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SPECIALTY COFFEES FERMENTED AT DIFFERENT ALTITUDES: INFLUENCE OF YEAST CO-INOCULATION ON CHEMICAL AND SENSORY COMPOSITION

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, área de concentração em Microbiologia de Alimentos e Processos Fermentativos, para a obtenção do título de Doutora.

Profa. Dra. Rosane Freitas Schwan
Orientadora

Prof. Dr. João Batista Pavesi Simão
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Coorientadores

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ANA PAULA PEREIRA BRESSANI

CAFÉS ESPECIAIS FERMENTADOS EM DIFERENTES ALTITUDES: INFLUÊNCIA DA CO-INOCULAÇÃO DE LEVEDURAS NA COMPOSIÇÃO QUÍMICA E SENSORIAL

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LAVRAS - MG
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Aos meus pais, exemplos de ética, perseverança e amor incondicional.

Amo vocês.

Dedico

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“A tarefa não é tanto ver aquilo que ninguém viu,
mas pensar o que ninguém ainda pensou sobre
aquilo que todo mundo vê.”
(Arthur Schopenhauer)

RESUMO GERAL

Os cafés especiais estão ganhando mais espaço no mercado brasileiro e as mudanças na produção, processamento, comercialização e cultura dos consumidores são perceptíveis. É indiscutível que o uso de culturas iniciadoras selecionadas no processo fermentativo incrementa a qualidade dos cafés. Entretanto, o comportamento desses microrganismos pode ser diferente dependendo da variedade, método de processamento e altitude onde o café é cultivado. Deste modo, o presente trabalho teve como objetivo: (i) utilizar metodologias metabolômicas, químicas e sensoriais para avaliar a qualidade de cafés fermentados da cultivar Catuaí vermelho IAC-44 em diferentes altitudes (600 e 1.200 m), localizados na região do Caparaó; (ii) analisar o conhecimento e as perspectivas dos consumidores sobre cafés e investigar como a informação pode influenciar a experiência sensorial dos consumidores. Para isso, cafés maduros foram colhidos manualmente e separados em bateladas de 20 Kg, representando sete tratamentos mais o controle em cada altitude. As culturas iniciadoras - *Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544 e *Torulospora delbrueckii* CCMA 0684 - foram inoculadas, isoladas e em co-inoculação. O controle não foi inoculado. A fermentação dos frutos inteiros foi conduzida por 72 h em biorreatores fechados. Então, os cafés foram colocados em terreiro suspenso até atingir umidade em torno de 11-12%. Amostras foram coletadas e congeladas até a realização das análises. Foi analisado a melhoria da qualidade da bebida do café cultivado em baixa altitude, por meio da inoculação de leveduras durante a fermentação por via seca. As análises foram realizadas por cromatografia líquida e gasosa, espectroscopia de infravermelho com transformada de Fourier (FTIR) e prova de xícara. Diferenças nos compostos químicos entre os cafés inoculados e o controle, tanto para o grão verde, quanto torrado, foram observadas. A fermentação aumentou a qualidade dos cafés de baixa altitude e a combinação de leveduras não *Saccharomyces* (*C. parapsilosis* CCMA 0544 e *T. delbrueckii* CCMA 0684) apresentou a maior nota sensorial (85), sendo a mais indicada para esse processo. O segundo tópico abordado neste trabalho foi a influência da fermentação com culturas iniciadoras em relação aos compostos bioativos, químicos e sensoriais de cafés produzidos em alta altitude. O teor de polifenóis total e antioxidantes estão fortemente relacionados com o fim da fermentação em biorreator fechado e após a torra. O teor de trigonelina apresentou correlação moderada e negativa com a fermentação e torra. Notas frutadas, cítricas e de vinho foram encontradas apenas nos tratamentos inoculados. O tratamento co-inoculado com as três leveduras apresentou maior nota sensorial (86.9). O terceiro aspecto abordado, foi compreender as perspectivas e desejos dos consumidores de café através de um questionário digital com 1.005 participantes. O sabor chocolate é o mais esperado no café especial. O teste Check-all-that-Apply foi realizado com 101 consumidores, utilizando o mesmo café (sem e com informação). A análise sensorial mostrou que os consumidores podem ser influenciados pelas informações. Portanto, a co-inoculação de leveduras na fermentação do café é uma alternativa altamente promissora para cafés de baixa e alta altitude. Além disso, os cafés especiais fermentados devem ser mais divulgados aos consumidores.

Palavras-chave: Fermentação. Cafés especiais. Co-inoculação de leveduras. Composição química. Consumidores.

GENERAL ABSTRACT

Specialty coffees are gaining more space in the Brazilian market, and the changes in production, processing, marketing, and consumer culture are noticeable. Unquestionably, the use of selected starter cultures in the fermentation process improves the coffee quality. However, the microorganisms' behavior can be different depending on the variety, processing method, and altitude. Thus, this paper aims to (i) use metabolomics, chemical, and sensory methodologies to evaluate the quality of fermented coffees of the cultivar Catuaí vermelho IAC-44 at different altitudes (600 and 1,200 m) located in the Caparaó region. (ii) analyze consumers' knowledge and perspectives on coffees and investigate how information can influence consumers' sensory experience. For this, the coffee cherries were harvested manually and separated into batches of 20 kg, representing seven treatments plus control at each altitude. The starter cultures - *Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544, and *Torulospora delbrueckii* CCMA 0684 - were inoculated, isolated, and in co-inoculation. The control was performed without inoculation. Fermentation of whole fruits lasted 72 h in closed bioreactors. Then, the coffees were transferred to the suspended terraces until 11- 12% moisture. Samples were collected and frozen for chemical analysis. We studied the improving coffee beverage quality cultivated at low altitudes through inoculation of yeast during fermentation in dry processing. The analyzes were performed using liquid and gas chromatography, Fourier transforms infrared spectroscopy (FTIR), and cup testing. Differences in chemical compounds were observed between the inoculated coffees and the control, both for green and roasted beans. Fermentation improved the quality of low-altitude coffees, and the co-inoculation of non-*Saccharomyces* yeasts (*C. parapsilosis* CCMA 0544 and *T. delbrueckii* CCMA 0684) had the highest sensory score (85), being the most suitable for this process. The second topic addressed in this work was the influence of fermentation with starter cultures concerning the bioactive, chemical, and sensory compounds of coffee produced at high altitudes. The content of total polyphenols and antioxidants is strongly correlated with the end of fermentation in a closed bioreactor and after roasting. The trigonelline content has a moderate and negative correlation with fermentation and roasting. Fruity, citric, and wine notes were found only in inoculated treatments. The co-inoculated with the three yeasts showed the highest sensory score (86.9). The third aspect addressed was understanding the perspectives and desires of coffee consumers through a questionnaire in a digital format with 1,005 participants. The chocolate flavor is still the most expected in specialty coffee. The Check-all-that-Apply (CATA) test was carried out with 101 consumers using the same coffee (without and with information). Sensory analysis showed that consumers could be influenced by information. Therefore, co-inoculation of yeasts in coffee fermentation is a promising alternative for low and high-altitude coffee. Also, specialty fermented coffees should be made more widely available to consumers.

Keywords: Fermentation. Specialty coffees. Yeast co-inoculation. Chemical composition. Consumers.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O café é uma das bebidas mais populares do mundo e o Brasil é o maior produtor e exportador mundial desse grão (VOLSI *et al*., 2019). Devido à bienalidade positiva, o país apresentou produção em torno de 63,08 milhões de sacas (60 kg) beneficiadas em 2020, correspondendo ao aumento de 27,9% em relação ao ano anterior. Os principais estados produtores de café no Brasil são: Minas Gerais, Espírito Santo, São Paulo e Bahia (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2020).

Os consumidores estão cada vez mais exigentes e conscientes dos processos de produção de seus alimentos, por isso, o consumo mundial de cafés especiais tem apresentado crescimento significativo nos últimos anos (UFER; LIN; ORTEGA, 2019). O mercado vem sendo afetado por novos produtos, pesquisas e cafeterias especializadas, incentivando mudanças na produção, processamento, comercialização, valorização e cultura dos consumidores (GUIMARÃES *et al*., 2019). Nesse sentido, uma compreensão da psicologia do consumidor e sua relação com as escolhas alimentares é essencial para atingir de maneira mais assertiva os diferentes nichos de consumidores de cafés.

Fatores como: origem geográfica, clima, espécie, métodos de colheita, altitude, processamento e armazenamento, influenciam a qualidade da bebida (ABREU *et al*., 2019; BRESSANELLO *et al*., 2017; RIBEIRO *et al*., 2016), apresentando diferentes sabores e aromas, que são utilizados como parâmetros na caracterização de cafés especiais (SUNARHARUM; WILLIAMS; SMYTH, 2014). Por exemplo, durante a maturação dos frutos de café, o clima e a altitude irão desempenhar um importante papel em decorrência da temperatura, luz e água disponível (BERTRAND *et al*., 2006; BODNER *et al*., 2019). A diferença de altitude pode estar relacionada às alterações fisiológicas e morfológicas do fruto (ZHU *et al*., 2010), diversidade microbiana e, consequentemente, interferir no perfil dos compostos relacionados ao sabor do café (MARTINS *et al*., 2020). Entretanto, cafés cultivados em baixas altitudes são pouco estudados.

Os frutos de café servem de substrato para o desenvolvimento de bactérias, leveduras e fungos filamentosos, suprindo-os de fontes de carbono e nitrogênio (SILVA *et al*., 2000). Durante a fermentação do café, microrganismos utilizados como culturas iniciadoras estão associados à degradação da pectina e outros carboidratos, produção de compostos voláteis desejáveis, e características sensoriais positivas e diferenciadas (BRESSANI *et al*., 2020; DE

BRUYN *et al*., 2017; ELHALIS *et al*., 2021; ESQUIVEL; JIMENEZ, 2012; EVANGELISTA *et al*., 2014b; SILVA *et al*., 2013). Além disso, algumas espécies de leveduras podem inibir o crescimento de fungos filamentosos produtores de toxinas através da inibição dos esporos e de ocratoxina A (SOUZA *et al*., 2017).

Estudos de Bressani *et al*. (2018; 2020), da Mota *et al*. (2020); Evangelista *et al*. (2014a; 2014b), Martinez *et al*. (2017) e Ribeiro *et al*. (2017) têm demonstrado que leveduras como *Saccharomyces cerevisiae*, *Candida parapsilosis* e *Torulospora delbrueckii* melhoram a qualidade sensorial do café, apresentando notas de caramelo, herbáceo, frutas vermelhas, frutas cítricas, caju, banana e vinhoso, podendo variar devido ao tipo de processamento, variedade e região produtora. Portanto, um estudo mais avançado sobre essas variáveis, juntamente com as interações que ocorrem durante o processo fermentativo, é necessário para o entendimento de como esses microrganismos podem afetar o sabor e aroma dos cafés de maneira positiva.

Desta maneira, os objetivos do presente trabalho foram: (i) utilizar metodologias metabolômicas, químicas e sensoriais para avaliar a qualidade de cafés da cultivar Catuaí vermelho IAC-44 fermentados sem e com culturas iniciadoras - *Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544 e *Torulospora delbrueckii* CCMA 0684 - isoladas e em co-inoculação, em duas altitudes (600 e 1.200 m), localizados na região do Caparaó; (ii) analisar o conhecimento e as perspectivas dos consumidores sobre cafés (commodity, especial e fermentado) e investigar como a informação pode influenciar a experiência sensorial dos consumidores.

2 REFERENCIAL TEÓRICO

2.1 Café: características gerais

O café é pertencente à família *Rubiacea*, gênero *Coffea*, sendo o *C. arabica* e *C. canephora* as principais espécies cultivadas (ESQUIVEL; JIMÉNEZ, 2012). A maioria das plantações de café no Brasil cultiva a *Coffea arabica* L., que fornece um produto de boa qualidade e é amplamente procurado pelo mercado consumidor (SILVA *et al*., 2016).

O fruto de café é constituído de seis partes: exocarpo (casca), endocarpo (polpa), mesocarpo (mucilagem), pergaminho, película prateada e endosperma (semente), apresentando composição química complexa. A casca é lisa e resistente, com coloração verde em frutos verdes e, dependendo do genótipo, pode apresentar coloração vermelha, amarela ou laranja quando maduro. A polpa apresenta característica amarelada, fibrosa e doce, representando, em base seca, em torno de 29% do peso integral. O café em pergaminho é composto por α-celulose (40-49%), hemicelulose (25-32%), lignina (33-35%) e cinzas (0,5-1%). Apenas o grão de café é torrado e utilizado na preparação da bebida (ESQUIVEL; JIMÉNEZ, 2012).

Os principais constituintes do café verde são carboidratos, compostos de nitrogênio - como proteínas, trigonelina e cafeína, lipídios, ácidos orgânicos e água, sendo que a maioria são precursores de aroma e sabor (BELITZ; GROSCH; SCHIEBERLE, 2009). Durante a torra, esses precursores de sabor/aroma são formados por meio de reações térmicas (WORKU *et al*., 2018), como a quebra de açúcares livres, aminoácidos, trigonelina e ácidos clorogênicos.

Além disso, o grão também é constituído por celulose, minerais (potássio, magnésio, cálcio, sódio, ferro, manganês, rubídio, zinco, cobre, estrôncio, crómio, vanádio, bário, molibdênio, e cádmio), açúcares (sacarose, glicose, frutose, arabinose, galactose e manose), lipídios, tanino, polifenóis, ácido clorogênico e aminoácidos (alanina, arginina, asparagina, cisteína, ácido glutâmico, glicina, histidina, isoleucina, leucina, lisina, metionina, fenilalanina, prolina, serina, treonina, tirosina e valina) (BELITZ; GROSCH; SCHIEBERLE, 2009).

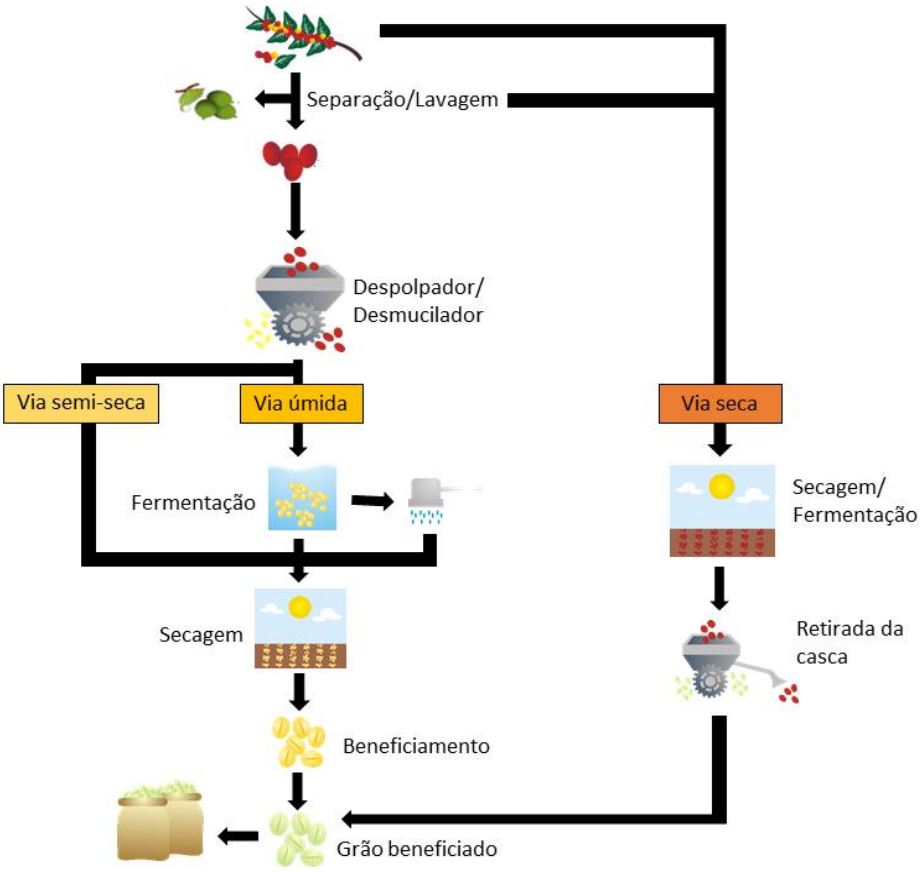
Muitos processos bioquímicos, fisiológicos e químicos que ocorrem durante o período de pré e pós-colheita podem resultar em cafés com grandes diferenças quanto à qualidade (ESQUIVEL; JIMÉNEZ, 2012). No que se refere às espécies, há uma diferenciação nas concentrações de diversos compostos interferentes na qualidade do café, como cafeína, ácido clorogênico, aminoácidos, entre outros. Além disso, o conhecimento dos compostos químicos do café pode permitir a detecção de adulterações após a moagem, bem como, a diferenciação das espécies arábica e robusta (MONAKHOVA *et al*., 2015).

2.2 Processamento pós-colheita do café

A colheita deve ser feita quando a maior parte dos frutos estiver maduros (BEZAME *et al*., 2021), a fim de evitar a presença de grãos imaturos (verdes), pretos e ardidos. Quando for realizada de forma não seletiva, é importante que se faça a separação dos frutos verdes/imaturos e secos, pois eles afetam negativamente a qualidade do café e o sabor da bebida (MIRANDA; DRUMOND; RONCHI, 2020). A maioria das características negativas da bebida são atribuídas ao controle inadequado durante a fermentação e secagem, promovendo a produção elevada de substâncias químicas, como ácido acético, butírico e propiônico, comprometendo a qualidade e gerando perdas econômicas adicionais (GONZALES-RIOS *et al*., 2007).

De acordo com Brando e Brando (2014), há três formas distintas de realizar o processamento do café. Por via seca, via úmida e via semi-seca (descascado natural), como descrito na Figura 1, podendo ocorrer algumas variações quanto ao método de processamento ao realizar fermentações controladas.

Figura 1 - Fluxograma dos principais tipos de processamento do café.



Fonte: Do Autor (2021).

O processamento por via seca, também chamado de café natural, é muito utilizado no Brasil (BRANDO; BRANDO, 2014; CHALFOUN; ANGÉLICO; DE RESENDE, 2018), principalmente, em regiões tropicais onde o clima é seco no período da colheita. Nesse processamento, os frutos de café são secos como todas as partes que os constituem (DE BRUYN *et al*., 2017).

"Os frutos colhidos em vários estádios de maturação (verde, cereja, passa e seco) podem, ou não, serem lavados e devem, preferencialmente, serem separados, a fim de melhorar a qualidade do café" (BORÉM, 2008, p. 129-156). O café deve ser espalhado em camadas finas, (5-8 cm) em terreiros de cimento ou suspensos, sendo amontoados à noite e revolvidos periodicamente durante o dia. Durante o período de secagem, também ocorre a fermentação microbiana natural, que influencia na qualidade final do produto. Cafés processados por via seca apresentam características sensoriais que os diferem dos cafés produzidos por via úmida, como sabor mais doce, mais corpo e maior complexidade (HAMEED *et al*., 2018; SELMAR; KLEINWÄCHTER; BITOF, 2014).

No processamento via úmida, o exocarpo e uma parte do mesocarpo são removidos mecanicamente. O processo de fermentação é realizado em tanques com um grande volume de água para a retirada do restante do mesocarpo que ficou aderido ao pergaminho. O tempo de fermentação pode variar, podendo ocorrer de 6 a 72 horas, dependendo da temperatura ambiente. Temperaturas mais altas e camadas mais espessas de mucilagem aceleram a fermentação (BRANDO; BRANDO, 2014). Então, os grãos são lavados com água limpa e secos em terreiros e/ou secadores rotativos (BRANDO; BRANDO, 2014; LEE *et al*., 2015). Os cafés produzidos com esse tipo de processamento são mais suaves, com acidez agradável e aroma complexo, se comparados aos naturais (SELMAR; KLEINWÄCHTER; BITOF, 2014).

O café ainda pode ser processado pelo método semi-seco. Os frutos de café são despolpados e a fermentação ocorre diretamente sob o sol, em terreiro e/ou secadores rotativos (BRANDO; BRANDO, 2014; VILELA *et al*., 2010). "A secagem visa diminuir a umidade dos grãos para 12 a 11%. Então, o café deve ser armazenado em locais com umidade e ventilação adequados para não ocorrer alteração da qualidade" (BORÉM, 2008, p. 129-156). Desta forma, os grãos já processados (cafés crus) podem ser armazenados durante meses, sem alteração significativa do sabor (BELITZ; GROSCH; SCHIEBERLE, 2009).

2.3 Fatores que influenciam a qualidade do café

"O café de boa qualidade pode ser considerado como a bebida que apresenta sabor e aroma agradáveis, bom corpo, acidez natural e suavidade ao paladar. Deve possuir poucos defeitos, cor e aspecto homogêneos e estar de acordo com as normas higiênico-sanitárias" (BORÉM, 2004, p. 127), além de atender aos gostos dos consumidores. Por este motivo, a qualidade do café é muito importante nas relações comerciais, com enorme influência sobre o preço do produto e crescente demanda por cafés especiais (GUIMARÃES *et al*., 2019).

O perfil químico dos compostos precursores de aroma e sabor do café varia em função de diversos parâmetros, como fatores genéticos, ambientais, de colheita e pós-colheita, que podem afetar diretamente a qualidade da bebida (WORKU *et al*., 2018). Além disso, a microbiota presente naturalmente no café é diversificada e tem influência direta na qualidade da bebida (HAILE; KANG, 2019). A ação dos microrganismos durante a fermentação pode resultar em diferentes tipos de bebidas de café, diferenciadas em termos de corpo, sabor, aroma, acidez e adstringência (ESQUIVEL; JIMÉNEZ, 2012; RUTA; FARCASANU, 2021). Portanto, culturas iniciadoras podem ser utilizadas na fermentação do café para melhorar o controle do processo fermentativo e a qualidade sensorial da bebida, através da produção de ácidos

orgânicos e compostos voláteis (BRESSANI *et al*., 2020; BRESSANI *et al*., 2018; DA MOTA *et al*., 2020; ELHALIS *et al*., 2021; WANG *et al*., 2020).

Dentre os componentes do grão, a cafeína é a mais conhecida. A quantidade deste constituinte pode variar de acordo com a variedade e espécie, sendo encontrados valores entre 0,8 e 1,4% (p/p) para café arábica e 1,7 a 4,0% (p/p) para robusta (BELITZ; GROSCH; SCHIEBERLE, 2009). A cafeína é um metabólito secundário nitrogenado, que influencia no corpo e amargor da bebida (SUNARHARUM; WILLIAMS; SMYTH, 2014). É o único composto termoestável, enquanto as outras substâncias como proteínas, arabinose, ácido clorogênico e trigonelina, podem ser transformados ou até mesmo destruídos pelo processo de torrefação (MUSSATTO *et al*., 2011). A presença de cafeína, trigonelina e compostos fenólicos, cujos principais representantes são os ácidos clorogênicos, interferem na atividade antioxidante e na qualidade da bebida do café (KIM *et al*., 2018).

Kim *et al*. (2018) observaram que a trigonelina está correlacionada com a qualidade do grão verde e da bebida, já que este é um precursor da formação de diferentes classes de compostos orgânicos voláteis durante a torrefação (como pirroles e piridinas). Ácidos clorogênicos também contribuem para o sabor da bebida. Durante o processo de torra, a alta temperatura promove a isomerização e degradação dos ácidos clorogênicos, contribuindo para a composição volátil no café torrado (RODRIGUEZ; GUZMAN; HERNANDEZ, 2020).

