	--	950	1000	1110	--	470
Ethanone, 1-(4-hydroxyphenyl)-	974	Heavy-floral, somewhat herbaceous or wood-like odor	--	--	--	--	--	--	--	--	--	--	3700	2700	3700	--	--	--
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	933	Sweet maple-like flavor	--	--	--	--	--	--	--	--	--	--	--	--	--	--	910	--

3-Hexen-2-one, 5-methyl-	1002	Nutty, blue cheese odor and mushroom flavor	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	630
4-Hydroxy-2-methylacetophenone	974	---	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	3500
Furane																		
Furfural	822	Caramelic, sweet, bread-like,toasted odor	--	--	--	--	--	--	--	--	4900	3200	4600	3300	3600	1800	4500	1500
2-Furanmethanol, acetate	845	Soft and floral flavor	--	--	--	--	--	--	--	--	5800	7400	14000	8000	12000	2900	4000	11000
Furan, 2,2'-methylenebis[5-methyl	865	Wintergreen note and mouthfeel sensation	--	--	--	--	--	--	--	--	590	1600	2600	1600	3000	--	--	--
2-Furanmethanol	884	Mild and slightly caramelized flavor	--	--	--	--	--	--	--	--	13000	13000	14000	6300	9400	2700	15000	7200
Furfuryl pentanoate	889	Fruity, green, pear fresh-refreshing flavor	--	--	--	--	--	--	--	--	710	1000	--	750	1100	--	--	400

Furan, 2-[(methyldithio)methyl]-	912	Coffee-like odor	--	--	--	--	--	--	--	--	2600	--	--	950	--	840	580	--
Furan, 2,2'-[oxybis(methylene)]bis-	941	Mushroom note	--	--	--	--	--	--	--	--	820	2300	1600	1800	2400	2100	800	480
Maltol	945	Fruit and caramel	--	--	--	--	--	--	--	--	1800	1800	1200	1600	1000	2400	1600	1000
Benzofuran, 2,3-dihydro-	1001	--	--	--	--	--	--	--	--	--	900	280	--	--	--	--	--	52
5-Hydroxymethylfurfural	1016	Sweet flavor	--	--	--	--	--	--	--	--	400	370	280				45	44
2-Furanmethanol, propanoate	862	The flavor is ruity, green, pear	--	--	--	--	--	--	--	--	--	--	730	--	--	--	--	--
2-Furancarboxaldehyde, 5-methyl-	857	--	--	--	--	--	--	--	--	--	--	6200	7000	5800	4700	2800	12000	380
Furan, 2,2'-methylenebis-	865	Wintergreen note	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	980
4-Methyl-5H-furan-2-one	930	Pungent, caramel and sweet flavor	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	180

Furanone

2(5H)-Furanone	904	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	240	--
2-Furanone, 2,5-dihydro-3,5-dimethyl	880	Freshly baked bread odor	--	--	--	--	--	--	--	--	--	590	--	540	130	--	--	400
Hidrocarbon																		
Tetradecane	876	--	--	--	--	--	--	--	8.2	--	--	--	--	--	--	--	--	--
Pentadecane	878	--	1.6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Heptadecane	897	--	20	17	18	14	93	--	5.3	--	--	--	--	--	--	--	--	--
Octadecane	948	--	--	--	--	--	--	1.6	3.6	--	--	--	--	--	--	--	--	--
Nonadecane	962		--	13	15	12	--	--	--	8.7	--	--	--	--	--	--	--	--
Tetracosane	976	--	2.2	--	2.7	4.4	--	--	5.2	--	--	--	--	--	--	--	--	--
Hexacosane	989	--	--	--	--	2.5	--	--	3.6	--	--	--	--	--	--	--	--	--
Lactone																		
Butyrolactone	873	Sweet flavor	--	--	--	--	--	--	--	--	--	--	--	2200	2600	2400	--	2400
2-Hydroxy-gamma-butyrolactone	973	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	96
Pantolactone	952	Bitter taste	--	--	--	--	--	--	--	--	--	--	--	--	--	--	280	92
Phenol																		