A acidez é um atributo importante do café e a presença de alguns ácidos é desejável, pois contribui para o sabor da bebida. Os ácidos cítrico, málico e succínico estão naturalmente presentes no café (EVANGELISTA *et al*., 2014b) e favorecem as características sensoriais. Outros ácidos como o acético, butanoico, fórmico, hexanoico, isovalérico e propanoico, também podem ser encontrados no café (PEREIRA *et al*., 2019), mas dependendo da concentração, podem se tornar indesejáveis. Entretanto, esses compostos se decompõem durante o processo de torrefação e geram metabólitos importantes, como piridinas e pirróis (POISSON *et al*., 2018; SUNARHARUM; WILLIAMS; SMYTH, 2014). Os ácidos também estão envolvidos na reação com os ácidos clorogênico e quínico durante a torrefação, para formar as lactonas, que também, podem influenciar o aroma do café (SUNARHARUM; WILLIAMS; SMYTH, 2014).

A sacarose é responsável por até 9% do peso seco dos grãos verdes de café arábica. Glicose, frutose e pectina são os principais componentes do mesocarpo. O endocarpo e a película prateada são ricos em celulose, hemicelulose e monossacarídeos. A semente também é rica em carboidratos solúveis (arabinose, frutose, galactose, glicose, sacarose, rafinose e estaquiose) e insolúvel (celulose e hemicelulose), com 59-61% de peso seco (ESQUIVEL;

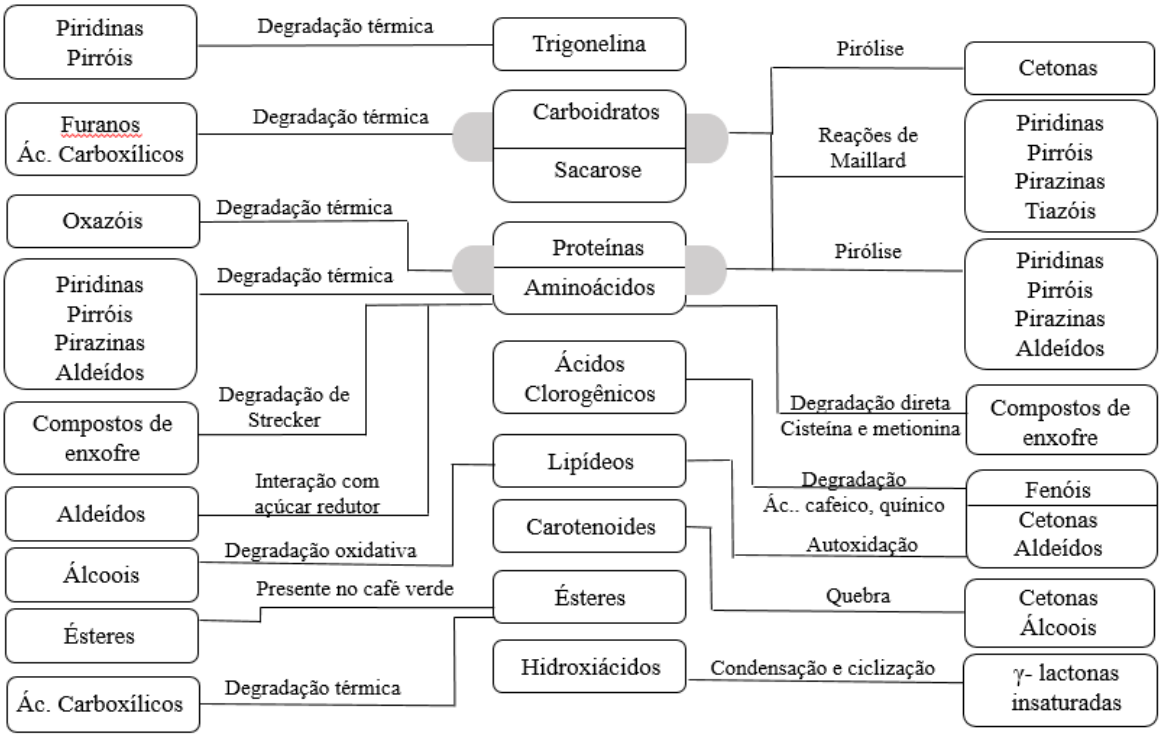
JIMÉNEZ, 2012; POISSON *et al*., 2018). As diferenças nas concentrações de frutose e glicose estão relacionadas aos diferentes processos. No entanto, outros açúcares de baixo peso molecular não são significativamente afetados pelos métodos de processamento (KNOPP; BYTOF; SELMAR, 2006). Os carboidratos são importantes para o desenvolvimento da cor, sabor e aroma do café, principalmente, por meio da reação de Maillard e da acidez da fermentação após a torrefação (WORKU *et al*., 2018).

A formação do aroma do café é atribuída às reações de Maillard (reações entre aminoácidos e açúcares redutores, que após uma complexa cascata de reações durante o aquecimento, resultam na formação de substâncias marrons (melanoidinas)). Nessa etapa são formados compostos voláteis que conferem aroma característico aos produtos termicamente processados, juntamente com outras reações catalisadas termicamente, que ocorrem durante a torrefação (BATISTA *et al*., 2016; SENINDE; CHAMBERS IV, 2020).

De acordo com Hwang, Chen e Ho (2012), os aminoácidos ligados à proteína podem contribuir para a formação volátil. Quantidades mais altas de proteína no café correlacionaram-se com uma maior concentração de pirazinas. Além disso, os autores relacionaram o café de coloração mais clara com maior teor de proteína, demonstrando a importante contribuição das proteínas na formação de voláteis e na cor do café.

Por isso, os precursores de aroma do café (como por exemplo, açúcares, aminoácidos, compostos fenólicos) são primordiais na formação de compostos voláteis durante a torrefação, sendo que alterações das concentrações desses precursores do aroma correspondem às diferenças nos perfis voláteis e aroma do café torrado, tornando-o responsável pela qualidade da bebida (LEE *et al*., 2015), além do desenvolvimento de características de sabor e cor (devido a pirólise de compostos orgânicos), como mostrado na Figura 2 (BELITZ; GROSCH; SCHIEBERLE, 2009).

Figura 2 - Representação das reações de formação de compostos voláteis ocorridas durante o processo de torra do café.



Fonte: Ribeiro *et al*. (2009).

As classes químicas voláteis mais abundantes em grãos verdes são álcoois, aldeídos e cetonas (BRESSANI *et al*., 2020; MARTINEZ *et al*., 2017). Então, a torra pode modificar a composição química do grão, formando compostos químicos com atributos desejáveis na bebida (BELITZ; GROSCH; SCHIEBERLE, 2009).

Furanos são geralmente produzidos por açúcares, ácidos e ácidos graxos insaturados durante a torrefação e conferem aromas maltados e doces (HAMEED *et al*., 2018), como notas herbais, frutadas, malte e caramelo, enquanto as cetonas estão relacionadas às notas amanteigadas, caramelo ou frutadas (BRESSANELLO *et al*., 2017). Piridinas surgem, principalmente, da reação de Maillard, pirólise direta de aminoácidos ou pela degradação da trigonelina (podem apresentar notas amargas, torradas e adstringentes). As pirazinas apresentam aromas de nozes, terrosos, torrados, verdes e doce (BRESSANELLO *et al*., 2017; FLAMENT, 2002). Compostos fenólicos são formados da degradação térmica do ácido clorogênico (CGA) e conferem um aroma apimentado em uma quantidade diretamente proporcional à quantidade de CGA nas sementes (HAMEED *et al*., 2018).

2.4 Características e importância do café na região do Caparaó

Na busca de um sistema de cultivo ambientalmente e economicamente sustentável, a produção de café brasileiro tem passado por diversas modificações. A agregação de valor, através da melhoria qualitativa, tem sido foco dos produtores nas últimas décadas. Neste contexto, a adição da indicação geográfica ao produto agrícola pode facilitar sua aceitação no mercado (RAMOS; FERNANDES; SOUZA, 2012). Além disso, diversificando os sistemas de produção do café, permite-se gerar produtos com características diferenciadas para diversos mercados consumidores (SILVA *et al*., 2016).

A região do Caparaó está localizada em torno do Parque Nacional do Caparaó, na divisa entre Minas Gerais (municípios de Alto Caparaó, Caparaó, Manhumirim, Martins Soares, Alto Jequitibá e Espera Feliz) e Espírito Santo (municípios de Alegre, Divino de São Lourenço, Dores do Rio Preto, Guaçuí, Ibatiba, Ibitirama, Irupi, Iúna, Muniz Freire e São José do Calçado) (SANTOS; SIMÃO, 2015). A produção de cafés em fazendas situadas na região é quase que, exclusivamente, realizada por pequenos produtores, com o uso da mão de obra familiar e da parceria com outros produtores (FREDERICO, 2013).

O Espírito Santo é o segundo maior estado produtor de café arábica do país e o principal produtor de café conilon, atingindo aproximadamente 64% da produção total do estado em 2020 (CONAB, 2020). Essa espécie é plantada em regiões ao norte e ao sul do estado, conhecida como Conilon Capixaba. Possui clima com temperatura média mais elevada, de 26 ºC, e altitudes menores que 500 m (FREDERICO, 2013).

O Estado de Minas Gerais é o maior produtor brasileiro de café, tanto em volume, quanto em valor de produção. O estado possui a maior área plantada com a espécie *Coffea arabica*, correspondendo a 72,1% da área brasileira ocupada com essa espécie. Em 2020, a produção foi em torno de 34.647,1 milhões de sacas de 60 kg, correspondendo a quase 60% da produção nacional (CONAB, 2020).

Em fevereiro de 2021, o Instituto Nacional da Propriedade Industrial (INPI, 2021) concedeu o selo de Indicação Geográfica aos cafés arábicas da região. Com foco na produção de cafés especiais, cada vez mais, a região do Caparaó vem se destacando em concursos de café de qualidade, como *Coffee of the Year* e *Cup of Excellence*, garantindo melhores preços aos produtores e maior visibilidade da região (APOSTÓLICO *et al*., 2017).

2.4.1 Efeito da altitude

Durante a maturação dos frutos de café, o clima e a altitude desempenham um importante papel, em decorrência da temperatura, luz e disponibilidade de água (BERTRAND *et al*., 2006). Plantas em altitudes mais elevadas possuem, por exemplo, maior exposição à irradiação e temperaturas médias mais baixas, alterando as respostas morfológicas e fisiológicas das plantas (ZHU *et al*., 2010).

Os cafés de baixa altitude estão relacionados à não uniformidade dos grãos e à bebidas de baixa qualidade, porém, são pouco estudados (BODNER *et al*., 2019; NUGROHO; BASUNANDA; YUSIANTO, 2020; SCHOLZ *et al*., 2018). Em um trabalho recente, realizado por Worku *et al*. (2018), foi constatado que, em geral, a cafeína e ácidos clorogênicos diminuíram, enquanto a sacarose, a acidez e o sabor aumentaram com o aumento da altitude. Entretanto, o efeito da altitude nas propriedades do grão de café arábica verde pode ser afetado pelo teor de sombra e pós-colheita. Além disso, a diversidade microbiana é distinta para diferentes altitudes, interferindo no perfil dos compostos relacionados ao sabor do café (MARTINS *et al*., 2020; VELOSO *et al*., 2020).

Em uma pesquisa realizada por da Paschoa *et al*. (2017), ficou evidenciado que, propriedades localizadas em terrenos de maior altitude e mais próximas ao Parque Nacional do Caparaó, produzem cafés mais bem pontuados, mesmo com mais dias de fermentação. Entretanto, para propriedades em menor altitude, um maior tempo de fermentação pode prejudicar a qualidade sensorial do café.

A produção cafeeira em pequenas propriedades rurais, situadas em áreas montanhosas, tem custos altos que dificultam a permanência das famílias no campo (SANTOS; SIMÃO, 2015). Dessa maneira, para agregar valor ao café, os produtores começaram a trabalhar com a agroindustrialização e/ou a valorização de atributos regionais, culturais e ecológicos do processo produtivo, bem como aspectos trabalhistas (SIQUEIRA; SOUZA; PONCIANO, 2011).

2.5 Processo fermentativo do café e a utilização de culturas iniciadoras

A fermentação do café ocorre logo após a colheita. O tempo necessário para que ocorra esta etapa irá depender do tipo de fermentação realizada. Entretanto, independentemente do processamento realizado, alterações físico-químicas, tais como, redução no teor de água e açúcares, e a formação de precursores voláteis irão ocorrer nos grãos (VAAST *et al*., 2006).

O objetivo da fermentação em todos os métodos de processamento é remover a mucilagem (LEE *et al*., 2015; PEREIRA *et al*., 2019) até a diminuição do teor de água ao final da secagem dos frutos de café e, consequentemente, gerar compostos que contribuam para a formação do sabor e aroma da bebida. Enzimas contidas nos frutos não são suficientes para degradar completamente a mucilagem. Dessa forma, é necessário que haja um crescimento microbiano que possa produzir enzimas (como poligalacturonases e pectina liases) para hidrolisar a pectina presente na mucilagem (SILVA *et al*., 2013).

A poligalacturonase (PG) catalisa a hidrólise de ligações glicosídicas α-1,4 em ácido péctico (ácido poligalacturônico); pectina liase (PL) atua catalisando a pectina, liberando ácidos galacturônicos insaturados e a pectinametilesterase (PME) é responsável pela desesterificação do grupo metoxila da pectina, formando ácido péctico e metanol (RUTA; FARCASANU, 2021; SILVA *et al*., 2013).

A microbiota presente naturalmente em frutos do cafeeiro é diversificada, incluindo bactérias, leveduras e fungos filamentosos, que influenciam na qualidade da bebida (MARTINEZ *et al*., 2019; PEREIRA *et al*., 2019; SILVA *et al*., 2000). De acordo com o tipo de processamento, a população microbiana de cada grupo pode ser alterada (DE BRUYN *et al*., 2017; EVANGELISTA *et al*., 2015; VILELA *et al*., 2010). Além disso, a falta de controle na fermentação pode impactar negativamente o aroma e sabor do café (LEE *et al*., 2015).

Espécies de microrganismos foram submetidos a testes com base nas atividades de poligalacturonase, pectina liase e pectinametilesterase e muitos apresentaram a capacidade de atuar como cultura iniciadora no processo de fermentação. Várias leveduras foram identificadas como potenciais culturas iniciadoras, como *Saccharomyces* sp., *Pichia* sp. e *Candida* sp. que mostraram uma melhor atividade da enzima pectinolítica para a degradação eficiente da mucilagem durante o processo fermentativo (SILVA *et al*., 2013).

Culturas iniciadoras podem atuar na melhoria da qualidade sensorial, agregando valor ao produto, reduzindo o tempo de processamento (SILVA *et al*., 2013) e inibindo fungos toxigênicos como *Aspergillus carbonarius* and *Aspergillus ochraceus* (SOUZA *et al*., 2017). Estirpes de *P. anomala* e *P. kluyveri* foram utilizadas como culturas iniciadoras, juntamente com bactérias do ácido lático em fermentação de café, e demonstraram capacidade de inibir o crescimento de fungos produtores de micotoxinas (MASSAWE; LIFA, 2010).

Leveduras pectinolíticas utilizadas durante a fermentação do café podem acelerar o processo e a produção de ácidos orgânicos, e compostos voláteis podem contribuir para a qualidade da bebida (BRESSANELLO *et al*., 2017; BRESSANI *et al*., 2020; MARTINEZ *et al*., 2019). De acordo com Evangelista *et al*. (2014a), ácidos orgânicos e compostos voláteis

produzidos por leveduras utilizadas em seu estudo contribuíram para a qualidade final da bebida. O café submetido ao processo de fermentação apresentou maiores concentrações de compostos voláteis e aromas mais agradáveis.

Pereira *et al*. (2015) estudaram o potencial da *Pichia fermentans* como cultura iniciadora em café da cultivar Catuaí pelo processamento via úmida. A inoculação aumentou a produção de compostos voláteis específicos (como acetaldeído, acetato de etilo e acetato de isoamila) e diminuiu a produção de ácido láctico durante o processo de fermentação. A análise sensorial das bebidas demonstrou que a utilização desta estirpe foi favorável para a produção de cafés de alta qualidade, obtendo percepção de sabor intenso de baunilha e aromas florais.

Bressani *et al*. (2018) e Martinez *et al*. (2017) avaliaram a qualidade de café da cultivar Catuaí amarelo pelo processamento seco e semi-seco, utilizando dois métodos de inoculação e três leveduras como culturas iniciadoras durante a fermentação do café. A qualidade sensorial dos cafés inoculados foi melhor, se comparados aos cafés produzidos a partir da fermentação com microbiota selvagem. Além disso, o café processado por via seca inoculado com *Saccharomyces cerevisiae* CCMA 0543 apresentou um aumento de 4 pontos na nota sensorial, se comparado com o controle (sem inoculação), sendo significativamente diferente das outras amostras e caracterizada por notas de banana, caju, acidez cítrica e bom corpo.

T. delbrueckii apresentou influência positiva na fermentação de cafés naturais, aumentando em 5 pontos a nota final, se comparado com a fermentação realizada sem inoculação de leveduras (DA MOTA *et al.*, 2020). Outros estudos recentes relatam resultados promissores usando leveduras e bactérias juntas durante a fermentação úmida do café (VALE *et al*., 2019; WANG *et al*., 2020b). No entanto, a aplicação de culturas starter combinadas em cafés processados a seco é escassa. A co-inoculação de leveduras ainda não foi estudada, entretanto, pode intensificar os atributos sensoriais, diversificar ainda mais os descritores sensoriais, além de serem uma alternativa para diferentes regiões e altitudes.

Estes resultados demonstraram que o emprego da fermentação controlada do café, através da utilização de culturas iniciadoras, promove a melhoria da qualidade sensorial da bebida, possibilitando melhor controle da fermentação e a previsibilidade do produto.

2.6 Análise sensorial de cafés especiais

A análise sensorial é a ciência que utiliza os sentidos humanos (como sabor, aroma, cor e textura) para avaliar características ou atributos de um alimento ou produto (PALERMO, 2015). É crucial para determinar a aceitação do produto, qualidade e prazo de validade

(MBELA *et al*., 2018; SHARIF *et al*., 2017). Com a chegada da "Terceira Onda do Café", o termo “qualidade” passou a ser mais frequente e é classificado, principalmente, por meio de protocolos definidos pela *Specialty Coffee Association* (SCA) (SAMOGGIA; RIEDEL, 2018).

As análises de cafés são realizadas por um painel de provadores treinados com certificação Q-Grader, que avaliam atributos de aroma/fragrância, sabor, gosto residual, acidez, corpo, uniformidade, equilíbrio, xícara limpa e doçura. Cada atributo recebe pontuação em uma escala de 6,0 a 10,0 pontos, com intervalos de 0,25. A pontuação final corresponde à soma total dos atributos avaliados. As amostras com pontuação total, igual ou superior a 80 pontos, são consideradas cafés especiais e podem ser subdivididas em muito bom (80-84,99), excelente (85-89,99) e excepcional (acima de 90 pontos). Os provadores também podem incluir descritores sensoriais para fornecer informações adicionais sobre cada café (SPECIALTY COFFEE ASSOCIATION - SCA, 2018).

A padronização da metodologia sensorial é extremamente útil para produtores, compradores e degustadores, pois auxilia na compra e venda do café. No entanto, muitas vezes, o protocolo é modificado e adaptado a diferentes estilos de trabalho (GUTIÉRREZ-GUZMÁN; CORTÉS-CABEZAS; CHAMBERS, 2018). A opinião dos avaliadores Q-Graders nem sempre está correlacionada com as preferências do consumidor (DI DONFRANCESCO; GUZMAN; CHAMBERS, 2014), principalmente, pelo fato de os consumidores serem influenciados por fatores extrínsecos, como embalagem, marcas, informações, entre outros (BRESSANI *et al*., 2021; LI; STRELETSKAYA; GÓMEZ, 2019; SAMOGGIA; RIEDEL, 2018; SPENCE; CARVALHO, 2020). Diferentes metodologias sensoriais podem expandir as informações sobre as escolhas e desejos dos consumidores de café. A soma dos resultados de provadores treinados e consumidores podem atingir efetivamente diferentes segmentos de mercado.

Diferentes testes sensoriais podem ser aplicados aos consumidores. Por exemplo, os testes de aceitação são utilizados com frequência e visam saber o quanto os provadores gostam ou não de uma amostra. Podem avaliar a aceitação global de diferentes processamentos, níveis de torra, métodos de preparo, entre outros, ou algum atributo específico (KWAK; JEONG; KIM, 2018; NGUYEN *et al*., 2016). Os testes de preferência podem expressar uma preferência entre duas (comparação emparelhada) ou mais amostras (teste de ordenação). Nesse caso, o avaliador saberá qual é a amostra mais/menos preferida e se há uma diferença significativa entre elas (LI; STRELETSKAYA; GÓMEZ, 2019). Além disso, essas metodologias podem ser aplicadas em conjunto com outros testes sensoriais descritivos para obter resultados completos.

A análise sensorial descritiva fornece resultados detalhados, precisos, confiáveis e consistentes. *Check-all-that-Apply* (CATA) é um método de análise sensorial descritiva usado

para discriminar amostras e melhorar produtos (VALENTIN *et al*., 2012). Esta análise consiste em uma lista de atributos a partir dos quais os provadores devem selecionar todos os atributos que considerarem adequados para descrever a amostra. Os atributos podem ser escolhidos pelos avaliadores por meio de um grupo de foco ou, no caso de cafés especiais, por meio de descritores fornecidos pelos avaliadores Q-Graders. Essa metodologia é considerada simples, porém, sua limitação é que a metodologia não permite a classificação das amostras e não fornece intensidade. Além disso, o CATA requer muitos avaliadores (VALENTIN *et al*., 2012). Vários estudos recentes utilizaram o CATA na avaliação sensorial de cafés com consumidores (GIACALONE *et al*., 2019; HEO *et al*., 2019; PRAMUDYA; SEO, 2018). Espitia-López *et al*. (2019) utilizaram CATA para descrever amostras de cafés especiais no México e obtiveram uma avaliação eficiente das diferenças obtidas pelos diferentes métodos de preparo (expresso e prensa francesa). Ao utilizar o teste CATA, Bressani *et al*. (2021) perceberam que os participantes não conseguiram perceber todos os atributos descritos pelos Q-Graders. Além disso, relacionaram o café fermentado ao sabor cítrico, sendo influenciados pelas informações.

Devido à complexidade do café, uma análise sensorial dinâmica, como Dominância Temporal das Sensações (*Temporal Dominance of Sensations* - TDS), poderia ajudar a entender os sabores percebidos ao longo do tempo (segundos). Esta análise permite avaliar os atributos percebidos como dominantes (a percepção mais marcante) (PINHEIRO; NUNES; VIETORIS, 2013). O TDS é uma ferramenta útil para avaliar diferentes produtos alimentícios e é amplamente utilizado em amostras de café. O método já foi aplicado na discriminação e caracterização de cafés fermentados (EVANGELISTA *et al*., 2014a, 2014b; RIBEIRO *et al*., 2017) e diferentes graus de torra (BARBOSA *et al*., 2019).

Devido à fadiga sensorial, o número de amostras pode ser limitante para a utilização de algumas metodologias. O retorno de participantes em várias sessões também pode dificultar a realização de análises sensoriais. Por isso, a escolha de um (s) teste (s) sensorial (s) depende do objetivo e da qualidade da medição sensorial. Os testes fornecem informações úteis sobre a percepção humana sobre as características de um produto, mudanças nos ingredientes, processamento, embalagem e prazo de validade. Eles são essenciais no controle de qualidade e na pesquisa de marketing. A análise sensorial pode ajudar a entender as preferências e desejos dos consumidores de cafés especiais, criando uma melhor comunicação entre o mercado e o consumidor final.

3 CONSIDERAÇÕES GERAIS

O processamento pós-colheita é uma etapa muito importante para garantir a qualidade do café. A fermentação melhora a qualidade da bebida e a inoculação de microrganismos selecionados, como culturas iniciadoras, contribui para obter características diferenciadas e com maior intensidade, padronização, além de inibir microrganismos indesejáveis. A presença de leveduras no café tem influenciado de forma positiva, principalmente, o perfil dos compostos voláteis e sensorial da bebida, aumentando a nota final dos cafés. A co-inoculação de leveduras ainda não foi estudada, entretanto, as informações obtidas sobre a composição química do café verde e torrado podem auxiliar na escolha da levedura mais indicada para cafés de diferentes altitudes.

Conhecer as expectativas e preferência dos consumidores é essencial para detectar os mercados que podem ser explorados com maior precisão e qualidade. Por isso, combinar técnicas sensoriais (como prova de xícara e *Check-all-that-Apply* - CATA) maximiza e complementa a descrição das características sensoriais dos cafés especiais.

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SEGUNDA PARTE

ARTIGO 1 - CO-INOCULATION OF YEASTS STARTERS: A STRATEGY TO IMPROVE QUALITY OF LOW ALTITUDE ARABICA COFFEE

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ABSTRACT

This study aimed to improve the beverage quality of coffee grown at low altitudes by inoculating three yeasts species (*Saccharomyces cerevisiae* CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, and *Candida parapsilosis* CCMA 0544) in a single and co-inoculation during the fermentation by dry processing. Important chemical compounds and groups were performed by liquid and gas chromatography and Fourier-transform infrared spectroscopy (FTIR). The inoculated coffees with yeast populations around 10^6 Log cell/g obtained the highest scores, and the co-inoculation with *C. parapsilosis* CCMA 0544 and *T. delbrueckii* CCMA 0684 had the highest score in the sensory analysis (85). Different descriptors were observed in each treatment, and the body, flavor, balance, and aftertaste are strongly related to *C. parapsilosis* CCMA 0544. The fermentation process improved the quality of low-altitude coffees, and the combination of non-Saccharomyces yeasts (*C. parapsilosis* CCMA 0544 and *T. delbrueckii* CCMA 0684) is the most indicated as starter cultures.

Keywords: coffee fermentation, co-inoculation, low altitude, FTIR, Caparaó region, quality.

1. Introduction

Coffee is a relevant commercial product consumed and appreciated globally. *Coffea arabica* reaches higher prices in the international market (ICO, 2020) due to its sensory properties, such as sweet, fruity, chocolate, floral, and caramel. Nowadays, specialty coffees have been gaining more market worldwide (Guimarães et al., 2019). Different aromas, flavors, and experiences demand coffee's from different processes, resulting in alternatives for coffee lovers (Guimarães et al., 2019).

The sensory analysis carried out by Q-Graders is one of the most important factors considered during specialty coffee vending. However, to unravel the sensory differences, key compounds (organic acids, carbohydrates, volatile compounds, proteins, and fatty acids) related to quality must be evaluated. For example, acidity is an attribute evaluated during cup tasting (Specialty Coffee Association, 2018), and some organic acids are correlated with the coffee acidity (Martins et al., 2019). However, not all acids are desirable (propionic and butyric acid have an unpleasant aroma and taste in coffee). During the different phases before and after harvesting, their concentration is susceptible to variations.

The coffee flavor complexity is mainly attributed to the volatile compounds, making their analysis essential. Volatile compounds are identified through Gas chromatography-mass spectrometry (GC–MS) techniques. However, other techniques exist, such as FTIR, which identifies quality parameters, defects, and bioactive compounds faster (Belchior et al., 2019); simultaneously, this technique can identify different volatile groups generated after fermentation. By implementing both techniques, the resulted data is maximized and complemented.

Coffee composition is affected by geographical origin, climate, altitude, species, harvesting methods, processing, and storage (Abreu et al., 2019; Bressanello et al., 2017). Many authors highlighted altitude as an essential factor in coffee quality. In Martins et al. (2020),

altitude affected the microbial diversity and interfered in the compounds profile related to coffee flavor. Veloso et al. (2020) observed a higher interaction between fungi-fungi and fungi-bacteria in low-altitude coffee.

Low-altitude coffees are associated with ununiform beans and lowquality beverages, making them poorly studied (Bodner et al., 2019; Tolessa et al., 2017). Considering the previous perspective and the influence of altitude, coffees cultivated at low altitudes would hardly have quality. Therefore, the post-harvest processes are also essential to maintain and increase coffee quality. Fermentation can occur regardless of the post-harvest processing (dry, semi-dry, or wet). Without control during this step may result in low-quality coffees (Lee et al., 2015). On the contrary, a controlled coffee fermentation is mainly performed to provide sensory experiences. Several studies have proven that starter cultures increase the coffee quality in different processing's (da Mota et al., 2020; Bressani et al., 2018; Martinez et al., 2017). However, different yeasts can result in coffees with different sensory profiles. Therefore, the co-inoculation of these microorganisms can intensify and increase the coffee sensory descriptors. Also, yeast co-inoculation in low-altitude arabica coffee fermentations has not yet been studied. The Caparaó National Park has the third highest peak in Brazil, Pico da Bandeira (2,892 m of altitude), situated between Minas Gerais and Espírito Santo states- Brazil. The Caparaó region is known for its specialty coffee production and striking sensory characteristics, making the region acquire its origin Caparaó designation. Besides, their coffee richness and high quality had allowed farmers to participate in national competitions (Apostólico et al., 2017). However, the quality is not the same in lower altitudes (below 700 m). In this context, we propose to use yeast co-inoculation as a strategy to innovate the fermentation process, improve the sensory characteristics, and overturn low-quality coffees from low-altitudes into higher quality coffees, mainly in the Caparaó region (both of which have been little studied). Consequently, the present study aimed to inoculate three yeasts (*Saccharomyces cerevisiae*

CCMA 0543, *Torulaspora delbrueckii* CCMA 0684, and *Candida parapsilosis* CCMA 0544) singly and with co-inoculation for fermentation using low-altitude coffee processed via dry to improve beverage. Further, this study aimed to performed compounds analysis to comprehend starter culture's influence on beverage quality.

2. Material and methods

2.1 Experimental design - coffee samples and yeasts inoculation

The Catuaí Vermelho IAC-44 coffee variety, growing at 600 m of altitude (20°47'15.58"S of latitude and 41°39'32.62"O of longitude) in the Caparaó region (Guaçuí-ES Brazil) was used for this study. The raw coffees had a soluble solid content average of 18.6 soluble solids (º Bx).

Selected coffee cherries (20 Kg for each treatment) were processed by the dry method, and fermentations were induced by yeast inoculation for 72 h (determined by temperature stabilization) in closed polypropylene bioreactors (self-induced anaerobic fermentation - SIAF). The average temperature during fermentation was 19.5 ºC (minimum temperature of 14.9 ºC and 24.8 ºC). The fermentations were divided into seven treatments carried out in triplicate and identified as: Sc treatment: inoculated only with *Saccharomyces cerevisiae* CCMA 0543; Cp treatment: inoculated only with *Candida parapsilosis* CCMA 0544; Td treatment: inoculated only with *Torulaspora delbrueckii* CCMA 0684; SC treatment: co-inoculation of *Saccharomyces cerevisiae* CCMA 0543 and *Candida parapsilosis* CCMA 0544; ST treatment: co-inoculation of *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora delbrueckii* CCMA 0684; CT treatment: co-inoculation of *Candida parapsilosis* CCMA 0544 and *Torulaspora delbrueckii* CCMA 0684; SCT treatment: co-inoculation of *Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544 and *Torulaspora delbrueckii*

CCMA 0684. The spontaneous fermentation (control) was performed under the same conditions without inoculation.

The yeasts were selected from the coffee environment (from dry and semi-dry processing). During their selection, they presented high pectin enzymatic activity and fermentative capacity, they produced desirable metabolites and increased the sensory notes of fermented coffees in different Brazilian regions (Bressani et al., 2018; da Mota et al., 2020; Evangelista et al., 2014; Martinez et al., 2017; Silva et al., 2013).

The strains were reactivated in 10 mL of YEPG broth (20 g/L glucose, 10 g/L yeast extract, and 10 g/L soya peptone (HiMedia); pH 3.5) at 28 °C for 24 h, in increasing volumes, until they are reaching a concentration of approximately 10^8 cells/g of coffee. Then cells were centrifuged, resuspended in 100 mL of distilled water, and inoculated in the coffee cherries. After fermentation, the cherries were transferred to the greenhouse with suspended terraces until 11- 12% moisture (33 days). Samples (200 g) were collected before and after fermentation and at the end of drying for chemical analysis.

2.2 Dynamic of yeast populations during fermentation

The yeast populations were monitored until the end of drying by real-time PCR (qPCR). DNA from strains and samples (0, 3, 9, and 36 days) were extracted by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the "DNA Purification from Tissues" protocol with the manufacturer's instructions. The starter cultures were cultivated separately in YEPG medium (pH 3.5) at 28 °C for 24 h. Cells were estimated using a Neubauer chamber and were serially diluted (1:10) from 10^9 to 10^3 cells/mL. Each dilution was measured in triplicate.

Specific primers were used for each species: SC-5fw 5′AGGAGTGCGGTTCTTTGTAAAG-3′ and SC-3bw 5′-TGAAATGCGAGATTCCCCT-3′

(*S. cerevisiae*); SADH-F 5′-GCTGCGGCTTCAACTGATGC-3′ and SADH-R 5′-CTTGGTCACGAGCCTCC-3′ (*C. parapsilosis*); Tods L2 5′-CAAAGTCATCCAAGCCAGC-3′ and Tods R2 5′-TTCTCAAACAATCATGTTTGGTAG-3′ (*T. delbrueckii*) (described in Bressani et al., 2018). The qPCR was carried out using the Rotor-Gene Q System (Qiagen, Hombrechtikon, ZH, Switzerland), according to Bressani et al. (2018), obtaining the following qPCR parameters: R^2=0.997, slope=−3.812 and efficiency of 0.83 (*S. cerevisiae*), R^2=0.991, slope=−3.628 and efficiency of 0.90 (*C. parapsilosis*), R^2=0.996, slope=−3.517 and efficiency of 0.92 (*T. delbrueckii*).

2.3 Chemical compounds

2.3.1 Organic acids

Citric, malic, succinic, oxalic, lactic, acetic, propionic, isovaleric, tartaric, and isobutyric acids were evaluated using a high-performance liquid chromatography system (Shimadzu Corp., Japan) in all treatments (0, 3, and 36 days). Three grams of each sample was homogenized in Falcon tubes with 20 mL of Milli-Q water by vortexing at room temperature for 10 min and were centrifuged twice at 12,745 RCF at 4 °C for 10 min. The supernatant pH was adjusted to 2.11 using a perchloric acid solution (16 mM) and filtered through a 0.22 μm cellulose acetate membrane. The resulting solution was then injected (20 μL) onto the chromatographic column (Shimpack SCR-101H -7.9 mm x 30 cm).

The analysis was performed at a temperature of 50 ºC, using ultrapure water and perchloric acid (pH 2.1) as a mobile phase, at a flow rate of 0.6 mL/min. The acids were detected by UV absorbance (210 nm). Calibration curves were constructed with standards to quantify the chemical compounds (Evangelista et al., 2014). Malic and citric acid were purchased from Merck (Germany), lactic was purchased from Acros Organic (Belgium), oxalic, isovaleric,

tartaric, acetic, and succinic acids were purchased from Sigma-Aldrich (Germany), and butyric acid was purchased from Riedel-de Haen (Germany).

2.3.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR analysis was conducted according to Liang et al. (2016) with minor modifications. The green and roasted ground coffee (2 g) was frozen for 24 h at -20 °C. The samples were subjected to a lyophilizing step for 24 h. FTIR spectra of coffee were recorded on a Digilab Excalibur, series FTS 3000 (United States), coupled to an attenuated total reflectance (ATR) accessory equipped with a ZnSe reflection crystal. The spectra were acquired at room temperature with 32 scans/samples in the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} using OriginPro software.

2.3.3 Volatile compounds characterization

Two grams of coffee samples (0, 3, 36 days, and roasted) were ground with liquid nitrogen and extracted by headspace solid-phase microextraction (HS-SPME) (Evangelista et al., 2014). The compounds were analyzed using a Shimadzu QP2010 GC model equipped with mass spectrometry (MS) and a silica capillary Carbo-Wax 20M (30m x 0.25mm x 0.25 mm) column.

The oven temperature was maintained at 50 ºC for 5 min. The temperature was then raised to 200 ºC in increments of 8 ºC/min and then maintained at 200 ºC for 15 min. Injector temperatures were kept at 230 ºC in splitless mode. The carrier gas (He) was maintained at a flow rate of 1.98 mL/min. The mass spectra detected was comparing with the NIST11/NIST11a database, and an alkane series (C10–C40) was used to calculate the retention index (RI); the RI values were compared with those found in the literature data (Amanpour & Selli, 2016; Toci et

al., 2020). The relative percentages of individual compounds were calculated from the total contents of volatiles on the chromatograms.

2.4 Sensory analysis

The samples were roasted carried out around 8:30 - 9:30 minutes with the final temperature between 185 and 190 ºC (Probat Leogap model TP2 2, Curitiba, Brazil), until obtaining beans with a coloration approximately 60 on the Agtron scale (Specialty Coffee Association, 2018). Coffee beans were ground in an electric mill (Mahlkönig, EK43 model, Hamburg, Germany) before sensory analysis and stored under controlled temperature until analysis was performed. A panel of five expert coffee tasters, with Q-Grader Coffee Certificate, performed the sensory analysis using five cups for each sample (predetermined ratio of 8.25 ± 0.25 g per 150 mL of water). The attributes evaluated were fragrance, flavor, aftertaste, acidity, body, balance, uniformity, sweetness, clean cup, overall, and the total score (Specialty Coffee Association, 2018), besides sensory descriptors.

2.5 Statistical analysis

Organic acid and qPCR data were analyzed using the Student-Newman-Keuls test (SNK) at 5% significance using the R statistics program. Heatmap and Tukey test at 5% significance were performed using the XLSTAT 2019 2.1 software for the volatile group and the sensory analysis's final scores, respectively. Pearson correlation coefficient was used for correlation tests through XLSTAT 2019 2.1 software.

3. Results

3.1 Yeasts population

The qPCR technique made it possible to monitor the population of *S. cerevisiae, C. parapsilosis,* and *T. delbrueckii* in the fermentation until the end of drying the coffees with and without inoculation (Figure 1).

At the beginning of fermentation, *S. cerevisiae*, *C. parapsilosis*, and *T. delbrueckii* populations in the spontaneous fermentation varied from 5 to 6 Log cell/g of coffee. *S. cerevisiae* and *C. parapsilosis* populations were lower in spontaneous fermentation when compared to inoculated treatments. *T. delbrueckii* population was the same in the spontaneous and inoculated treatments.

The inoculated treatments with *S. cerevisiae* showed no significant change after 3 days of fermentation in a closed bioreactor. However, there was an increase in the *S. cerevisiae* population from drying until day 9 in all treatments. From that point on (day 9), the Sc and SC treatments showed no significant difference (Figure 1A).

C. parapsilosis population was constant throughout the process, ranging from 5.82-6.41 Log cell/g of coffee. The CT and SCT treatments showed similar population dynamics of *C. parapsilosis* until the end of drying. Co-inoculation treatments showed a small increase in the population of *C. parapsilosis* on day 9, except for SC treatment. The Cp treatment had the largest population (6.3 Log cell/g of coffee), with a significant difference from the other treatments. The population of *C. parapsilosis* showed a significant increase from the beginning of day 9th of drying (6.41 Log cell/g of coffee) in spontaneous fermentation. However, at the end of drying, Cp treatment had the highest population (6.25 Log cell/g). The SC and SCT treatments showed no significant difference at the end of fermentation (6.09 and 6.06 Log cell/g) (Figure 1B).

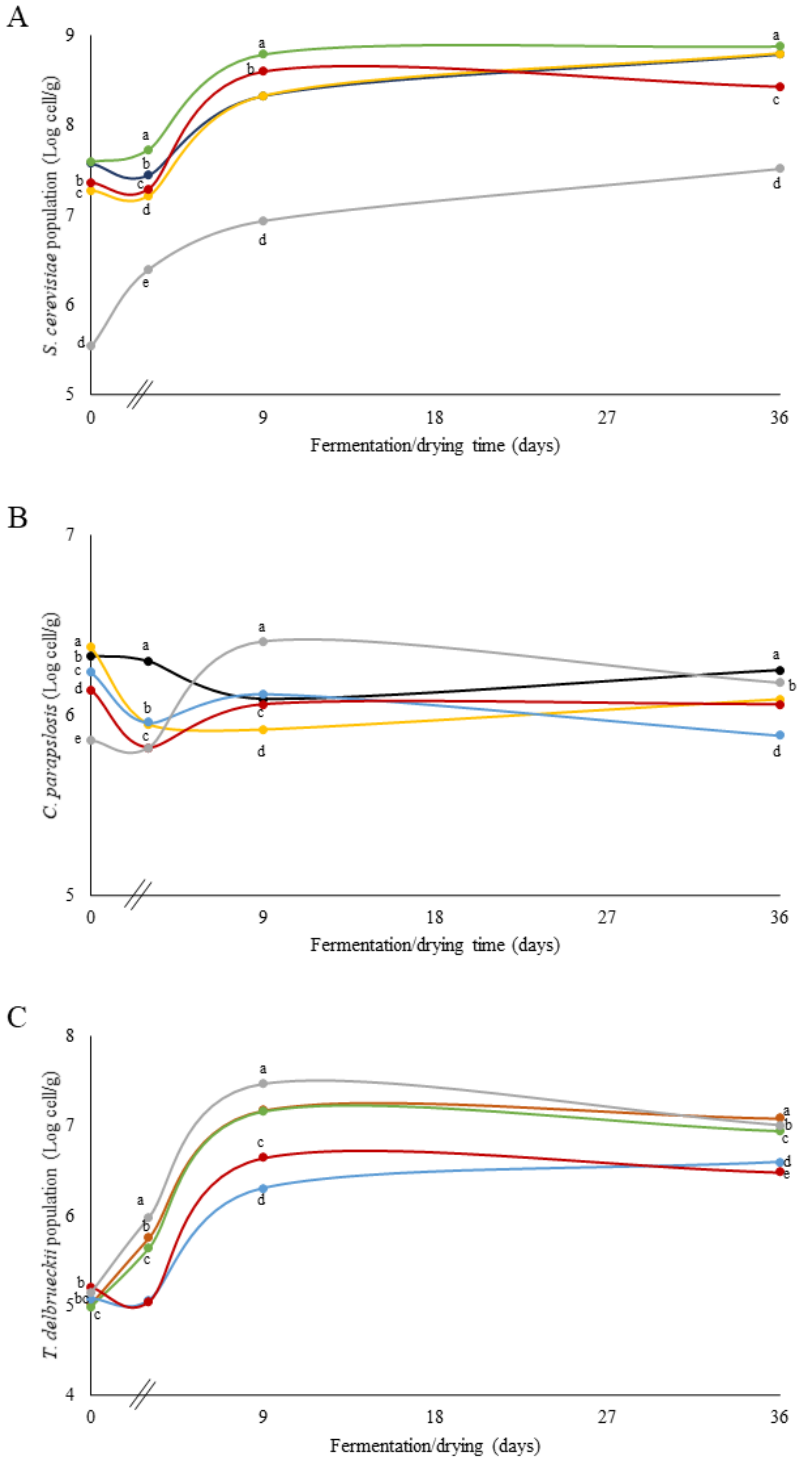

Fig. 1. The monitoring of the yeast population (A) *S. cerevisiae*; (B) *C. parapsilosis*, and (C) *T. delbrueckii* was carried out from the beginning of fermentation in a closed bioreactor until the end of drying by qPCR. Treatments: ● Sc - *S. cerevisiae*; ● Cp - *C. parapsilosis*; ● Td - *T. delbrueckii*; ● SC - *S. cerevisiae* + *C. parapsilosis*; ● ST - *S. cerevisiae* + *T. delbrueckii*; ● CT - *C. parapsilosis* + *T. delbrueckii*; ● SCT - *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; ● Spontaneous fermentation. Lower case letters indicate a significant difference in means between treatments at each stage. at $p < 0.05$ by Student-Newman-Keuls.

CT and SCT treatments presented a similar dynamic behavior of the *T. delbrueckii* population throughout the processing. Compared to the other treatments, CT and SCT had evident differences at the end of fermentation. The treatments Td and ST showed no significant difference between them at the beginning of fermentation and drying 9th. The Td treatment had the highest population of *T. delbrueckii* (7.08 Log cell/g), and the SCT treatment had the lowest population (6.48 Log cell/g) at the end of drying (Figure 1C).

3.2 Organic acids determination

Citric, malic, and succinic acids were detected in all treatments at the beginning of fermentation and remained until the drying (Table 1). From the beginning to the end of fermentation and drying, citric acid decreased except in Cp treatment. There was no significant difference in any treatment of citric and succinic acid production, and the malic acid content decreased in all treatments at the end of fermentation, except for spontaneous fermentation and Td treatment. The Sc and SC treatments showed the lowest concentrations of this compound (0.67 and 0.61 mg/g, respectively). Td treatment presented the highest lactic acid concentration at the end of the fermentation (1.83 mg/g), showing a significant difference from the other treatments.

Among the treatments, the spontaneous fermentation presented the highest citric acid concentration (1.83 mg/g), followed by all the treatments inoculated with *S. cerevisiae* CCMA 0543 at the end of drying. Also, Cp treatment presented the highest succinic acid concentration (0.87 mg/g). Meanwhile, acetic acid was the primary acid detected. Moreover, the highest acetic acid concentration was found in the spontaneous fermentation (7.59 mg/g) and SCT treatment (7.21 mg/g), showing a significant difference among the other treatments (Table 1).

Table 1. Organic acids detected in coffee during fermentation with and without yeasts inoculation and drying.

Acids	Treatments (mg/g)							
	Sc	Cp	Td	SC	ST	CT	SCT	Spontaneous fermentation
Citric^{ns}								
IF	2.22±0.10	1.22±0.05	2.10±0.12	1.88±0.02	2.07±0.15	1.65±0.25	1.74±0.27	1.98±0.33
FF	1.81±0.24	1.41±0.07	1.81±0.04	1.58±0.32	1.39±0.24	1.27±0.17	1.59±0.44	1.95±0.09
FD	1.40±0.27	1.11±0.22	0.93±0.06	1.34±0.18	1.34±0.16	1.00±0.16	1.24±0.15	1.83±0.05
Malic								
IF	3.21±0.38^{aB}	1.53±0.20^{bC}	2.70±0.37^{aC}	2.59±0.26^{aC}	4.49±0.19^{aA}	3.39±0.04^{aB}	2.31±0.17^{aC}	2.55±0.11^{bC}
FF	0.67±0.09b^{CB}	1.23±0.30^{bB}	2.87±0.13^{aA}	0.61±0.15^{cC}	1.28±0.24^{bB}	1.18±0.16^{bB}	1.49±0.13^{bB}	2.78±0.05^{bA}
FD	1.78±0.07^{cC}	2.45±0.00a^{BC}	2.90±0.13^{aB}	2.02±0.22^{bC}	1.90±0.17^{cC}	2.03±0.06^{cC}	2.02±0.19^{aC}	3.40±0.03^{aA}
Succinic^{ns}								
IF	0.74±0.22	0.42±0.18	0.69±0.02	0.73±0.33	0.90±0.03	0.66±0.10	0.71±0.02	0.77±0.35
FF	0.87±0.17	0.56±0.19	0.60±0.04	0.60±0.12	0.49±0.15	0.43±0.01	0.58±0.23	1.05±0.12
FD	0.61±0.15	0.87±0.38	0.71±0.07	0.68±0.03	0.58±0.09	0.50±0.01	0.56±0.11	0.86±0.01
Lactic								
IF	0.00±0.00^{bB}	0.00±0.00^{cB}	0.00±0.00^{cB}	0.57±0.06^{bA}	0.00±0.00^{cB}	0.16±0.01^{cB}	0.00±0.00^{cB}	0.00±0.00^{cB}
FF	1.16±0.28^{aB}	1.27±0.15^{bB}	1.83±0.28^{bA}	0.91±0.33^{bB}	0.77±0.00^{bB}	1.09±0.13^{bB}	0.75±0.07^{bB}	1.27±0.12^{bB}
FD	1.02±0.12^{aE}	2.77±0.31^{aB}	3.24±0.31^{aA}	1.43±0.14^{aD}	1.70±0.24^{aCD}	3.34±0.07^{aA}	2.02±0.04^{aC}	3.43±0.09^{aA}
Acetic								
IF	11.62±0.18^{aA}	0.00±0.00^{bC}	9.39±1.61^{aB}	0.00±0.00^{cC}	0.00±0.00^{bC}	8.56±0.11^{aB}	0.00±0.00^{bC}	0.00±0.00^{bC}
FF	8.07±0.11^{bA}	3.73±0.25^{aD}	6.02±0.08^{bC}	7.56±0.44^{aAB}	6.33±0.33^{aC}	4.46±0.52^{bD}	7.36±0.13^{aAB}	6.97±0.13^{aAB}
FD	5.16±0.22^{cB}	3.38±0.27^{aC}	5.05±0.02^{bB}	5.96±0.09^{bAB}	6.24±0.05^{aAB}	4.46±0.18^{bB}	7.21±0.05^{aA}	7.59±0.10^{aA}

Data are shown as the mean. ± = standard deviation. Equal lowercase letters do not differ significantly ($p < 0.05$) from each other in the column by the Student-Newman-Keuls test. Equal capital letters do not differ significantly ($p < 0.05$) from each other on the line by the Student-Newman-Keuls test. Initial fermentation (IF); Final fermentation (FF); Final drying (FD). ^{ns} there was no significant difference in any of the treatments.