Phenol, 2-methoxy-	923	--	--	--	1.1	--	--	--	--	--	3600	3400	3700	2600	4800	2500	1600	1400
Phenol, 4-ethyl-2-methoxy-	950	Soy sauce flavor	12	--	7	4.1	4.3	6.7	--	--	4600	4100	7400	5700	3100	5700	2700	1800
Phenol	946	--	--	--	2.6	--	--	4.4	--	--	2200	--	2200	1800	2100	--	--	830
Phenol, 2-methyl-	960	--	--	--	1.1	--	--	--	--	--	--	--	--	--	--	--	--	--
Phenol, 4-methyl-	962	--	--	--	--	--	--	1.1	--	1.6	--	--	--	--	--	--	--	--
Hydroquinone	1084	Sweet taste	--	--	--	--	--	--	--	--	4.3	--	--	--	--	--	--	--
Phenol, 2-methoxy-4-(1-propenyl)-)	995	Phenolic flavor,	--	--	--	--	--	--	--	--	--	37	--	--	--	--	--	--
Phenol, 4-ethyl-	971	Rather Sweet odor	--	--	--	--	--	--	--	--	--	--	--	--	420	--	--	--
Pyrazine																		
2,6-dimethylpyrazine	801	Sweet	--	--	--	--	--	--	--	--	--	--	--	240	--	--	--	--
2-ethyl-6-methyl-pyrazine	803	Roasted hazelnut-like taste	--	--	--	--	--	--	--	--	--	1900	3600	9700	1700	710	1000	2300
2-ethyl-5-methyl-pyrazine	800	A coffee-like taste	--	--	--	--	--	--	--	--	6800	4300	7300	3100	1700	1500	2800	3700
Trimethylpyrazine	818	--	--	--	--	--	--	--	--	--	3700	3000	4900	3200	4000	--	3400	3500

2-ethyl-3-methyl-pyrazine	822	Nutty and roasted	--	--	--	--	--	--	--	--	4300	5200	8200	1500	1900	300	1300	1200
3-ethyl-2,5-dimethyl-pyrazine	840	Hazelnut-like, earthy, baked, potato-like	--	--	--	--	--	--	--	--	3900	1800	1400	5300	8700	3900	5300	4200
2-Acetyl-3-methylpyrazine	884	Caramel, bready and roasted flavor	--	--	--	--	--	--	--	--	2500	2800	--	2100	--	2100	2000	--
1-(6-Methyl-2-pyrazinyl)-1-ethanone	894	Popcorn flavor	--	--	--	--	--	--	--	--	570	1300	1900	1400	--	1600	1800	760
2-methyl-5-(1-propenyl)-, (E)-pyrazine	836	--	--	--	--	--	--	--	--	--	1500	1500	1600	1200	1000	1200	1000	5600
3,5-diethyl-2-methyl-pyrazine	840	Nutty notes	--	--	--	--	--	--	--	--	1400	1800	2800	--	--	--	1200	--
2,3,5-Trimethylpyrazine	850	--	--	--	--	--	--	--	--	--	9700	1800	--	--	--	--	--	--
Pyrazine, (1-methylethenyl)-	870	--	--	--	--	--	--	--	--	--	510	1300	1700	1000	--	--	--	--

5H-Cyclopentapyrazine, 6,7-dihydro-5-methyl-	880	Baked and potato-like odor	--	--	--	--	--	--	--	--	2700	950	--	--	--	--	--	--
1,4-Diazine	1014	Sweet odor	--	--	--	--	--	--	--	--	--	--	1300	--	--	--	--	520
Pyrazine, 2-ethyl-3,5-dimethyl-	830	---	--	--	--	--	--	--	--	--	--	--	--	2000	--	1500	1900	1900
Pyridine																		
4(H)-Pyridine, N-acetyl-	896	--	--	--	--	--	--	--	--	--	--	2300	2100	1600	--	2200	1800	1200
3-Pyridinol	909	--	--	--	--	--	--	--	--	--	19	810	500	780	--	660	530	150
Pyrroles																		
1H-Pyrrole, 1-(2-furanylmethyl)-	915	--	--	--	--	--	--	--	--	--	5700	4900	5100	3500	4000	3500	2900	1800
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	871	Mild flavor	--	--	--	--	--	--	--	--	--	2700	2600	1700	2000	1600	1300	1300