Oxalic, tartaric, isovaleric, isobutyric, and propionic acids were not detected in any sample. The presence of isovaleric, isobutyric, and propionic acid provided unpleasant flavors, indicating no lack of control or overfermentation.

3.3 fTIR

FTIR's spectral infrared analysis described the main functional groups from green and roasted coffee beans (Figure 2). The visual examination of ATR-FTIR spectra revealed that the intensity of some bands was changed. The absorbance bands observed at ≈ 3300, 2920, and 2850 cm^{-1} are assigned to –OH stretching, symmetrical and asymmetric vibrations of -CH groups of carbohydrates and caffeine, respectively (Tavares et al., 2012).

The 1780-1700 cm^{-1} range is characteristic of vibrational stretch of C = O bond for carbonyl lipids, aliphatic esters, and carboxylic acids (Belchior et al., 2019; Krause et al., 2019; Tavares et al., 2012). All samples showed a peak of 1743 cm^{-1}, with a visible increase in roasted samples.

Band 1637 cm^{-1} represents the bond C = C ethylenic stretching. Liang et al. (2016) designated the region 1700−1600 cm^{-1} to the chlorogenic acid isomer composition in coffee. Belchior et al. (2019) associate caffeine and trigonelline with this region.

The 1457 cm^{-1} wavelength is associated with C–H bending vibration to CH_2 and CH_3 aliphatic groups. 1157 cm^{-1} was associated with C–O ester group stretching and rocking vibration, for example, CH_2 (Raba et al., 2015). These two bands were identified only in the roasted samples. The region between 1000 and 1100 cm^{-1} corresponds to the C–O and C–C–O bonds (Krause et al., 2019).

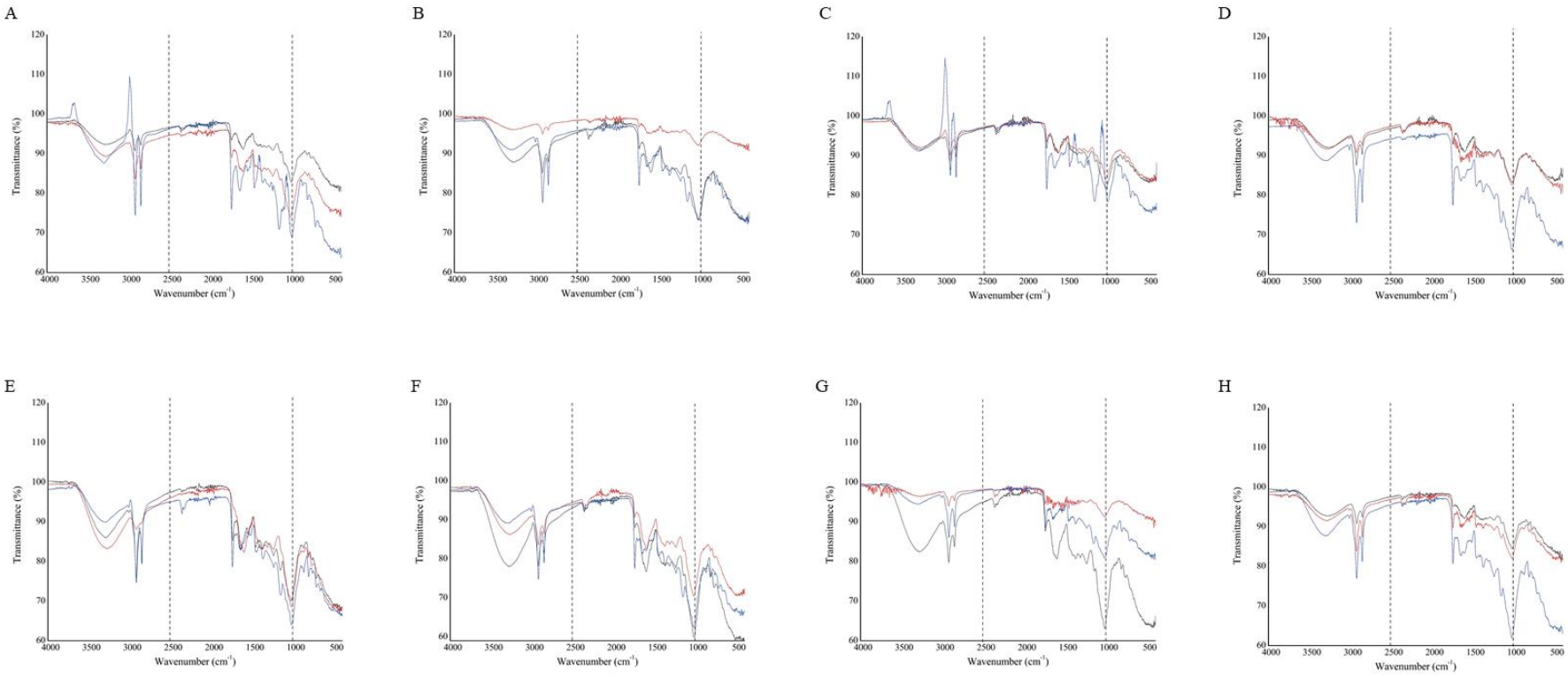

Fig. 2. FTIR-ATR spectra from green and roasted coffee produced by spontaneous and inoculated fermentation (single yeasts and co-inoculation). — beginning of fermentation; — final fermentation; — after roasted. Treatments: (A) Sc - *S. cerevisiae*; (B) SC - *S. cerevisiae* + *C. parapsilosis*; (C) Cp - *C. parapsilosis*; (D) CT - *C. parapsilosis* + *T. delbrueckii*; (E) Td - *T. delbrueckii*; (F) ST - *S. cerevisiae* + *T. delbrueckii*; (G) SCT - *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; and (H) Spontaneous fermentation.

3.4 Volatile Compounds

Different volatile compounds (164) were detected in green and roasted coffee by SPME–GC–MS (Supplementary material - Table 1 and Table 2). Alcohols and aldehydes were the main chemical classes found before fermentation in the closed bioreactor. 2-heptanol, 3-ethyl-4-methyl pentanol, and hexanal are the most abundant compounds (15.42, 12.97, and 11.05%, respectively) found in coffee before fermentation. The only acid detected was acetic acid (2.60%).

Other chemical classes (mainly esters and lactones) aroused after 72 h of fermentation. Octanoic acid, ethyl ester (fruity), was more abundant among the esters after fermentation in a closed bioreactor, except for the SC treatment. The treatments that showed the highest abundance of this compound were ST and Cp (17.57 and 16.40%, respectively). The compound pentanol acid production, ethyl ester, presents the sensory perception of juicy, blueberry, and apple. Its presence was observed in Cp (12.68%), CT (18.09%), Td (6.06%), ST (7.61%), SCT (14.7%), and spontaneous fermentation (15.32%). Spontaneous fermentation and Td treatment showed lower concentrations (both 0.25%) of 2-phenylethyl acetate (fruity, honey, floral). The treatment inoculated with Td showed a higher concentration of alcohol and fewer pyrazines production after 72 h of fermentation. The compounds 2-methyl pyrazine (nutty, roasted, sweet, almond, biter) and 2,6-dimethyl pyrazine (cocoa, coffee, roasted nutty) were present in only Sc treatment (Supplementary material- Table 1). Only the Sc and SC treatments showed much ketones production in the final fermentation (Figure 3).

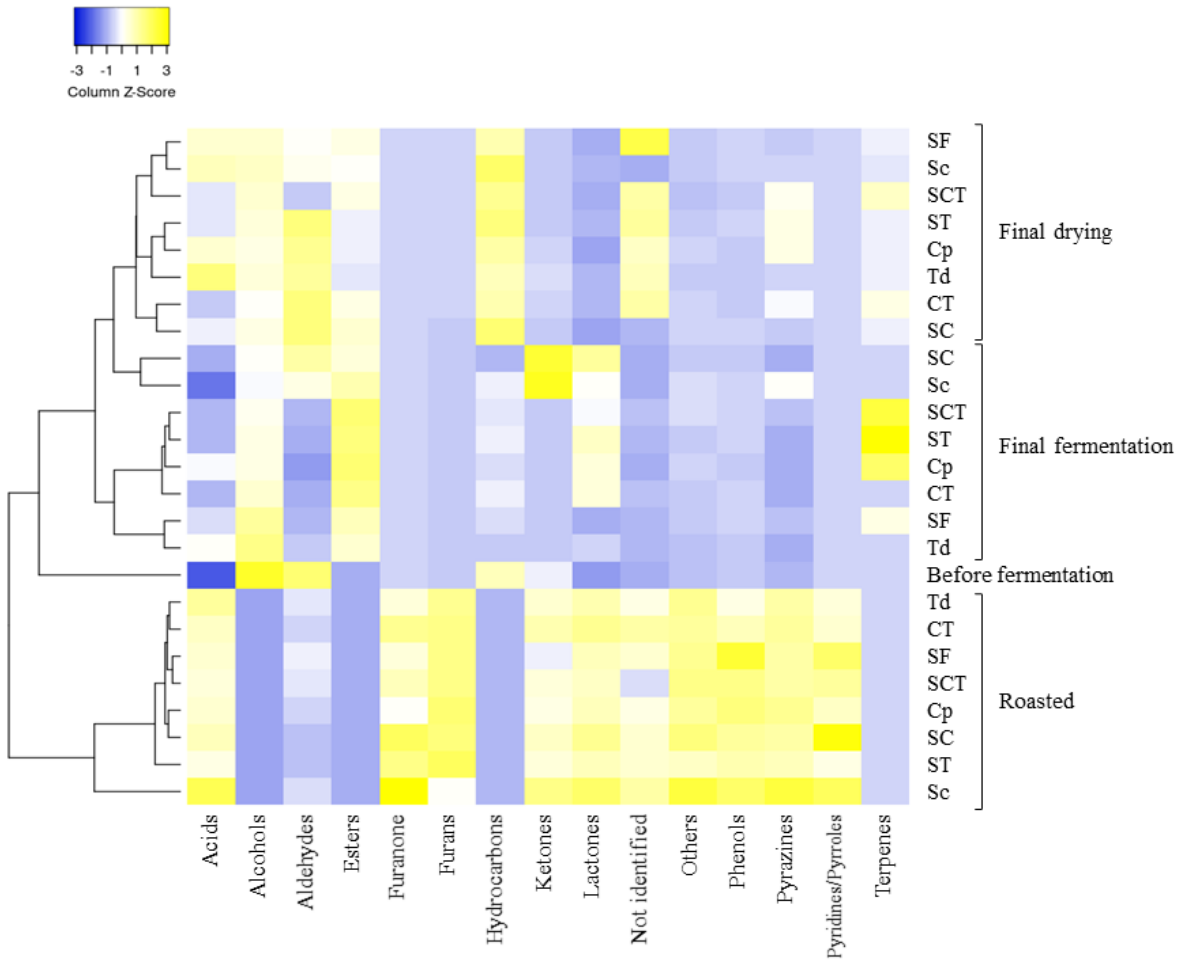

Figure 3. Heatmap with normalized intensities of the volatile group showing a significant difference between spontaneous and inoculated fermentation (single yeasts and co-inoculation). Sc - *S. cerevisiae*; Cp - *C. parapsilosis*; Td - *T. delbrueckii*; SC - *S. cerevisiae* + *C. parapsilosis*; ST - *S. cerevisiae* + *T. delbrueckii*; CT - *C. parapsilosis* + *T. delbrueckii*; SCT - *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; SF - Spontaneous fermentation.

Until the end of drying, there were modifications of the chemical groups, from which hydrocarbons, aldehydes, and pyrazines were the most abundant. The Sc, SC, and ST treatments showed the highest hydrocarbons (9.56; 9.02; 8.84%, respectively) (Supplementary material-Table 1). The highest concentrations of pyrazines were found in the treatments Cp, CT, ST, and SCT (Figure 3), being the most abundant 2-ethyl-5-methyl pyrazine and 2-methoxy-3-(2-methyl propyl)-) pyrazine. Methyl salicylate (sensory descriptor: caramel and peppermint) was produced after 72 h of fermentation and increased until the end of drying in all treatments.

After roasting, the concentration of alcohols, aldehydes, and esters decreased (more than 90, 60, and 90%, respectively). The only hydrocarbon detected was tetradecane (only Sc treatment (0.26%)) (Supplementary material - Table 2). Furanones, furans, lactones, pyrazines, pyrroles/pyridines were produced (Figure 3). Among these groups, the main compounds found after roasting were: 5-methyl-2-furancarboxaldehyde; 2-furanmethanol; furfural; 2-ethyl-5-methyl pyrazine. However, more compounds belonging to the lactones and pyrroles/pyridines classes were detected in less abundance (less than 3 and 1%, respectively). All treatments showed considerable acetic acid abundance after roasting, with higher concentrations in the Sc (22.84%) and Td (18.62%) treatments (Supplementary material- Table 2). However, this detected acid does not seem directly related to the coffee's acidity and final score.

3.5 Cupping

All coffees were classified as specialty coffees. Figures 4 and 5 show the sensory analysis results of fermented coffees with and without inoculation. The scores for all attributes ranged from 7.4 to 8.1 (Figure 4). When compared to other treatments, Td had the lowest scores for flavor, aftertaste, and overall. The CT treatment obtained the highest scores for fragrance/aroma (8.1), acidity (7.9), body (7.8), balance (7.8), and overall (7.8). This treatment obtained a lower score (7.9) only than the SCT treatment (8.0). Moreover, the body, overall, flavor, aftertaste, and acidity were robust positive correlation with a population of *C. parapsilosis* (0.924, 0.792, 0.793, 0.786, 0.738, respectively) and weak correlation between *T. delbrueckii* and *S. cerevisiae* (> 0.3) (Supplementary material- Table 3).

Cp treatment showed higher scores when compared to the other treatments inoculated with one yeast. It is possible to notice that the most significant difference between this treatment and the spontaneous fermentation was overall fragrance/aroma and flavor attributes. Sc, SC,

and ST treatments obtained lower scores (83.9; 84.0; 83.7, respectively) than the spontaneous fermentation (84.4) (Figure 4). These results show that *S. cerevisiae* CCMA 0543 in low altitude coffee is unfavorable compared to spontaneous fermentation (Figure 4). Td treatment was also unfavorable in low-altitude coffee in the Caparaó region.

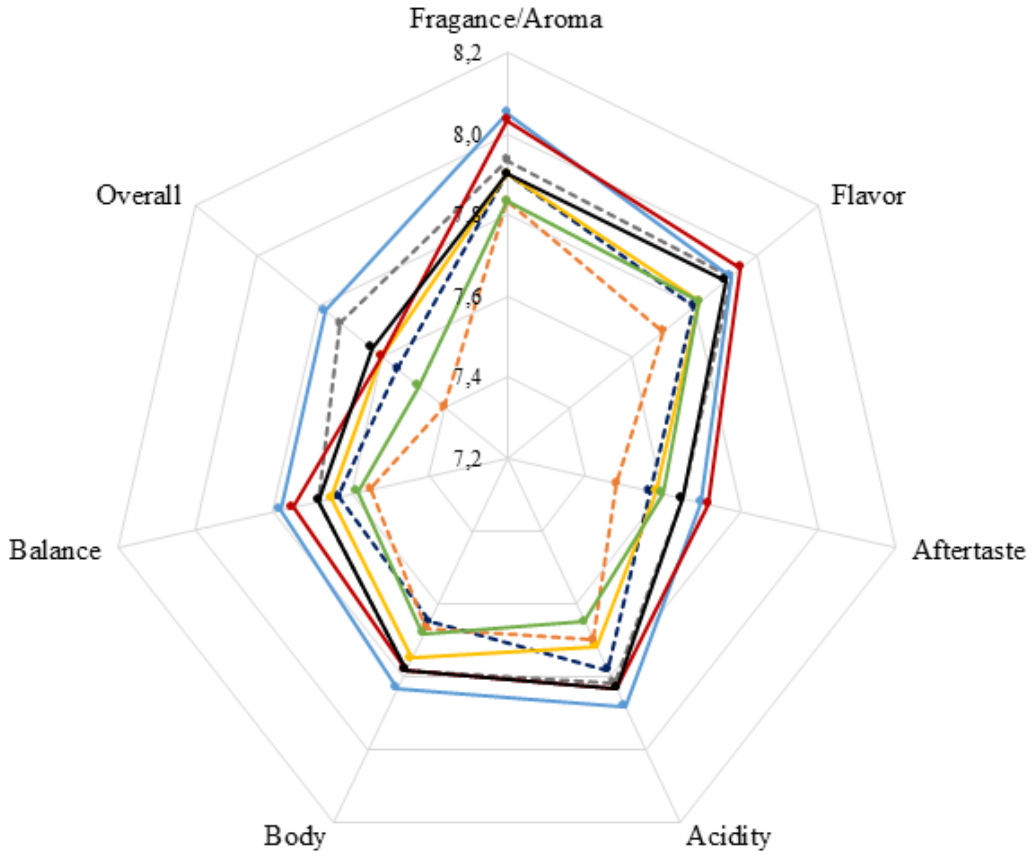

Figure 4. The average score of attributes evaluated by protocol cupping SCA (5 Q-graders). The treatments were evaluated in triplicate. All treatments scored 10 for uniformity. Sweetness. and clean cup. Treatments: - - - Sc - *S. cerevisiae*; - - - Cp - *C. parapsilosis*; - - - Td - *T. delbrueckii*; — SC - *S. cerevisiae* + *C. parapsilosis*; — ST - *S. cerevisiae* + *T. delbrueckii*; — CT - *C. parapsilosis* + *T. delbrueckii*; — SCT - *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; — Spontaneous fermentation.

Sensory descriptors were also defined by the tasters (Figure 5). All samples had chocolate and caramelized descriptors (except the Td treatment, which did not have the last descriptor). The descriptor nut/almond was perceived only in the treatments inoculated with *S. cerevisiae* CCMA 0543 (Sc, SC, ST, and SCT). The fruity descriptor was not detected in CT,

Td, ST, and spontaneous fermentation. Besides, the descriptor cucumber was perceived when *T. delbrueckii* CCMA 0684 (Td) was inoculated individually (Figure 5) and had the lowest final score (83.3), significantly different from the other treatments. Despite not having a different descriptor from the spontaneous fermentation, CT treatment had the highest final score (85), showing a significant difference from the other treatments (Fig. 5). This difference can be attributed to the scores of the other attributes evaluated (Fig. 4).

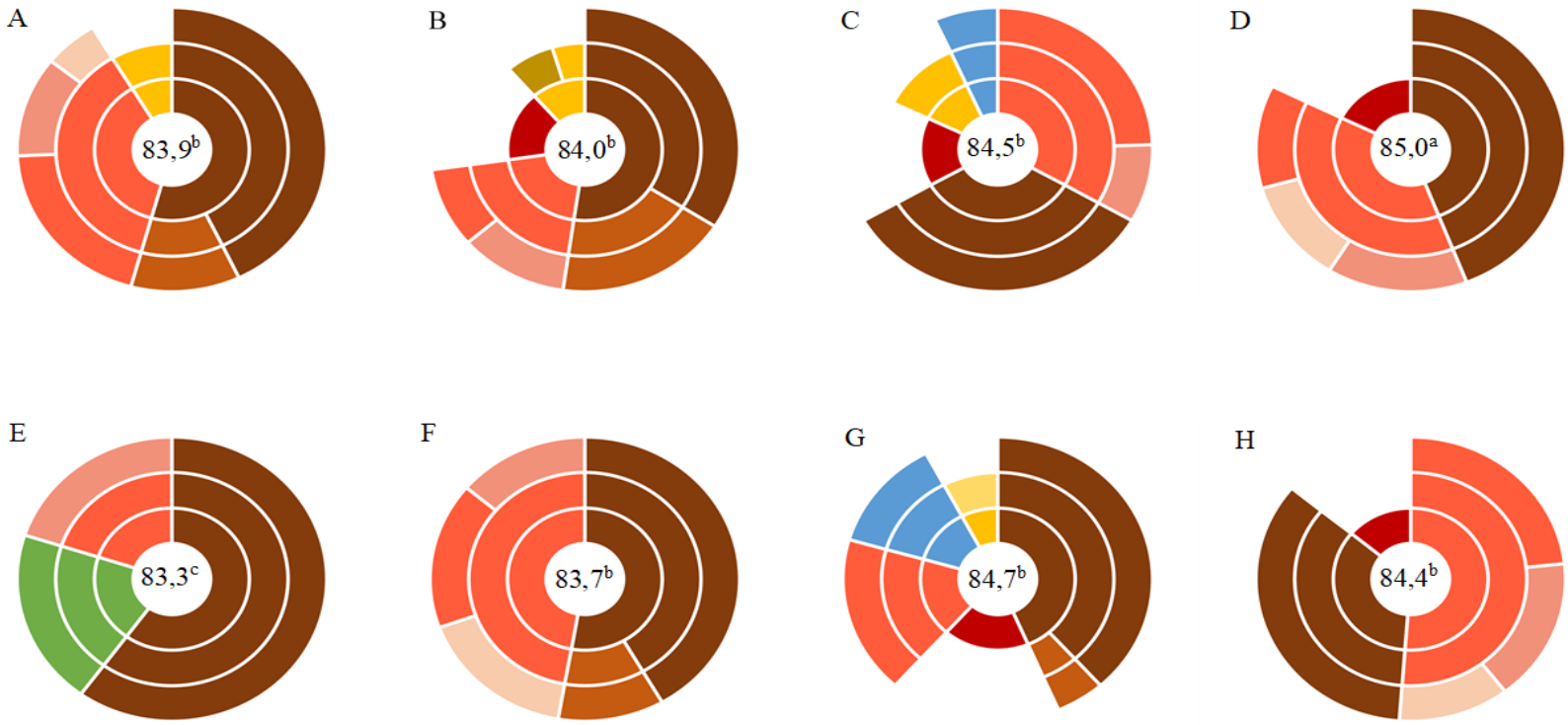

Fig. 5. Sensorial descriptors and the final score of fermented coffee. Treatments: (A) Sc - *S. cerevisiae*; (B) SC - *S. cerevisiae* + *C. parapsilosis*; (C) Cp - *C. parapsilosis*; (D) CT - *C. parapsilosis* + *T. delbrueckii*; (E) Td - *T. delbrueckii*; (F) ST - *S. cerevisiae* + *T. delbrueckii*; (G) SCT - *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; and (H) Spontaneous fermentation. The groupings of the descriptors follow the SCA flavor wheel (■ cocoa/chocolate; ■ nutty/almond; ■ caramelized; ■ molasses; ■ honey; ■ fruit; ■ dried fruit; ■ yellow fruit; ■ citric acidity; ■ spice; ■ cucumber). Different lower case letters indicate a statistically significant difference ($p < 0.05$) by the Tukey test.

4. Discussion

S. cerevisiae, *C. parapsilosis*, and *T. delbrueckii* populations have already been monitored by qPCR during coffee fermentation in different processes (Bressani et al., 2018; da Mota et al., 2020; Martinez et al., 2017). However, it is the first time that the technique has been used to monitor the population dynamics of yeasts in co-inoculation during coffee fermentation. The population increases at the beginning of drying until day 9 due to its facultative anaerobic condition (Lagunas, 1981).

The inoculation of starter cultures during the fermentative process can enhance preservation, nutritional value, and sensory qualities and increase the final product's economic value. Besides, its population should be capable of conducting the fermentation, colonizing the product, and dominating over other microorganisms from the beginning to the end of the process (Silva et al., 2013).

Coffee fermentation is complex and relies on a succession of microbial populations that play a specific role. Exothermic reactions during the fermentation process promote an increase in the coffee mass (19.5 °C; ambient temperature: 17 °C). Temperature combined with an acidic environment contributes to the proteolysis of seed and the formation of flavor precursors for later coffee aroma (Muñoz et al., 2019).

Organic acids, as citric, malic, and succinic acids, are produced by the coffee plant. Their concentrations can be affected by genetic aspects, growth conditions (altitude, agricultural methods, degree of fruit maturity), choice of postharvest method, roast degree, and the type of extraction (Diviš et al., 2019; Martinez et al., 2017; Evangelista et al., 2014). In complex fermentative processes, those acids are used as a carbon source by the microbiota present, such as citric acid fermentation by lactic acid bacteria (LAB). Furthermore, acidity is one of the parameters that interferes with beverage quality and essential for the classification of specialty coffees (Specialty Coffee Association, 2018). The presence of citric acid in coffee

is desirable as it also contributes to the tartness, fruity, berry flavor, and the perceived brightness in taste (Vandenberghe et al., 2018). This acid may have contributed to the perception of citric acidity in Cp and SCT treatments and fruity in Sc, SC, Cp, and SCT treatments (Fig. 5).

Succinic acid production depends on the yeast strain genetic trait, fermentation temperature, chemical composition of the growth medium, and aeration. The production of succinic acid at the end of fermentation in a closed bioreactor (Sc, Cp, SF treatments) and at the end of drying (Cp, Td, SC, ST, and CT treatments) may indicate that the yeasts used the glyoxylate cycle and the citric acid-reducing cycle to balance the NADH/NAD (Ferreira & Mendes-Faia, 2020). Lactic acid bacteria (LAB) are a heterogeneous group distributed in different habitats known as O_2-tolerant anaerobes (Zotta et al., 2018). Lactic acid production indicated lactic acid bacteria's growth during fermentation in closed bioreactors until final drying since yeasts produce low lactic acid contents (Ferreira & Mendes-Faia, 2020). LAB can promote malolactic fermentation through decarboxylation of L-malic acid in L-lactic acid (Ramírez & Velázquez, 2018). Yeasts like *S. cerevisiae* can use the Krebs group (-) using one or more of the TCA cycle intermediates only in the presence of glucose or other assimilable carbon sources (Ferreira & Mendes-Faia, 2020).

Citric acid could also be converted into lactic acid and acetic acid, following pyruvate metabolism, or succinic acid through the tricarboxylic acid cycle (Gänzle, 2015). Acetic acid also contributes to the beverage's final acidity (Bressani et al., 2018) and is responsible for the fruity, winey, and fermented aroma (Seninde & Chambers IV, 2020). Yeasts can also produce acetic acid during the fermentation of coffee mucilage (Pereira et al., 2019) and were verified in all treatments after fermentation and final drying (Supplementary material- Table 2).

The different spectrum regions detected in the FTIR are correlated to the compounds that give different coffee aromas and corroborate the wide variety of volatile compounds identified by the SPME-GC–MS technique. The spectrum region of 1700 cm^{-1} is crucial

because it is related to compounds that give different coffee aromas (Belchior et al., 2019). The aliphatic carboxylic acid content contributes to the beverage's acidity and the palate's perceived brightness (Lyman et al., 2003). Compounds derived from trigonelline contribute to the coffee's sensory quality, especially for roasted coffee's aroma (Belchior et al., 2019).

Different compounds were produced at the end of fermentation in a closed bioreactor, mainly alcohols, and esters. Yeasts can catabolize amino acids through the Ehrlich pathway or use the acetyl-CoA produced in glycolysis to generate esters (Pereira et al., 2019). 2,3-butanediol (natural odor of cocoa butter, sweet chocolate) was not found in the Sc and SC treatments. 2-phenyl ethanol (rose-honey-like odor, pleasant floral-woody) was found in considerable concentrations in all treatments (Supplementary material- Table 1).

In the same way, higher alcohols, aldehydes, ketone, and terpenoids can also be produced, and different molecules may be generated through the metabolism of carbon and nitrogen (Dzialo et al., 2017; Silva et al., 2013).

During the aerobic phase, compounds of pyrazines, hydrocarbons, and aldehydes are most intensely produced. A significant increase in pyrazines was observed mainly for CT treatment (Fig. 3). Muñoz et al. (2019) affirmed that drying could significantly affect the cocoa's final quality, modulating the flavor. These authors consider that drying helps obtain maximum flavor development, producing alcohols, aldehydes, ketones, esters, acids, and pyrazines (Rodriguez-Campos et al., 2012). However, it is necessary to study with more detail the yeast metabolisms in dry coffee.

Coffee is a complex matrix and contains many volatile compounds (principally after roasted), so it is unlikely that a specific chemical compound can determine the beverage's flavor/aroma. The carbonyl group is essential because it constitutes different chemical classes, such as aldehydes, esters, ketones, carboxylic acids, and amides. This group's presence can affect the aroma and taste centers in the nose and mouth, giving different sensory qualities. For

example, depending on this group's type and concentration, aldehydes may have sharp odors, ranging from woody to cucumber, cooked fruit, and nuts. Ketones have the same odors but are less accentuated. Acids can exhibit aromas ranging from vinegar to chocolate to burnt caramel, and some have no odor (Lyman et al., 2003).

The formation of furans after roasting is partly caused by sugar caramelization or a sugar and an amino acid reaction. Furans can reach a maximum concentration in a medium roast and assign sweet aromatics, caramel, and burnt aromas to coffee beverages (Seninde & Chambers IV, 2020). Pyrazines and ketones can be formed using the Maillard reaction (sugars and amino acids) (Gonzalez-Rios et al., 2007). 2-ethyl-5-methyl pyrazine (coffee-like taste to a sugar syrup) was a relevant compound in the group of pyrazines. This compound was found in all samples in a lower concentration in spontaneous fermentation.

The decrease in esters' concentration after roasting may be related to increased carboxylic acids (mainly acetic acid) in all treatments. This acid showed a higher concentration when inoculated with *S. cerevisiae* CCMA 0543 (Sc) and *T. delbrueckii* CCMA 0684 (Td) (Supplementary material- Table 2).

The final score is calculated by adding the scores for each attribute, subtracting the defects. The overall score is based on the sensory experience of the individual taster as a personal appraisal. In this case, balance and body principally were more correlated with the overall score. The co-inoculation of *T. delbrueckii* and *C. parapsilosis* with a population of around 10^6 cell/g showed the highest notes of acidity, body, fragrance, balance, and overall. Unlike other studies (da Mota et al., 2020; Bressani et al., 2018; Evangelista et al., 2014), the Sc, Td, and ST treatments had lower sensory scores than the control, although they were classified as specialty coffees. This result may indicate the particularity of the Caparaó region, of the microbiota, and modification of the metabolism of the microorganisms studied. However, the differentiation observed in each treatment's sensory descriptors is essential because it can

serve different consumer niches. Besides increasing the number of descriptors, fermentation can bring differentiated notes, such as fruity, nuts/almond, and citric acid (Figure 4). The effect of terroir, quantity, and different yeasts combination should also be considered when evaluating the fermentation's influence on the coffee quality.

5. Conclusions

The inoculation of yeasts separately or together in the fermentation process impacts the quality of low-altitude coffees. The inoculated coffees with yeast populations around 10^6 cell/g obtained the highest sensory scores. Chemical analyzes such as chromatography and FTIR are essential to determine the formation/loss of metabolites (such acids, volatile compounds, and caffeine) throughout the postharvest process and after roasting. Yeasts inoculation results in different sensory descriptors and differences in the other attributes evaluated. The spice flavor was noticed in all treatments inoculated with *C. parapsilosis* CCMA 0544, and nuts/almond notes are present in treatments inoculated with *S. cerevisiae*. The combination of non-*Saccharomyces* yeasts (*C. parapsilosis* CCMA 0544 and *T. delbrueckii* CCMA 0684) is the most indicated as starter cultures for low altitude coffee (600 m) as it presents the best final score by the Q-graders (score 85). Due to the promising results, fermentation with yeast co-inoculation must be included in the postharvest processing of low-altitude coffees. Besides, further studies should be carried out on coffees of different varieties and altitudes.

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ARTIGO 2 - CHARACTERIZATION OF BIOACTIVE, CHEMICAL, AND SENSORY COMPOUNDS FROM FERMENTED COFFEES WITH DIFFERENT YEASTS SPECIES

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ABSTRACT

Selected yeasts influence the types of compounds produced during coffee fermentation and correlate with the beverage sensory characteristics. This work aimed to evaluate the chemical and sensory modifications of coffee fermented with one yeast (*Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544, or *Torulaspora delbrueckii* CCMA 0684) and in co-inoculation (from two to two and the three together) by dry processing. Real-time PCR analyzes, total phenolic content and antioxidant activity (DPPH, ABTS, and FRAP), liquid and gas chromatography, as well as sensory analysis, were performed. Caparaó coffees showed a higher *C. parapsilosis* (6.14 Log cell.g^{-1}) population followed by *S. cerevisiae* (5.85 Log cell.g^{-1}) and *T. delbrueckii* (4.64 Log cell.g^{-1}). The total phenolic content has a strong and positive correlation with the fermentation time and the roasted beans and a moderate and positive correlation with DPPH, FRAP, and ABTS. Coffee inoculated with *T. delbrueckii* reduced caffeine concentration during the fermentation process. The trigonelline concentration showed the most significant decrease (around 4 mg.g^{-1}) when inoculated with *S. cerevisiae* and *T. delbrueckii* in co-cultivation. Detection of some organic acids and volatile compounds during fermentation may indicate that the starter cultures used different metabolic routes. All co-inoculation treatments presented the best sensory scores (greater than 86 points). In the inoculated fermentation, fruity, citric, molasses, freshness, and wine notes appeared. The co-inoculated treatment with *S. cerevisiae* CCMA 0543, *C. parapsilosis* CCMA 0544, and *T. delbrueckii* CCMA 0684 was the best, considering the sensory notes' diversity descriptors and the final concentration of organic acids.

Keywords: Specialty coffees; co-inoculation; yeasts; anaerobic fermentation; designation of origin Caparaó; dry processing.

1. Introduction

Post-harvest processing is essential to maintain and increase the coffee beverage's quality, influencing the coffee's chemical and sensory composition. Several researchers worldwide have extensively studied natural coffee fermentation (de Bruyn et al., 2017; Elhalis, Cox, & Zhao, 2020; Evangelista, Miguel, Silva, Pinheiro, & Schwan, 2015). Controlled coffee fermentation mainly provides positive sensory experiences, resulting in a differentiated and complex beverage with striking sensory descriptors. However, due to climatic, management, and microbiota differences, fermentation processes can become complicated and have low reproducibility with each harvest (de Bruyn et al., 2017; Elhalis et al., 2020).

The use of selected starter cultures for coffee fermentation is a viable alternative for a more standardized and controlled processing, resulting in higher-quality beverages (Elhalis, Cox, Damian, & Zhao, 2021). It is widely known that the implementation of starter cultures increases the production of volatile compounds and consequently improves the beverage flavor and aroma. Volatile compounds with 6,10-dimethyl-5,9-undecadien-2-one, heptadecanol, 4-hydroxy-2-methyl acetophenone, and 2-phenyl ethanol have been related to the metabolism of some yeast strains (Bressani, Martinez, Sarmento, Borém, & Schwan, 2020; Haile & Kang, 2019; Lee et al., 2017; Martinez, Bressani, Dias, Simão, & Schwan, 2019).

Different starter cultures isolated from coffee - mainly pure yeasts - have been studied for their use in improving quality (Bressani et al., 2020; Bressani, Martinez, Evangelista, Dias, & Schwan, 2018; da Mota et al., 2020; Elhalis et al., 2021; Lee et al., 2017; Martins, Ribeiro, Miguel, Evangelista, & Schwan, 2019; Wang, Sun, Lassabliere, Yu, & Liu, 2020a). Yeasts influence the types of compounds produced during fermentation and after roasting and correlate with the sensory characteristics perceived during the cup tasting (Ruta & Farcasanu, 2021). Various reports highlighted *Saccharomyces cerevisiae* CCMA 0543 and *Torulaspora*

delbrueckii CCMA 0684 as suitable species for coffee fermentation under different conditions (Bressani et al., 2020; Bressani et al., 2018; da Mota et al., 2020; Martins et al., 2019).

Some more recent studies report promising results using yeasts and bacteria during wet coffee fermentation (Vale et al., 2019; Wang, Sun, Lassabliere, Yu, & Liu, 2020b). However, the application of combined starter cultures in dry-processed coffees is limited, mainly for yeast co-inoculation. Moreover, the influence of these microorganisms on the bioactive compounds, antioxidant activity, and other chemical compounds of coffee is narrow (Haile & Kang, 2019; Kwak, Jeong, & Kim, 2018; Rochín-Medina, Ramírez, Rangel-Peraza, & Bustos-Terrones, 2018).

Given the above, the objective of the present work was to evaluate the chemical and sensory modifications of coffee fermented via natural singly inoculated with yeasts (*Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544, or *Torulaspora delbrueckii* CCMA 0684) and in co-inoculation (from two to two and the three together).

2. Material and methods

2.1. Yeast starters and coffee fermentation experiment

The yeasts *Saccharomyces cerevisiae* CCMA 0543, *Candida parapsilosis* CCMA 0544, and *Torulaspora delbrueckii* CCMA 0684 were isolated from coffee. Each microorganism was obtained from the Culture Collection of Agricultural Microbiology – CCMA (Federal University of Lavras, Lavras, MG, Brazil) and reactivated separately in 10 mL of YEPG broth (20 $g.L^{-1}$ glucose, 10 $g.L^{-1}$ yeast extract, and 10 $g.L^{-1}$ soya peptone (HiMedia); pH 3.5) at 28 °C for 24 h, in increasing volumes until reaching a concentration of approximately 10^8 $cells.g^{-1}$. Then cells were centrifuged, resuspended in 100 mL of distilled water.

Coffee cherries from Catuaí Vermelho IAC-44 (480 kg) were harvested at an altitude of 1,200 m in Espera Feliz – MG, Caparaó region, Brazil. The average content of soluble solids in the fruits was 18.9 ºBx. Each fermentation used 20 kg of coffee. Fermentations were performed without (control) and with yeast inoculation, as described in Table 1. The fermentation was carried out in closed polyethylene containers for 72 h in triplicate and at an average temperature was 21.9 ºC (minimum of 13 ºC and a maximum of 30 ºC). The average ambient temperature was 17.0 - 27.6 °C, and the mean relative humidity was 67.65. After fermentation, the cherries were transferred to suspended terraces until obtaining 11.5 – 12.0% moisture. Samples of cherries (100 g) were collected before and after fermentation, initial and final drying, and frozen until chemical analyses.

Table 1. Detailed information of the treatments used in the experiment.

ID	Starter cultures
1Sc	*S. cerevisiae* CCMA 0543
1Cp	*C. parapsilosis* CCMA 0544
1Td	*T. delbrueckii* CCMA 0684
2SC	*S. cerevisiae* CCMA 0543 + *C. parapsilosis* CCMA 0544
2CT	*C. parapsilosis* CCMA 0544 + *T. delbrueckii* CCMA 0684
2ST	*S. cerevisiae* CCMA 0543 + *T. delbrueckii* CCMA 0684
3SCT	*S. cerevisiae* CCMA 0543 + *C. parapsilosis* CCMA 0544 + *T. delbrueckii* CCMA 0684
Control	None (spontaneous fermentation)

ID = identification of treatments.

2.2 Monitoring of the population of yeast starters

The starters yeast population was monitored from fermentation until the end of drying by real-time PCR (qPCR). The calibration curves were constructed by cultivating each strain separately, according to Bressani et al. (2020). DNA from strains and samples (0, 3, 9, and 36 days) was extracted with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the "DNA Purification from Tissues" protocol.

The Rotor-Gene Q System (Qiagen, Hombrechtikon, ZH, Switzerland) was used according to Batista et al. (2015) with specific primers SC-5fw 5′AGGAGTGCGGTTCTTTGTAAAG-3′ and SC-3bw 5′-TGAAATGCGAGATTCCCCT-3′ (*S. cerevisiae*); SADH-F 5′-GCTGCGGCTTCAACTGATGC-3′ and SADH-R 5′-CTTGGTCACGAGCCTCC-3′ (*C. parapsilosis*); Tods L2 5′-CAAAGTCATCCAAGCCAGC-3′ and Tods R2 5′-TTCTCAAACAATCATGTTTGGTAG-3′ (*T. delbrueckii*) (Díaz et al., 2013; Hays et al., 2011; Zott et al., 2010). The equipment parameters were R^2=0.997, slope=−3.812 and efficiency of 0.83 (*S. cerevisiae*), R^2=0.991, slope=−3.628, and efficiency of 0.90 (*C. parapsilosis*), R^2=0.996, slope=−3.517 and efficiency of 0.92 (*T. delbrueckii*).

2.3 Polyphenol and antioxidant content

2.3.1 Preparation of the extract

Coffee cherry and roasted coffee samples were defatted following the methodology described by Batista et al. (2015). 4 g of each sample was extracted three times with 20 mL of n-hexane (Merck, Germany) to eliminate the lipids. Then, the fat-free samples were air-dried for 24 hours to evaporate the residual organic solvent.

The polyphenol and antioxidant were extracted according to Kim et al. (2018) with minor modifications. The proportion used was 2.75 g of coffee ground in 50 mL of distilled

water at 90 °C. The extract was left by standing at room temperature for 20 min, followed by filtration through Whatman No. 2 filter paper.

2.3.2 Determination of total polyphenol content (TPC)

Colorimetric assay by spectrophotometry (UV-VIS Spectrum SP-2000 UV, Biosystems) was used to determine the total phenolic content by the Follin – Ciocalteau methodology with minor modifications (Kim et al., 2018). In brief, 500 μL of coffee extract, 2.5 mL of Folin–Ciocalteau reagent (10%), and 2.0 mL of Na_2CO_3 (4% w/v) were homogenized and incubated at room temperature, in the dark for 120 min. The absorbance of the samples was measured at 750 nm. Through the standard curve of gallic acid (ranging from 10 to 100 μg.mL^{-1}), the TPC concentrations were calculated and expressed as milligrams of gallic acid equivalents per gram of ground coffee (mg GAE.g^{-1}). The analyses were performed in triplicate.

2.3.3 Antioxidant Activity Assays

Three different methodologies measured the antioxidant activity of coffee extracts. For the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, 0.1 mL of coffee extract was added to 3.9 mL of the DPPH radical solution (0.06 mM) and incubated at room temperature in the dark for 120 min. The absorbance was measured at 515 nm. Trolox was used as standard, and results were expressed as μM Trolox Equivalents (TE) per gram of ground coffee (μM TE.g^{-1}). A calibration curve ($y = -0.0004x + 0.6636$), in the range of 10, 20, 30, 40, 50 and 60 μM with linearity $R^2 = 0.9999$ (Batista et al., 2016).

The ABTS (2,2′-Azinobis(3-ethylbenzothiazoline6-sulfonic acid) assay was performed from the ABTS stock solution reaction (7 mM) with potassium persulphate (140 mM) and allowing the mixture to stand in the dark at room temperature for 16 h before use. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. 30 μL of extracts

coffee was added to 3.0 mL of the ABTS radical solution 6 min the absorbance readings were taken. A calibration curve (y = -0.0003x + 0.6802) was developed using a range of 100, 500, 1,000, 1,500 and 2,000 μM and linearity of R^2 = 0.9983. The results were expressed as μM Trolox Equivalents (TE) per gram of ground coffee (μM $TE.g^{-1}$).

The third analysis performed to determine the antioxidant activity of coffee used the ferric reducing ability (FRAP assay) described by Pulido, Bravo, Saura-Calixto (2000) with modifications. Aliquots (90 μL) of coffee extracts were mixed with 2.7 mL of FRAP reagent (10 mM TPTZ prepared in 40 mM HCl, 20 mM $FeCl_3$, and 0.3 M acetate buffer at pH 3.6) and 270 μL distilled water. After 30 min at 37 ºC, the absorbance was measured at 595 nm. The calibration curve (y = 0.0006x – 0.0062) was performed with ferrous sulfate, in the range of 500, 1,000, 1,500 and 2,000 μM with linearization of R^2 = 0.9988. The results were expressed as μM ferrous sulphate (FS) per gram of ground coffee (μM $FS.g^{-1}$). All the analyses were performed in triplicate.

2.4 Chromatographic analysis

2.4.1 Caffeine, Chlorogenic acid, and trigonelline by high-performance liquid chromatography

HPLC was used to determine caffeine, chlorogenic acid [5-CQA], and trigonelline. Treatments were evaluated at 0 and 3 days of fermentation and roasted coffee beans, and the extraction was performed following Bressani et al. (2018). A Shimadzu HPLC system with 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 $mL.min^{-1}$, 30 °C. The identification and quantitative analysis were done using standard curves for each

compound in different concentrations. All chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri).

2.4.2 Organic acids

The HPLC was used to detect organic acids in coffee cherry samples (0, 3, and 36 days). Citric, succinic, malic, oxalic, lactic, acetic, propionic, isovaleric, tartaric, and isobutyric acids were evaluated.

The extraction was carried out according to Bressani et al. (2020), and the extracts were injected into the chromatographic column (Shimpack SCR-101H -7.9 mm x 30 cm) from the HPLC system (Shimadzu Corp., Japan) equipped with a dual detection system consisting of a UV–vis detector (SPD 10Ai). The analysis condition for organic acids was: 50 ºC, ultrapure water, and perchloric acid (pH 2.1) as a mobile phase, at a flow rate of 0.6 $mL.min^{-1}$, and detected by UV absorbance (210 nm).

Calibration curves were constructed with standards at different concentrations [malic and citric acid were purchased from Merck (Germany), lactic was purchased from Acros Organic (Belgium), oxalic, isovaleric, tartaric, acetic, and succinic acids were purchased from Sigma-Aldrich (Germany), butyric acid was purchased from Riedel-de Haen (Germany)].

2.4.3 Gas chromatography-mass spectrometry

GC-MS detected coffee cherry (0, 3, and 36 days) volatile compounds and roasted coffee. Two grams of coffee samples were ground with liquid nitrogen and extracted by headspace solid-phase microextraction (HS-SPME), according to da Mota et al. (2020). The compounds were analyzed using a Shimadzu QP2010 GC model equipped with mass spectrometry (MS) and a silica capillary Carbo-Wax 20M (30 m x 0.25 mm x 0.25 mm) column.