1H-Pyrrole-2-carboxaldehyde-5methyl	964	--	--	--	--	--	--	--	--	--	8400	--	--	1200	870	1400	980	--
1H-Pyrrole, 1-butyl-	895	Earthy, green, woody flavor	--	--	--	--	--	--	--	--	--	--	--	--	3200	--	--	--
Indole	1006	Floral notes	--	--	--	--	--	--	--	--	1000	360	380	270	--	--	280	59
1H-Indole, 3-methyl-	1011	Sweet odor	--	--	--	--	--	--	--	--	--	--	--	250	--	--	--	--
Thiazole																		
Benzothiazole	941	--	--	1.4	3.1	--	--	--	--	--	--	--	--	--	--	--	--	--
Thiopenes																		
2-Thiophenemethanol	937	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	600	290
1-Hexanone, 1-(2-thienyl)-	908	Mettalic and bitter notes	--	--	--	--	--	--	--	--	--	--	--	--	570	--	--	--
Others																		
Caffeine	1134	--	440	590	450	340	300	430	750	550	240	420	390	960	930	3900	4100	270
Kaur-16-ene	1004	--	--	--	--	4.3	11	7.4	6.2	6.8	--	--	--	--	--	--	--	--

4-Methylpyrrolo[1,2-a]pyrazine	969	--	--	--	--	--	--	--	--	--	290	560	--	380	--	680	390	--
Ethanone,1-(4-hydroxyphenyl)	974	Herbaceous odor	--	--	--	--	--	--	--	--	660	3800	--	--	--	3400	--	--
Piracetam	989	--	--	--	--	--	--	--	--	--	880	--	--	--	--	--	--	76
Apocynin	1032	Sweet odor	--	--	--	--	--	--	--	--	190	130	--	--	--	--	--	--
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	955	--	--	--	--	--	--	--	--	--	--	900	260	880	570	1000	1200	350
4-Methylpyrrolo[1,2-a]pyrazine	969	Slightly burnt flavor	--	--	--	--	--	--	--	--	--	--	--	--	--	380	--	--

Control: Spontaneous fermentation; **M**: *Leuconostoc mesenteroides*; **P**: *Lactiplantibacillus plantarum*; **MP**: *Leuconostoc mesenteroides* + *Lactiplantibacillus plantarum*; **MD**: *Leuconostoc mesenteroides* + *Torulaspora delbrueckii*; **MC**: *Leuconostoc mesenteroides* + *Saccharomyces cerevisiae*; **PD**: *Lactiplantibacillus plantarum* + *Torulaspora delbrueckii*; **PC***: Lactiplantibacillus plantarum* + *Saccharomyces cerevisiae***.**

LRI: Linear Retention Index;

* References of sensory attributes: [1]Flament, & Bessière-Thomas, (2002); [2]Lee et al., (2015; 2017); [3]Bressani et al., (2018); [4]Martínez et al, (2017); [5]Ribeiro et al., (2018); [6]da Mota et al., (2020).

Table S3. Sensory attributes of conilon coffee

Fermentations	Score	Fragrance/Aroma	Flavor	Aftertaste	Salinity/Acidity	Bitterness/Sweetness	Mouthfeel
Control	75.33^{c}	7.10^{c}	7.000^{b}	6.67^{b}	7.00^{a}	7.08^{b}	6.83^{b}
M	81.00^{a}	7.75^{a}	7.667^{a}	7.67^{a}	7.50^{a}	7.67^{a}	7.50^{a}
P	79.67^{b}	7.50^{b}	7.500^{a}	7.50^{a}	7.58^{a}	7.58^{a}	7.33^{a}
MD	80.00^{b}	7.58^{b}	7.583^{a}	7.50^{a}	7.33^{a}	7.67^{a}	7.50^{a}
PD	79.00^{b}	7.33	7.333^{a}	7.00^{b}	7.33^{a}	7.08^{b}	7.00^{b}
MP	80.33^{a}	7.50^{b}	7.500^{a}	7.17^{b}	7.50^{a}	7.67^{a}	7.50^{a}
MC	81.33^{a}	7.83^{a}	7.583^{a}	7.67^{a}	7.58^{a}	7.92^{a}	7.50^{a}
PC	79.00^{b}	7.57^{b}	7.417^{a}	7.42^{a}	7.33^{a}	7.50^{a}	7.33^{a}
SEM	0.47	0.47	0.076	0.16	0.11	0.19	0.14

SEM: standard error of the mean

Mean values followed by the same letter in column (capital) do not differ significantly by the Scott-Knott test ($p > 0.05$).