The oven temperature was maintained at 50 ºC for 5 min. The temperature was then raised to 200 ºC in increments of 8 ºC.min^{-1} and then maintained at 200 ºC for 15 min. Injector temperatures were kept at 230 ºC in splitless mode. The carrier gas (He) was maintained at a flow rate of 1.98 mL.min^{-1}. The detected mass spectra were compared with the NIST11 library, and an alkane series (C10–C40) was used to calculate the retention index (RI); the RI values were compared with those found in the literature data. The relative percentage for each compound was calculated from the total volatiles content found in the chromatograms.

2.5 Sensory analysis

The coffee samples were roasted 24 h before sensory analysis in a Probat Leogap model TP-2 roaster, Curitiba, Brazil for a time of 8 - 12 min, inlet, and outlet temperature equal to 175 ºC and 190 ºC, respectively (reference color number 65 for ground beans and 55 for whole beans - SCA/Agtron Roast Color Classification System).

Each sample was prepared with five cups using the predetermined ratio of 8.25 ± 0.25 g per 150 mL of water. Samples were presented randomly, coded with 3 digits numbers, and of monadic way. A panel of five expert coffee tasters, with Q-Grader Coffee Certificate, performed the sensory analysis evaluating the attributes of fragrance/aroma, flavor, acidity, body, balance, aftertaste, uniformity, sweetness, clean cup, the overall and total score of 6.0 to 10.0 (Specialty Coffee Association, 2018), besides sensory descriptors.

2.6 Statistical analysis

Real-time PCR, bioactive compounds, antioxidant activity, organic acids were analyzed by the Scott-Knott test with a 5% significance level. Principal Component Analysis (PCA) was performed to assess volatile chemical groups' concentration. The cup test scores were evaluated

by the Tukey test with a 5% significance level using XLSTAT software. The sensory beverage profiles were analyzed considering each evaluated attribute's relative frequency.

3. Results and discussion

3.1 qPCR

qPCR is a fast and important technique that allows population dynamics monitoring of each yeast studied during coffee fermentation and drying. The control results showed that the coffee fruit microbiota in the Caparaó region contained a high *S. cerevisiae* and *C. parapsilosis* population (Table 2) differentiating it from other producing regions (Bressani et al., 2018; Bressani et al., 2020; da Mota et al., 2020).

S. cerevisiae population in control remained smaller, ranging from 5.85 - 6.65 Log cell.g^{-1} (Table 2). *S. cerevisiae* population showed a significant difference ($p > 0.05$) at the end compared to the beginning of fermentation in all treatments, except for the control. The 1Sc treatment had the largest population (8.01 Log cell.g^{-1}) at the end of fermentation. After six days of drying, the *S. cerevisiae* population increased in all treatments, above 8 Log cell.g^{-1} for the inoculated treatments. At the end of drying, it was detected that the 3SCT treatment had the highest population of this yeast (8.74 Log cell.g^{-1}), and as expected, the control had the lowest population (6.65 Log cell.g^{-1}) (Table 2). These results indicate that *S. cerevisiae* showed excellent adaptation and competition with the endophytic microbiota.

There was no significant difference in the *C. parapsilosis* population ($p < 0.05$) compared to the initial and final time of fermentation of all treatments. The treatments 1Cp and 3SCT showed no significant difference between them, with a smaller population (5.86 and 5.75 Log cell.g^{-1}, respectively). After six days of drying, only the 1Cp treatment showed a significant increase. However, the largest population detected at the end of drying was for the 2CT

treatment (6.30 Log cell.g^{-1}) (Table 2). The *C. parapsilosis* seems to be sensitive to variations in coffee fermentation and co-inoculation. Despite this, singly and co-inoculation of *C. parapsilosis* resulted in positive and differentiated sensory characteristics.

Table 2. Yeast populations (Log cell.g^{-1}) in coffee fermentation measured by qPCR.

	Fermentation time (days)		Drying time (days)			
	0	3	6	36	SEM*	P value
Primer *S. cerevisiae*						<0.001
1Sc	7.82^{cA}	8.01^{dB}	8.42^{cC}	8.38^{bC}	0.027	
2SC	8.02^{dB}	7.72^{bA}	8.61^{dD}	8.33^{bC}	0.027	
2ST	7.35^{bA}	7.69^{bB}	8.28^{bC}	8.44^{cD}	0.027	
3SCT	8.12^{eB}	7.90^{cA}	8.33^{bC}	8.74^{dD}	0.027	
control	5.85^{aA}	5.86^{aA}	6.50^{aC}	6.65^{aD}	0.027	
Primer *C. parapsilosis*						<0.001
1Cd	5.87^{aB}	5.86^{aB}	6.21^{cC}	5.67^{aA}	0.054	
2SC	6.27^{cB}	6.19^{bB}	5.76^{bA}	5.72^{aA}	0.054	
2CT	6.07^{bB}	6.04^{bB}	5.20^{aA}	6.30^{cC}	0.054	
3SCT	5.87^{aA}	5.75^{aA}	5.87^{bA}	5.88^{bA}	0.054	
control	6.14^{bB}	6.10^{bB}	5.86^{bA}	6.15^{cB}	0.054	
Primer *T. delbrueckii*						<0.001
1Td	4.60^{bA}	5.90^{bB}	6.60^{dC}	6.60^{bC}	0.053	
2ST	4.32^{aA}	4.91^{aB}	6.16^{cC}	6.40^{aD}	0.053	
2CT	4.98^{cA}	4.94^{aA}	5.68^{bB}	6.39^{aC}	0.053	
3SCT	4.70^{bA}	4.89^{aB}	5.41^{aC}	6.30^{aD}	0.053	
control	4.64^{bA}	6.55^{cB}	7.34^{eD}	7.00^{cC}	0.053	

Data are presented as mean. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. Mean values with different lowercase letters are significant at $p < 0.05$ by Scott-Knott test in the column. Mean values with different capital letters are significant at $p < 0.05$ by Scott-Knott test in the line. *Standard error of the means. *P* value = Treatment, Time, and Treatment x Time.

T. delbrueckii population at the beginning of the fermentation was around 4.64 Log cell.g^{-1} for all treatments. All treatments increased the *T. delbrueckii* population at the end of the fermentation, except for the 2CT treatment. *T. delbrueckii* developed best during coffee drying, increasing population in all treatments on the sixth day of drying. At that time, the

control still has a larger population of *T. delbrueckii* to other treatments (7.34 Log cell.g^{-1}). Nevertheless, at the end of drying, the *T. delbrueckii* population decreased in control compared to the sixth day of drying. The treatments 2ST, 2CT, and 3SCT showed no significant difference between them at the end of drying (6.40, 6.39, 6.30 Log cell.g^{-1}, respectively) (Table 2).

3.2 Total Polyphenol Content and antioxidant properties

Coffee is a source of bioactive compounds, including polyphenols, caffeine, chlorogenic acid, among others, which are beneficial to consumers' health (Pérez-Burillo et al., 2019).

As summarized in Table 3, it is possible to verify that there was a variation in TPC between treatments before fermentation (167.56 - 297.40 mg GAE.g^{-1}) at the end of fermentation (154.67 - 294.73 mg GAE.g^{-1}) and after roasting (1,858.85 - 2,503.55 mg GAE.g^{-1}). Only the 1Sc treatment showed an increase in TPC content after fermentation. The enzymatic activity of *S. cerevisiae* CCMA 0543 (Silva et al., 2013) can help release some phenolic compounds adhered to the coffee fiber, causing an increase in TPC. The same behavior was observed by Rochín-Medina et al. (2018) through inoculation of *Bacillus clausii* in coffee fermentation and Kwak et al. (2018) when they used different *S. cerevisiae* strains for coffee fermentation.

The treatments 1Td, 2SC, 2ST, and 3SCT, showed no statistical difference between the evaluated times. The treatments 1Cp, 2CT, and control showed a decrease in TPC content. The polyphenol oxidase enzyme is responsible for catalyzing the oxidation of polyphenols to condensed polyphenols of high molecular weight, which may be one reason for the loss of TPC (Álvarez, Álvarez, García, & Salazar, 2017), reducing of TPC in these treatments. Also, at the end of the fermentation, it is possible to observe that the 3SCT treatment had a higher TPC content (294.73 mg GAE.g^{-1}) with a significant difference ($p > 0.05$) when compared to the

other treatments. However, yeasts' quantity is not correlated with TPC and antioxidant activity (Supplementary material – Table 1).

The TPC content strongly correlates with the fermentation time (0 and 72 h) and the roasted beans (Supplementary material - Table 1). All roasted treatments showed an increase of at least 8.5 times greater when compared to the respective treatment at the end of fermentation. The smallest increase in TPC was observed for control, and the highest was observed in treatment 2ST (as a 13-fold increase) (Table 3). The Spearman correlation matrix showed a moderate and positive TPC correlation with DPPH, FRAP, and ABTS, respectively.

There is no standardized method for performing these analyses, so it is recommended at least two methods to provide comprehensive information about the total antioxidant capacity of food, considering the different antioxidants' mechanisms (Batista et al., 2016; Dudonné et al., 2009).

Correlations among the results obtained for antioxidant activity (DPPH, ABTS, and FRAP) were highly positive, indicating that all assays were similar. Besides, there was a high correlation of these analyzes with the fermentation time and the roasted beans ($p > 0.05$) (Supplementary material - Table 1). There was a variation of DPPH between treatments before fermentation (49.67 - 91.60 μM Trolox.g^{-1}) at the end of fermentation (50.33 - 113.95 μM Trolox.g^{-1}) and in the roasted beans (2,200.57 - 4,809.93 μM Trolox.g^{-1}) (Table 3).

Table 3. The total phenolic compounds (TPC) and antioxidant capacity evaluated using the DPPH, ABTS, and FRAP methods, of green coffee (before and after fermentation) and roasted coffee.

Treatments	Before fermentation	Final fermentation	Roasted
TPC (mg GAE.g^{-1})			
1Sc	174.52±0.17^{aA}	228.05±0.02^{bB}	2,157.19±0.07^{cC}
1Cd	290.53±0.21^{bB}	235.87±0.29^{bA}	2,503.55±0.07^{dC}
1Td	189.47±0.19^{aA}	201.46±0.09^{aA}	1,906.01±0.05^{cB}
2SC	193.21±0.27^{aA}	185.24±0.09^{aA}	1,858.85±0.01^{aB}
2CT	264.76±0.50^{bB}	154.67±0.11^{aA}	1,940.90±0.09^{aC}
2ST	167.56±0.42^{aA}	178.23±0.29^{aA}	2,344.28±0.11^{dB}
3SCT	274.13±0.10^{bA}	294.73±0.06^{cA}	2,084.88±0.06^{bB}
control	297.40±0.06^{bB}	220.27±0.60^{bA}	1,896.78±0.07^{aC}
DPPH (μM trolox.g^{-1})			
1Sc	52.02±0.01^{cA}	82.44±0.16^{fB}	2,812.04±0.14^{cC}
1Cd	49.67±0.14^{aA}	55.34±0.12^{bB}	2,845.53±0.11^{dC}
1Td	49.67±0.11^{aA}	50.33±0.55^{aB}	3,427.03±0.64^{fC}
2SC	53.47±0.08^{dA}	107.77±0.58^{gB}	2,200.57±0.08^{aC}
2CT	49.69±0.01^{bA}	55.38±0.37^{cB}	2,688.55±0.01^{bC}
2ST	49.71±0.15^{bA}	113.95±0.64^{hB}	4,709.35±0.59^{gC}
3SCT	91.60±0.11^{fB}	80.83±0.19^{eA}	4,809.93±0.55^{hC}
control	75.38±0.20^{eB}	55.61±0.14^{dA}	3,126.99±0.05^{eC}
ABTS (μM trolox.g^{-1})			
1Sc	280.58±0.28^{aA}	516.44±0.08^{aB}	5,455.21±0.01^{dC}
1Cd	204.80±0.05^{aA}	274.92±0.31^{aA}	3,566.05±0.01^{aB}
1Td	148.82±0.03^{aA}	306.86±0.24^{aA}	5,835.66±0.01^{eB}
2SC	353.94±0.05^{aA}	400.87±0.02^{aA}	3,565.01±0.03^{aB}
2CT	166.16±0.42^{aA}	366.91±0.35^{aA}	3,438.93±0.01^{aB}
2ST	231.55±0.85^{aA}	371.95±0.13^{aA}	5,388.33±0.00^{dB}
3SCT	316.05±0.01^{aA}	232.92±0.04^{aA}	4,204.69±0.01^{bB}
control	217.84±0.19^{aA}	144.69±0.01^{aA}	4,434.86±0.01^{cB}
FRAP (μM FS.g^{-1})			
1Sc	198.26±0.16^{aA}	263.66±0.66^{aA}	5,654.12±0.00^{cB}
1Cd	106.00±0.22^{aA}	125.05±0.02^{aA}	3,072.33±0.00^{aB}
1Td	146.37±0.08^{aA}	183.67±0.02^{aA}	6,836.68±0.01^{dB}
2SC	232.54±0.04^{aA}	214.88±0.08^{aA}	4,227.94±0.01^{bB}
2CT	161.38±0.05^{aA}	181.75±0.09^{aA}	6,714.72±0.00^{dB}
2ST	180.36±0.05^{aA}	233.72±0.20^{aA}	5,555.08±0.00^{cB}
3SCT	203.02±0.09^{aA}	161.55±0.60^{aA}	5,781.55±0.01^{dB}
control	175.14±0.25^{aA}	158.03±0.03^{aA}	5,572.19±0.01^{cB}

Data are presented as mean. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. Mean values with different lowercase letters are significant at $p < 0.05$ by Scott-Knott test in the column. Mean values with different capital letters are significant at $p < 0.05$ by Scott-Knott test in the line.

DPPH assay showed minor activity in all treatments before and at the end of fermentation. This result may be related to the mechanism of action since DPPH is more suitable for samples with lipophilic antioxidants or those characterized by a high lipid content (Jeszka-Skowron, Stanisz, & de Peña, 2016). After roasting, all treatments showed increased antioxidant activity, regardless of the analysis (DPPH, ABTS, and FRAP).

ABTS also evaluates the antioxidant capacity in food based on the scavenging of stable free radicals by antioxidants. However, the ABTS assay is related to lipophilic and hydrophilic antioxidants (Jeszka-Skowron et al., 2016). Only 1Sc treatment presented an increase at the end of fermentation with a significant difference ($p > 0.05$) compared to the same treatment before fermentation. Rochín-Medina et al. (2018) also observed an increase in antioxidant activity after fermentation and suggested that this increase was due to the enzymatic activity of *Bacillus sp.*, able to break down and release phenolic compounds linked to the coffee fiber. As in the DPPH assay, only the 3SCT and control treatments decreased the ABTS value (316.05 - 232.92, 217.84 - 144.69 μM Trolox.g^{-1}, respectively). It was possible to detect a significant difference between treatments in the roasted beans, ranging from 3,438.93 – 5,835.66 μM Trolox.g^{-1} (Table 3). The differences observed may be related to phenols' solubility (which are hydrophilic) (Jeszka-Skowron et al., 2016; Rochín-Medina et al., 2018).

The ferric ion reducing antioxidant power (FRAP) assay measures a sample's ability to participate in electron redox reactions (Liang & Kitts, 2014). It was not possible to observe a significant difference between the FRAP values before (106.00 – 232.54 μM FS.g^{-1}) and at the end of the fermentation (125.05 – 263.66 μM FS.g^{-1}). However, for roasted beans, the FRAP analysis showed greater antioxidant power than DPPH and ABTS (Table 3). The increase in antioxidant activity after roasting may be related to the Maillard reaction, which contributes to the formation of reducing substances (for example, melanoidins) whose reducing power is

responsible for its free radical scavenging activity, increasing the antioxidant effect of the beans (Batista et al., 2016).

3.3 Caffeine, chlorogenic acids, and trigonelline

These bioactive compounds are responsible for the bitterness and astringency of the final coffee beverage (de Bruyn et al., 2017). The caffeine content can be influenced by the species, variety, and origin of the coffee (Rodriguez, Guzman, & Hernandez, 2020).

Caffeine levels in green beans before fermentation ranged from 5.2 - 9.5 $mg.g^{-1}$. Treatments that showed values close to 5 $mg.g^{-1}$ (1Sc, 1Cp, and control) before fermentation showed a significant increase in caffeine after fermentation (6.80, 6.64, and 7.08 $mg.g^{-1}$, respectively) (Figure 1a). In contrast, the other treatments showed a significant decrease after fermentation, probably due to its degradation. The caffeine levels ranged from 3.84 - 7.52 $mg.g^{-1}$, with the highest range for the 3SCT treatment (from 9.01-3.84 $mg.g^{-1}$). According to Oktavianawati, Arimurti, & Suharjono (2020), filamentous fungi or bacteria can degrade caffeine in xanthine or dimethylxanthine during fermentation. However, there are no reports on this mechanism in yeasts.

After roasting, treatments 1Sc, 1Cp, 2CT, and 2ST showed no significant difference in caffeine content compared to the same treatments at the end of fermentation (Figure 1a). The caffeine content is not significantly altered during coffee roasting due to its thermal stability, but slight losses can occur due to sublimation. In terms of percentage composition, an increase in caffeine content can be observed due to the loss of thermolabile compounds (Lima & Farah, 2019).

Chlorogenic acids (CGAs) are generated during the esterification of quinic acid, resulting in some cinnamic acid derivatives (such as caffeic acid, ferulic and p-coumaric acid),

where 5-O-caffeoylquinic acid is the most abundant compound (Jeszka-Skowron et al., 2016). CGAs levels before fermentation ranged from 7.43 - 26.75 $mg.g^{-1}$. The treatments 1Cp, 1Td, 2ST, and 3SCT showed significantly high contents at the end of fermentation. The highest increased was in the 3SCT treatment (14.65 - 26.07 $mg.g^{-1}$). The other treatments showed a decrease in this content after fermentation. Treatments with more than 15.00 $mg.g^{-1}$ before fermentation decreased after fermentation (Figure 1b). Microorganisms can decarboxylate CGAs and produce various alkyl and vinyl phenols (Matei, Seung-Hun, & Kuhnert, 2019).

During roasting, CGAs are degraded or incorporated into new bioactive compounds, mainly by the Maillard reaction (Liu & Kitts, 2011). These new compounds will be responsible for the beverage characteristics such as color, aroma, and flavor (phenol compounds) (Kim et al., 2018; Vignoli, Viegas, Bassoli, & Benassi, 2014). Kamiyama, Moon, Jang, & Shubamoto (2015) correlated the decrease in chlorogenic acids with the potentiation of antioxidant activity. The roasting degree and speed also influence the content of CGAs (Rodriguez et al., 2020). As expected, chlorogenic acid levels decreased significantly in all treatments after roasting (Figure 1b).

The trigonelline data were strongly correlated with caffeine (Supplementary material - Table 1). Trigonelline levels ranged from 8.22 - 13.38 $mg.g^{-1}$ in green coffee before fermentation. After fermentation, all treatments showed a decrease in this compound, except for the 1Sc treatment (Figure 1c). Although some treatments did not present a significant difference, all treatments decreased the trigonelline content after roasting due to its thermal instability.

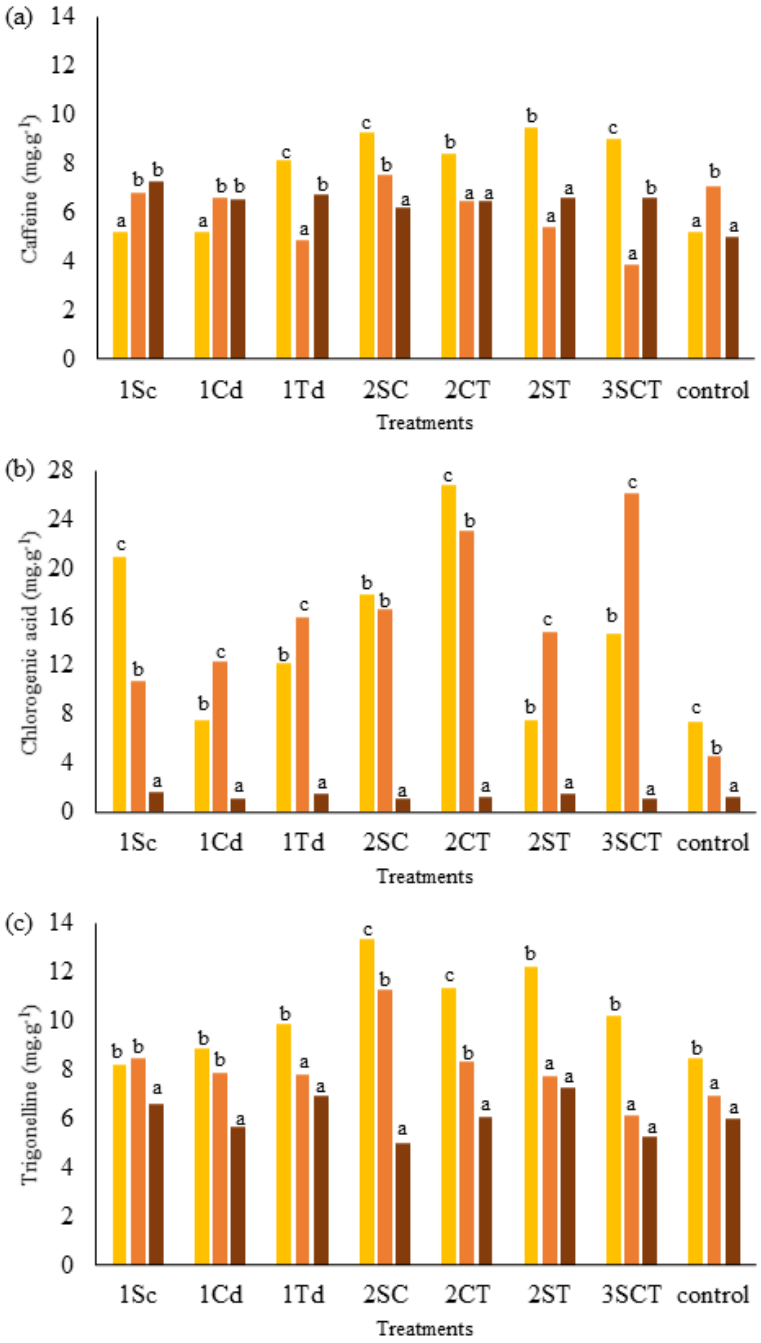

Figure 1. Caffeine, chlorogenic acid, and trigonelline present in inoculated coffee fermentation and control by HPLC. Mean values of caffeine (a), chlorogenic acids (b), and trigonelline (c) before fermentation (■), final fermentation (■), and roasted (■). Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. Different lowercase letters are significant at $p < 0.05$ by Scott-Knott test in each treatment (before and at the end of fermentation) and after roasting.

According to Nogueira & Trugo (2003), trigonelline degradation is more related to the roasting temperature than the processing time. As the roasting of all treatments was standardized and ended at around 210 to 217 ºC, the trigonelline content showed slight variation between treatments (5.02 - 7.25 $mg.g^{-1}$) (Figure 1c). Trigonelline degradation during roasting relates to compounds (furans, pyrazine, alkyl-pyridines, and pyrroles) that provide the beverage's flavor and aroma (Kim et al., 2018; Perrone, Don Angelo, & Farah, 2008). Furthermore, trigonelline is the only one that can form an important compound for human metabolism (Vignoli et al., 2014). Niacin is a vitamin responsible for participating effectively in energy metabolism and DNA repair so, it is relevant (Nogueira & Trugo, 2003).

3.4 Organic acids

Acidity is an important attribute evaluated in the beverage and contributes to flavor in different ways and intensities (Farah & de Lima, 2019). Citric, succinic, and malic acids were found in all treatments, from before fermentation to the end of drying (Figure 2).

Citric acid contributes to acidity, fruity flavor, fresh tartness, and the perceived brightness in taste (Vandenberghe et al., 2018). The concentration of citric acid in the coffee before fermenting ranged from 2.78 - 4.65 $mg.g^{-1}$. After fermentation in a closed bioreactor, only 1Sc, 1Td, and control treatments showed a significant decrease ($p < 0.05$) (Figure 2a) in the concentration of citric acid, indicating this acid's consumption under anaerobic conditions. The consumption of citric and malic acid may indicate a change in microbial metabolism by using other metabolic pathways, as in vegetable and fruit fermentations (Zhang et al., 2019). Bressani et al. (2020) and Zhang et al. (2019) also detected a decrease in citric acid at the end of dry and semi-dry coffee fermentation.

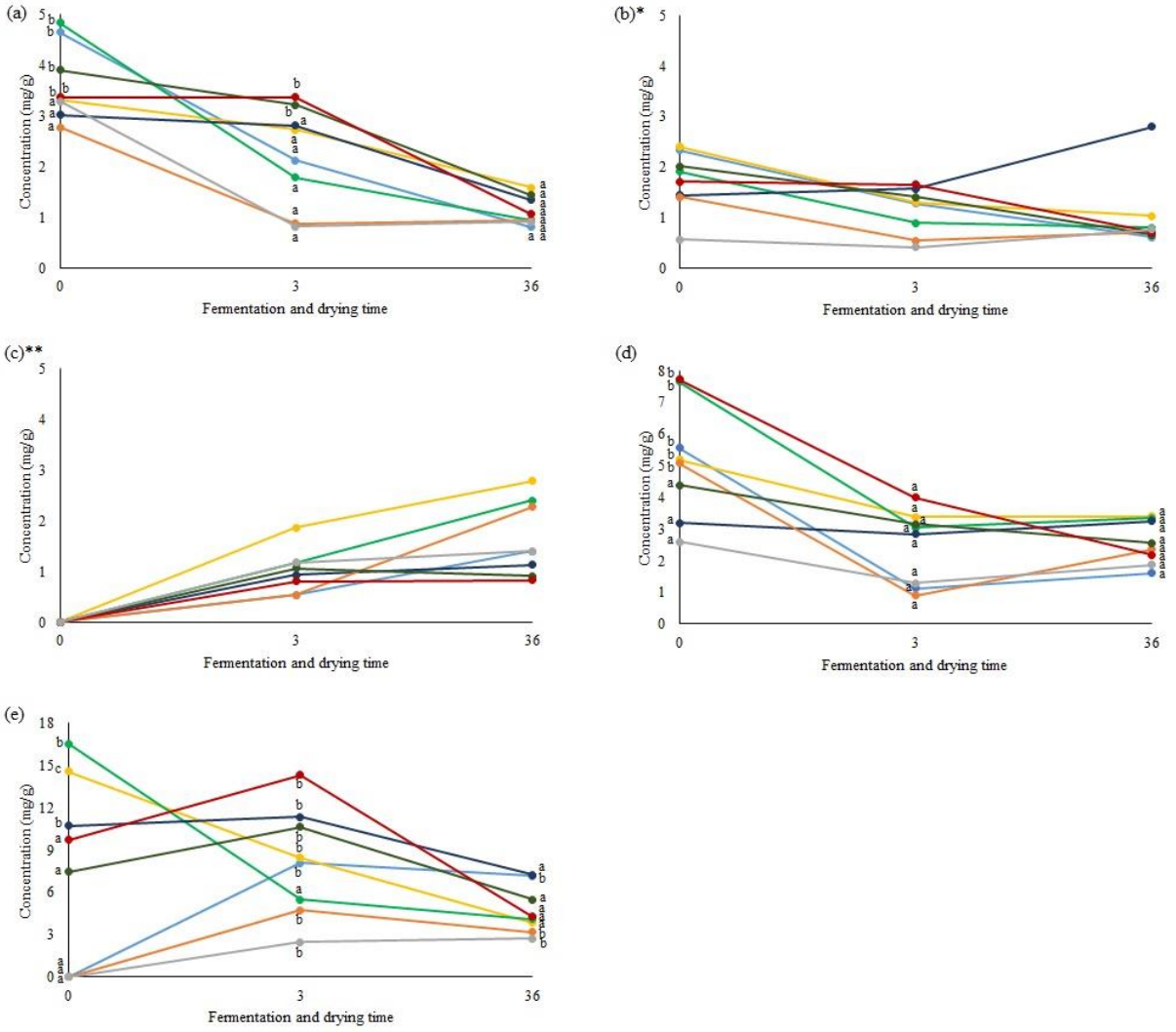

Figure 2. Organic acids detected in inoculated coffee fermentation and control by HPLC. Data are presented as mean. Mean values with different lowercase letters are significant at ($p < 0.05$) by the Scott- Knott test in the line. *The data showed no significant difference at ($p < 0.05$) by the Scott- Knott test in any treatment. ** The data showed no significant difference at ($p < 0.05$) by the Scott- Knott test in 3 and 36 days. Acids: (a) Citric; (b) Succinic; (c) Lactic; (d) Malic; (e) Acetic. ● 1Sc (*S. cerevisiae*); ● 1Cp (*C. parapsilosis)*; ● 1Td (*T. delbrueckii)*; ● 2SC (*S. cerevisiae* + *C. parapsilosis*); ● 2CT (*C. parapsilosis* + *T. delbrueckii*); ● 2ST (*S. cerevisiae* + *T. delbrueckii*); ● 3SCT (*S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*); ● Control (without inoculation).

Succinic acid showed concentrations between 0.58 - 2.40 $mg.g^{-1}$ before fermentation. Despite the increase observed at the end of drying for treatment 2SC, no significant difference was observed in any treatment during fermentation/drying (Figure 2b). The succinic acid

production by yeast is done mainly by the glyoxylate cycle and the citric acid reduction cycle (Jayaram et al., 2014). These results suggested that only 2SC treatment followed this path.

Lactic acid was detected only at the end of fermentation and drying. However, there was no significant difference between these times in any treatments (Figure 2c). The detection of lactic acid suggests that lactic acid bacteria may have developed during fermentation until the end of drying (Ramírez & Velázquez, 2018) (Table 2). The lactic acid can give buttery notes (Farah & de Lima, 2019), which was not noticed in any treatment.

Malic acid has a mild flavor, less acid than citric acid, and more persistent, with fruity notes (mainly green apple) (Farah & de Lima, 2019). Malic acid concentrations varied widely before fermentation (2.61 - 7.65 $mg.g^{-1}$). Only treatments 2SC, 2ST, and control showed no significant difference compared to the end of fermentation. The minor decrease observed was for the 1Cp treatment (5.18 - 3.38 $mg.g^{-1}$) (Figure 2d). Besides, yeasts also can consume malic acid by converting it into ethanol through malo-ethanolic deacidification (Vilela, 2017).

In moderate concentrations, acetic acid also contributes to fruity, wine, fermented aromas (Seninde & Chambers IV, 2020). Acetic acid presented the highest variation in its content before fermentation (7.45 -16.55 $mg.g^{-1}$), and for treatments 1Sc, 2CT, and control, this acid was not detected in this step. After the fermentation, treatments 1Sc, 2CT, 2ST, 3SCT, and control showed an increase with a significant difference ($p < 0.05$). At the end of drying, all treatments in co-inoculation with *S. cerevisiae* CCMA 0543 (2SC, 3SCT, and 2ST) and 1Cp showed decreased acetic acid content significant difference (Figure 2e).

The desirable acidity (as citric and malic acids) contributes to the perception of sweetness, the freshness of the flavor, and even the fruity taste (Farah & de Lima, 2019). However, the concentration of the principal acids does not seem to be directly correlated with this attribute's perception. The highest scores given by the Q-Graders for acid were for the 3SCT, 2SC, and 2CT treatments (which also had the highest final scores in the cup test).

However, while the 3SCT and 2CT treatments had acid content near the end of drying, the 2SC treatment had higher malic, succinic, and lactic acid levels.

Tartaric acid was detected at the end of fermentation in all treatments (0.02 - 0.93 $mg.g^{-1}$), with a higher concentration for the 1Sc treatment and lower control. However, only the 1SC and 1Cp treatments showed this acid at the end of the drying (0.03 $mg.g^{-1}$) (data not shown).

3.5 GC-MS Analysis

The aroma of coffee is one of the most complex sensory profiles. It is formed by many volatile compounds (Ruta & Farcasanu, 2021) with different aroma qualities, intensities, and concentrations. The yeasts were chosen because they present pectinolytic activity, fermentative capacity, production of desirable volatile compounds, and high sensory notes in fermented coffees from different regions (Bressani et al., 2018; da Mota et al., 2020; Martinez et al., 2019; Silva et al., 2013).

Volatile compounds detected in coffee were grouped in the main chemical classes. The main chemical groups found in green coffee were alcohols, aldehydes, carboxylic acids, ketones, and esters. Furans, ketones, carboxylic acids, and others were found in greater abundance in roasted coffees (Supplementary material - Table 2).

The data in Figure 3 clearly show the influence of different chemical classes after fermentation, drying, and roasting. Through different metabolic routes, the selected starter cultures contribute to the biotransformation of the flavor and aroma of green coffee and consequently modify the production of volatile compounds during roasting (Martinez et al., 2019; Wang et al., 2020a; Zhang et al., 2019).

Chemical groups in the negative quadrant of main component 1 (PC1) differentiate green beans from roasted beans (positive quadrant of PC1). Also, for main component 2 (PC2),

the negative quadrant may differentiate treatments at the end of drying from treatments before inoculation and most treatments at the end of fermentation (except for treatments 1Td FF, 3SCT FF, and control FF) (Figure 3).

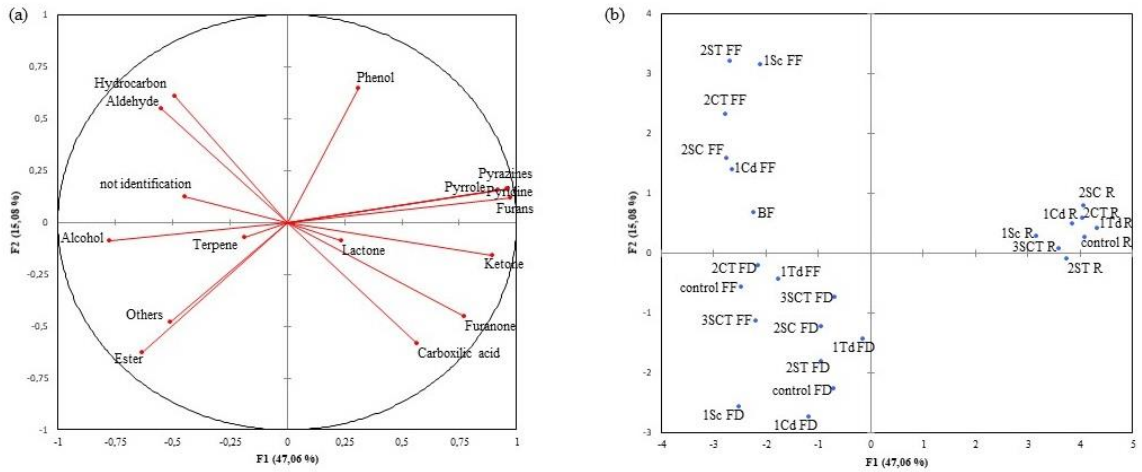

Figure 3. Loading plot (a) and score plot (b) of the principal components analysis (PCA) performed on the analytical data of the percentage of the classes of compounds from the volatile compounds detected in fermented and control coffees. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. BF: Before fermentation; FF: Final fermentation; FD: Final drying; R: Roasted.

The 3SCT treatment showed the highest increase in esters compared to coffee before fermenting (Supplementary material - Table 2). The production of esters can be through the use of lipid and acetyl-CoA metabolism by yeasts or by the chemical esterification of alcohols (Dzialo et al., 2017). The treatment 3SCT also showed a higher decrease of aldehydes after fermentation. In particular, the 3-methyl-1-butanol was found only in the treatments 1Sc, 1Cp, and 3SCT with much higher concentrations for the last treatment (20.92%) (Supplementary material - Table 2). The 3-methyl-1-butanol is derived from leucine and valine and has been described as a yeast metabolite during coffee fermentation and has sensory descriptors of banana and pear (Elhalis et al., 2020; Wang et al., 2020a).

The percentage of esters increases in all treatments, except for the 3SCT treatment at the end of drying. During the drying, ketones were produced mainly by 1Cp, 1Td, and 3SCT (11.07%, 23.52%, and 24.48%, respectively). The highest decrease of aldehydes was found for treatments inoculated with one yeast (1Sc, 1Cp, and 1Td) (Supplementary material - Table 2).

Pyrrole, pyridines, and pyrazines were detected only in roasted coffees. Alcohols, aldehydes, hydrocarbons, and esters showed a significant decrease after roasting (Supplementary material - Table 2).

Although aroma and flavor are formed mainly by the Maillard reaction, other chemical reactions (such as Strecker degradation, sulfur amino acid degradation, acids, sugars, and fats) are also associated with the compound formation during roasting. The caramelization of sugars or the reaction between sugar and amino acids during roasting can form furans (Seninde & Chambers IV, 2020). The production of furfural by the 3-deoxiosone from the Maillard reaction can be converted into other furfural and furan derivatives (Wang et al., 2020b). Furans are assigned to assign sweet, caramel, and burnt aromas to coffee beverages (Seninde & Chambers IV, 2020). Therefore, the compounds formed in green coffee during fermentation can participate in these reactions and present different sensory profiles. Although the treatments showed a higher difference of volatile compounds at the end of fermentation and drying, the yeast strains and their combinations impacted the sensory perception of the beverage.

3.6 Cupping

A sensory analysis performed by the SCA methodology is the most used to evaluate specialty coffees (Gutiérrez-Guzmán et al., 2018). Five certified tasters participated in the analysis to obtain a more reliable result. All treatments received a score of 10 in uniformity, clean cup, and sweetness. The scores for the other attributes are shown in Figure 4.

Although no significant difference between treatments, it is possible to observe that the treatments 3SCT, 2CT, and 2SC presented the highest scores for all evaluated attributes. The 3SCT treatment had the highest scores for the attributes of fragrance/aroma, body, balance, and overall. In contrast, the 1Td, 1Sc, and control treatments had the lowest scores in almost all attributes, being that 1Td treatment had the lowest scores for the flavor, aftertaste, acidity, body, balance, and overall attributes) (Figure 4).

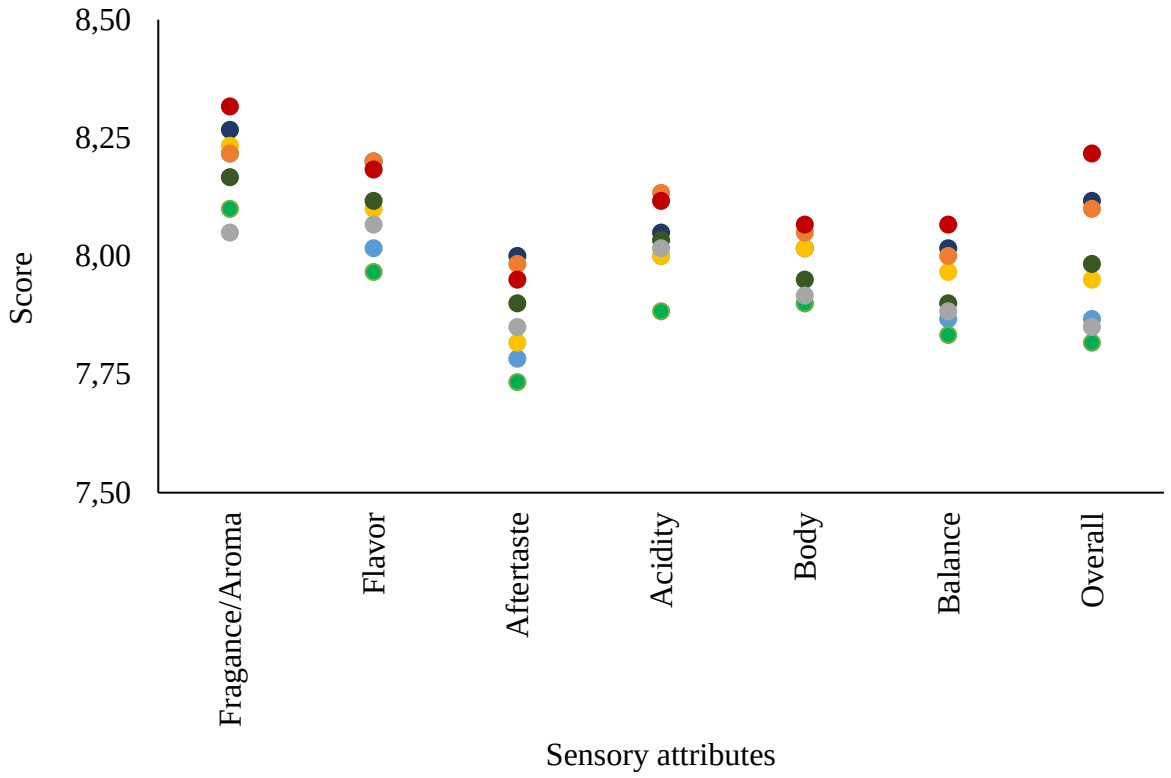

Figure 4. Score of sensory attributes according to SCA methodology. Scores showed no significant difference ($p < 0.05$) by the Tukey test. ● 1Sc (*S. cerevisiae*); ● 1Cp (*C. parapsilosis*); ● 1Td (*T. delbrueckii*); ● 2SC (*S. cerevisiae* + *C. parapsilosis*); ● 2CT (*C. parapsilosis* + *T. delbrueckii*); ● 2ST (*S. cerevisiae* + *T. delbrueckii*); ● 3SCT (*S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*); ● Control (without inoculation).

All treatments obtained a final score greater than 85 points, classifying them as excellent specialty coffees (SCA, 2018). The treatments with co-inoculation had the highest scores (Figure 5). The final score and sensory characteristics are becoming increasingly important in

the purchase decision of connoisseurs of specialty coffees due to changes in consumer behavior about specialty coffees.

The fermentative process modulates, differentiates, and increases the sensory descriptors of coffee. In Figure 5, the descriptors of chocolate and caramel are typical in all treatments. Honey, molasses, fruity, red fruits, wine, citric, and freshness were noticed in inoculated treatments, and milk was noticed in control.

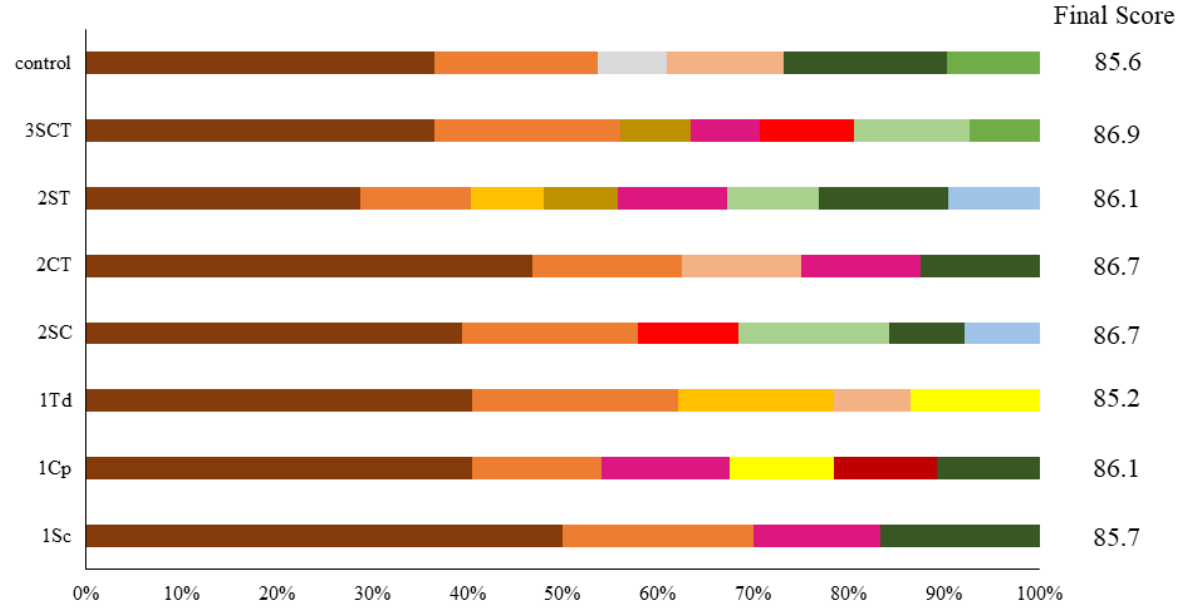

Figure 5. Sensory descriptors and final score of fermented coffees with and without yeast. The final score showed no significant difference ($p < 0.05$) by the Tukey test. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. ■ Chocolate; ■ Caramel; ■ Milk; ■ Honey; ■ Molasses; ■ Yellow fruit; ■ Fruity; ■ Mild; ■ Red fruit; ■ Winey; ■ Spices; ■ Mint; ■ Citric; ■ Freshness.

All treatments showed fruity notes (yellow or red fruits), except the control. The citric descriptor is present only in treatments in which *S. cerevisiae* was combined with other yeasts (2SC, 2ST, and 3SCT). The spice descriptor was not noticed in the treatments 1Td and 3SCT. Only the 1Cp treatment showed a wine descriptor. The inoculation with *T. delbrueckii* may be

related to sweetness since treatments 1Td, 2ST, and 3SCT showed descriptors of honey and molasses. Besides presenting attributes with higher notes, treatments with co-inoculation presented greater complexity of the coffee sensory profile. Different descriptors were perceived, such as red fruity (2SC and 3SCT), citric (2SC, 2ST, and 3SCT), molasses (2ST and 3SCT), and freshness (2SC and 2ST) (Figure 5).

4. Conclusions

All yeasts used as starter cultures showed potential for coffee fermentation. All treatments were classified as excellent specialty coffees. In the inoculated fermentation, fruity, citric, and wine notes appeared. Differences were found in caffeine, chlorogenic acids, and trigonelline in green and roasted coffees. However, they do not seem to be related to yeast inoculation. The total phenolic and antioxidant levels by different methods are correlated and increase after roasting. All co-inoculated treatments (3SCT, 2SC, 2CT, and 2ST) and the 1Cp treatment had higher sensory scores than the control, with different descriptors. However, the co-inoculated treatment with *S. cerevisiae* CCMA 0543, *C. parapsilosis* CCMA 0544, and *T. delbrueckii* CCMA 0684 was the best, considering sensory notes, diversity of descriptors, the final concentration of organic acids.

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ARTIGO 3 - INTO THE MINDS OF COFFEE CONSUMERS: PERCEPTION, PREFERENCE, AND IMPACT OF INFORMATION IN THE SENSORY ANALYSIS OF SPECIALTY COFFEE

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Abstract

This study aimed to analyze consumers' knowledge, perspectives, and preferences about specialty coffees and investigate how information can influence the perception of taste and the sensory characteristics of consumers. A descriptive-analytic survey was conducted through a questionnaire in a digital format with 1005 respondents. Four trained Q-Grader tasters evaluated a sample of cherry coffee fermented. The Specialty Coffee Association developed the cupping protocol used. According to the described descriptors, a sensory analysis was performed with the same coffee with 101 consumers to evaluate the influence of information received before the analysis. The chocolate flavor is the most expected in coffee. However, the participants are willing to try different specialty coffees. The coffee has been considered excellent (85.15 points) by Q-Grader tasters and is widely accepted by consumers. Check-all-that-Apply (CATA) test showed that consumers could be influenced by information. When the coffee has been presented without information, the consumers noticed a more caramel flavor. However, when the information has been added to the same coffee, the citrus flavor was more noticeable. In conclusion, information on specialty coffees should be more widespread. Consumer's expectations can be influenced by information, which in turn can modify their sensory perception.

Keywords: coffee consumers; the influence of information; specialty coffee; CATA; fermented coffee.

Practical Application: Consumers’ knowledge about specialty coffees helps the market understand and serve them assertively.

1 Introduction

Coffee is one of the most popular beverages worldwide, and changes in production, processing, trading, appreciation, and consumers' culture are noticeable (Guimarães et al., 2019). Specialty and high-quality coffees are gaining space in the market, meeting consumers' demands (Giacalone et al., 2019; Ufer et al., 2019). The worldwide consumption of specialty coffee is growing (with an increase of 1.5%), and this growth in the market is affected by new products, research, and specialized coffee shops (Guimarães et al., 2019). For these reasons, it is necessary to evaluate consumers' behavior and desires (Wang & Yu, 2016), detecting many markets that can be explored with greater accuracy and quality.

Market surveys are essential for understanding consumer intentions. The use of the internet (Twitter, e-mail, among others) in the application of questionnaires using different qualitative sensory methods (such as completion task) has become very important to discover the motivations, perceptions, and attitudes of consumers about a product (Sass et al., 2020; Torres et al., 2020). However, there is not enough information about the specialty coffee market, especially about consumers' characteristics and buying behavior (Guimarães et al., 2019).

Several studies have shown that extrinsic factors, such as packaging, brand, information, emotion, and atmospheric, influence food products' sensory perception (Spence, 2015; Li et al., 2019; Samoggia & Riedel, 2019; Spence & Carvalho, 2020). Acceptance testing (e.g., hedonic scale) and descriptive analysis are commonly used in sensory analysis (Wang & Yu, 2016). Although the DQA method and Focus Group are techniques that generate robust data, they are considered laborious, expensive, and demand highly trained tasters. Therefore, innovative methods using untrained tasters [such as Preferred Attribute Elicitation (PAE), Temporal Dominance Sensation (TDS), and Free Listing Task] are being studied and use as a replacement (Costa et al., 2020; Schuch et al., 2019; Silva et al., 2018; Vieira et al., 2020). Thus, different

sensory methodologies can select specific markets and then undergo specific, targeted marketing techniques (Carvalho et al., 2015).

Objective and subjective sensory information can positively impact consumer preference (Giacalone et al., 2016; Wang & Yu, 2016; Li et al., 2019; Sales et al., 2020). In particular, the Check-all-that-Apply (CATA) methodology has been widely used and is considered efficient for describing and discriminating between products by consumers. In this way, CATA responses are directly linked to consumers' perception of the product's characteristics, maximizing and complementing product acceptance results (Alcantara & Freitas-Sá, 2018).

This study aimed to analyze consumers' knowledge and perspectives about coffees (commodity, specialty, and fermented), measure the acceptance of a specialty coffee and investigate how information can influence consumers' sensory experience.

2 Materials and methods

2.1 Consumer knowledge of commodity, specialty, and fermented coffees

A descriptive-analytic survey was conducted through a digital format questionnaire to measure consumer knowledge of commodity, specialty, and fermented coffees (Supplementary material – Appendix C). Participants were invited to participate in the survey via a hyperlink. The survey lasted approximately 5 minutes. All participants (1005) who agreed to participate in the survey were over 18 years old and were adequately informed about the study objectives. The survey was divided into three main sections. The first section aimed to correlate data on socio-demographic aspects—gender and age—with coffee consumption frequency. The second section's questions aimed to uncover the behavior and perspectives of commodity and specialty coffee consumers, such as the characteristics observed when buying coffee, which flavors the commodity and specialty coffee consumers expect to find, besides preference, price, and others.

The last section was directed toward fermented and inoculated coffees—to try to find out to try to find the reasons behind each consumers' preference.

2.2 Coffee processing

Coffee cherries of the Catuaí Vermelho IAC-44 variety, grown at an altitude of 1200 m in the Caparaó region-Brazil, were fermented by a natural processing method (whole coffee fruit) for 72 hours in a closed polypropylene container, without adding water. Then, the cherries were transferred to suspended terraces until they obtained 11.5% moisture and stored under temperature-controlled conditions (15 °C ± 2 °C) until roasting.

The coffee beans were roasted according to the Specialty Coffee Association (Specialty Coffee Association, 2018) in a laboratory roaster (TP-2, Probat Leogap model, Curitiba, Brazil) for no longer than 24 h before tasting. The roasting lasted 9 minutes and 39 seconds, with a final temperature of 189 °C. The coffee beans were ground in an electric mill (Mahlkönig, EK43 model, Hamburg, Germany) before sensory analysis.

2.3 Sensory analysis (Q-grader tasters and consumers)

A sensory analysis was performed according to the SCA protocol (Specialty Coffee Association, 2018). Five cups from the same sample were used with a pre-determined ratio of 8.25 ± 0.25 g per 150 mL of water (92.2-94.4 °C). During cupping, four expert coffee tasters with a Q-Grader Coffee Certificate evaluated fragrance, flavor, after taste, acidity, body, balance, uniformity, clean cup, sweetness, and overall impression following the assessment sheet of the SCA. They also described the flavor and sensory descriptors of coffee.

The acceptance test followed by the CATA test was performed with the participation of 101 consumers. The primary sensory descriptors described by expert coffee tasters were used for the CATA list. Thus, twelve descriptors were selected: chocolate, caramel, spice, mild

flavor, milk, mint, almonds, sweet, citric, honey, fruity, and floral. The beverage preparation was every 30 minutes, in a ratio of 80 g of ground coffee in 1 L of water. The samples were coded with three-digit numbers (Palermo, 2015) and were served monadically at a warm temperature of 60 °C (± 1 °C). The participants were instructed to rinse their mouths out with water between samples to clean their palates.

Sensory analysis was divided into two sessions and was performed at the Sensorial Analysis Laboratory of the Federal University of Lavras-UFLA (Lavras, MG-Brazil). In Session 1 (S1), the sample was served together with the sensory evaluation sheet without any consumers' information. The participants were invited to taste the sample and mark the sensory descriptors they perceived based on the CATA list. Besides, the hedonic scale of nine points (9-" extremely like" to 1- "extremely dislike") has been used to evaluate the flavor and global impression of the coffee. The same sample was served in Session 2 (S2) with the information, "This coffee is a specialty coffee, 85 points, obtained by spontaneous fermentation". The sensory evaluation sheet was the same as in the first session. This study (CAAE: 00147118.4.0000.5148) was reviewed and approved by the Ethics Committee of the Federal University of Lavras (UFLA), Lavras-MG, Brazil.

2.4 Data analysis

Statistical analyses were performed using the XLSTAT software. Survey data were evaluated by a k-proportions test, with a 5% significance level and frequency responses. The CATA test's frequency responses with consumers, the acceptance test with consumers, and the cupping with the Q-Graders were evaluated using a Cochran test, t-test, and Scott-Knott test, each with a 5% significance level. The correlation between the frequency of consumers' coffee consumption and the scores obtained for taste and the overall impression was evaluated by a principal component analysis (PCA).

3 Results

3.1 Descriptive-analytic survey

The characteristics of the participants and their frequencies of consumption are described in Table 1.

Table 1. Population characteristics, according to coffee intake groups from questionnaire participants (N= 1005).

Parameters	Frequency of coffee consumption				Total (%)
	Never	1-2 per week	3-5 per week	Daily	
Sex					
Male	6	34	47	377	46.17
Female	15	63	58	405	53.83
Age (years)					
18-40	19	79	80	496	67.07
41-50	0	9	15	105	12.83
≥ 51	2	9	10	181	20.1
Country					
Brazil	20	91	105	771	98.2
Other country[1]	1	6	1	10	1.8
Education					
High school[2]	0	9	0	65	7.37
Undergraduate[2]	10	51	50	316	42.5
Postgraduate	11	37	55	401	50.13
Consumption[3]*					
Home	-	69	82	720	-
Work/University	-	42	68	526	-
Coffee shop	-	27	33	346	-

[1] Countries: Argentina, Colombia, China, Guatemala, United States, London, Nepal, United Arab Emirates, Venezuela;
[2]Studying/complete;
[3]Local of consumption;
*May have more than one answer.

Over 77% of participants consume coffee daily, demonstrating that coffee is a product that is appreciated. The most common location for consumption remains the home, followed by work/university, and, lastly, coffee shops. Only 21 of 1005 participants answered that they do not consume the beverage. The majority of participants are from Brazil (98.2%), and 1.8% are from other countries.

The out of the total, 31.2% of participants do not know what specialty coffee is. However, 91.2% said they would like to know more about specialty coffee, demonstrating receptivity to new sensory experiences. However, the knowledge about specialty coffees has increased from 68.8-72.7% after participants read a statement, “Special coffees are beans free of impurities and defects that have different sensory attributes, including clean and sweet,

balanced body, acidity, and scores 80 or higher by a certified Q-Grader on the Specialty Coffee Association's quality scale. In addition to intrinsic quality, specialty coffees must have certified traceability and environmental, economic, and social sustainability criteria at all stages of production" (Associação Brasileira de Cafés Especiais, 2019), showing that there are gaps in communication between the market and consumer.

When asked why they would like to consume specialty coffees, the most cited reasons were: knowing/sensory experience and curiosity (51.1%). Flavor and quality had 37.2% of the answers, while the aroma, pleasure, and sustainability represented 11.7%.

More than half of the participants (56.67%) reported having difficulty finding specialty coffees or do not know places that sell these coffees. Besides, the price of specialty coffee influences the consumption of 21.46% of the participants. And 80.4% of participants who consume specialty coffees are willing to pay up to approximately US $ 6 for 250 g of coffee, pointing out that the price is not such a limiting factor.

Sales et al. (2020) reported that the Brazilian coffee consumers associated commodity coffee beans with higher quality than coffee powder and in capsules. Also, they correlated coffee beans with a more powerful aroma, flavor, and freshness, generating greater satisfaction and pleasure. The characteristics observed when buying commodity coffee and the reasons for consuming specialty coffees are the same. However, there is a difference in the priority of these characteristics (Figure 1).

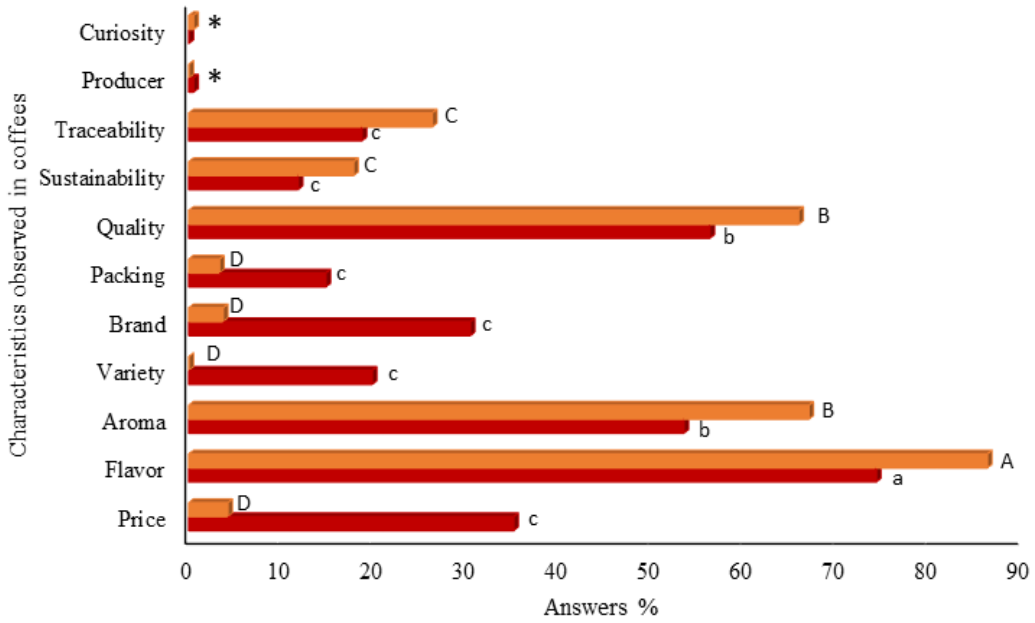

Figure 1. Characteristics observed by respondents when buying coffee. Different lowercase letters show a statistical difference at $p < 0.05$ by k-proportions test between the characteristics observed when buying commodity coffee. Different capital letters show statistical difference at $p < 0.05$ by k-proportions test between the characteristics observed when buying specialty coffee. *There was no statistical difference at $p < 0.05$ by k-proportions test in each characteristic observed between commodity and specialty coffee.

Flavor, aroma, and quality characteristics were the most mentioned. However, mentions of these factors increase, on average, 12% when talking about the consumption of specialty coffees and become essential. This increase also has been observed in traceability and sustainability. In contrast, the price, variety, brand, and packing decreased in importance when buying specialty coffees—up to a 30% difference compared to commodity coffees (for price and brand). Curiosity about the producer showed no significant difference between commodity and specialty coffee (Figure 1).

Flavor (86.3%) was the most-cited attribute for specialty coffee, significantly more frequently than the others. Aroma (62.2%) and aftertaste (56.1%) were also highly cited but did not significantly differ amongst themselves. The least-considered attributes were the degree of

roasting (29.4%) and body (31.6%)- there was no significant difference between them. Chocolate is the flavor that consumers most expect to find in specialty coffees (presenting a significant difference). Caramel, fruit, nuts, and almonds were also frequently cited and showed no significant difference. Further, 28.6% of the participants expected the coffee to have a roasted flavor (Figure 2).

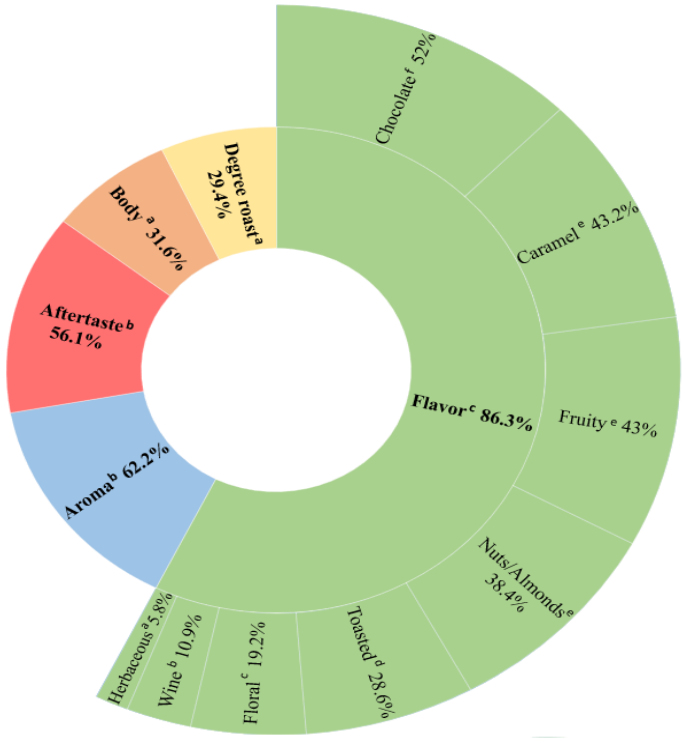

Figure 2. Important attributes evaluated when consuming specialty coffee and flavors that respondents expect to find in a specialty coffee. Different bold lowercase letters show a statistical difference at $p < 0.05$ by k-proportions test between the following characteristics: flavor, aroma, aftertaste, body, and degree of roast characteristics—different lowercase letters without bold show statistical difference at $p < 0.05$ by k-proportions test between flavors.

Of the five basic tastes, sweet is what consumers expect most (71.4%). Bitter is the second basic taste they expect to find in specialty coffee (41.6%), even though this type of coffee has a lower bitterness than commodity coffee.

The last part of the questionnaire focuses on fermented coffees. 48.5% of participants did not know that coffee can undergo a controlled fermentation process, and only 28.6% had

ever consumed this type of coffee. This result shows that, despite the growth of specialty coffees, information about fermented coffee needs to be spread to consumers since 89.6% of participants would like to consume this type of coffee, mainly for the best taste.

According to the survey, only 36.7% of participants knew about fermented coffee with microorganism starters. Only 16.2% had consumed this type of coffee, and the main attributes of coffee inoculated with microorganisms that pleased the interviewees were flavor (most cited: chocolate (51%), caramel (24%), floral (7%), fruity (6%), red fruits (5%), and wine(5%)), aroma, being different/exotic, and acidity. Besides, the acceptance rate of participants who have not tasted fermented coffees is much higher when the known inoculum (90.5%) than when the inoculum is unknown (46.1%).

3.2 Sensory analysis

Four trained tasters evaluated the fermented coffee sample according to the SCA protocol. The coffee received an average score of 85.15 (Table 2), and the most cited sensory characteristics of flavor were: chocolate, caramel, almonds, spices, soft flavor, milk, mint, citrus, fruity, honey, sweet, and floral. The score was used in the information presented to consumers in the second session of the sensory analysis to verify its influence on the acceptance test and the sensory characteristics perceived in the CATA test.

Table 2. The total score of each expert taster for coffee beverages (scale Specialty Coffee Association, 2018).

	Expert coffee tasters				
	T1	T2	T3	T4	MV
Final score	85.08^{a}± 0.88	85.17^{a} ± 0.52	85.08^{a} ± 1.18	85.25^{a} ± 1.25	85.15^{a} ± 0.08

MV = mean value of all tasters. Mean values ± standard deviation within the same lowercase superscript letters no differs significantly ($p < 0.05$) by the Scott-Knott test between the grades of each Q-Grader taster. All analyses were carried out in triplicate.

The panel of 101 untrained consumers was 62 females and 39 males, aged 18-60. Almost 47% of the participants drink coffee with sugar, and of these, 57% are women. Also, 75% of tasters drink coffee daily.

Acceptance was classified between “moderately like” and “very much like” (7.0-7.1 and 7.0-7.2, respectively) according to the hedonic scale (in the acceptance test) of taste and overall impression (in Sessions 1 and 2).

The PCA analysis (Figure 3) allowed the correlation between the frequency of coffee consumption by participants and the scores obtained for taste and overall impression. The information less influenced the tasters who consume coffee rarely (R). Those tasters were characterized by lower scores for flavor (between 3 and 4). The same happened with tasters who drink coffee 1-2 times a week; however, they scored the coffee higher, at 8. Although the group of tasters who consume coffee 3-5 days a week are in the same quadrant in S1 and S2, it can be observed that the information had a significant influence on the taste score. In S1, this group was characterized by a score of 7, while in S2, the score was closer to 9. Unlike expected, the information had a negative effect on the taste score of the tasters who drink coffee daily (D) (S1 was characterized by a score of 8, and S2 by a score of 7) (Figure 3A1). Thus, the cluster analysis separated consumers into three main groups. Only those who consumed coffee rarely (R) had the same grouping in S1 and S2 (Figure 3A2).

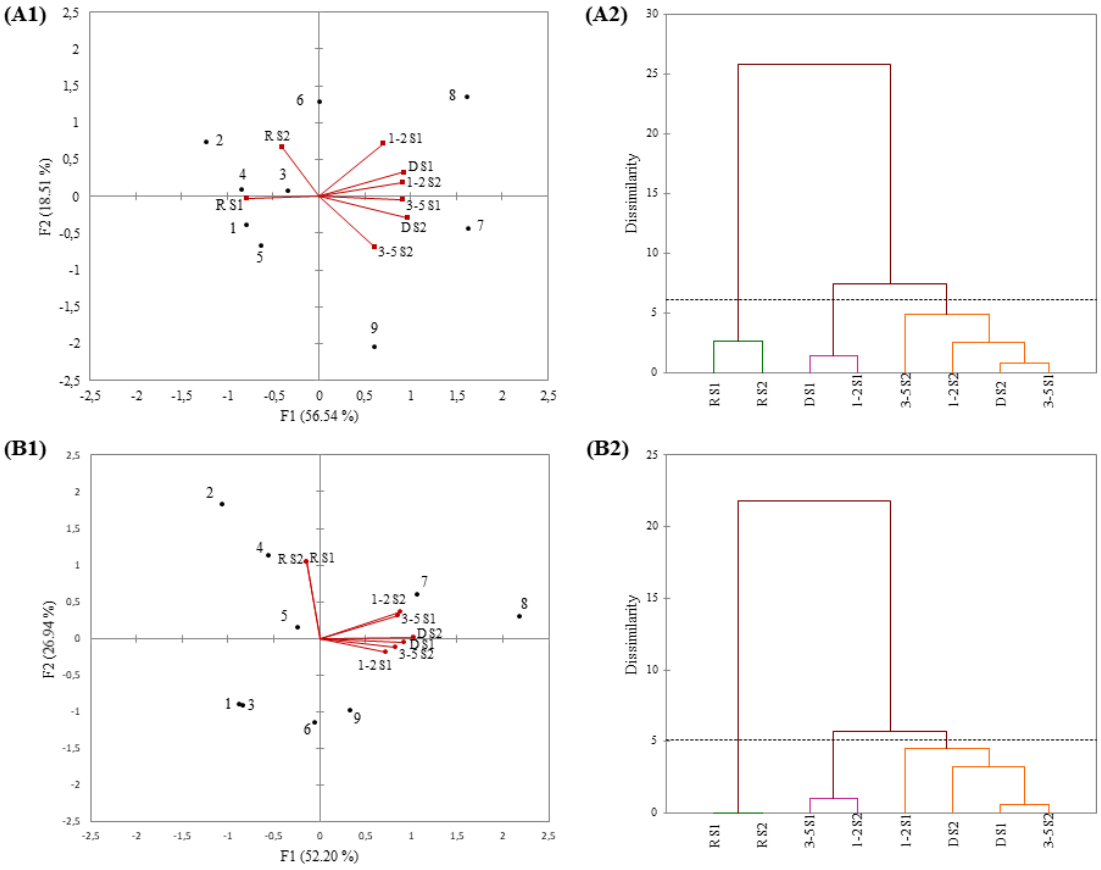

Figure 3. Correlation between the frequency of coffee consumption by consumers and the scores obtained for taste and overall impression were evaluated by principal component analysis (PCA) and cluster through dendrogram. A = Score evaluated for flavor; B = Score evaluated for global impression. Frequency of coffee consumption: D = Daily; 3-5 = 3 to 5 times a week; 1-2 = 1 to 2 times a week; R = Rarely; S1= Session 1
and S2 = Session 2. (—) Frequency of coffee consumption in each session.

In Figure 3B1, it can be observed that 79.14% of the total variance for the global impression scores was explained. The tasters who rarely consume coffee (R) were characterized by the same score (4) in S1 and S2. In this case, the information negatively influenced the scores of those who consumed coffee 1-2 times a week —in S1. S1 group was closer to a score of 9, and in S2, a score of 7. The tasters who drink coffee 3-5 times a week were also influenced by the information and had the highest overall impression score in S2 (closer to a score of 9). Tasters who drink coffee daily (D) did not seem to be influenced by the information and were characterized by a score of 8 in both sessions (Figure 3B1).

As for taste, Figure 3B2 shows that those who rarely consumed coffee were in the same group for both sessions. Those who drink coffee 3-5 times a week in S2 and those who drink coffee 1-2 times a week in S1 are in the same group, most closely related by a higher score. A dissimilarity of Groups 5-3 can be observed in Figure 3B2.

In S1, participants perceived sweeter and more caramel descriptors. After the information (S2) that the coffee scored 85 points and was fermented, the perception changed, and participants described the coffee as more citric and fruity. The perception of honey and floral descriptors showed no difference between S1 and S2 (Figure 4). These results indicate that untrained consumers perceived some attributes described by Q-Graders. However, they could be influenced by the information provided.

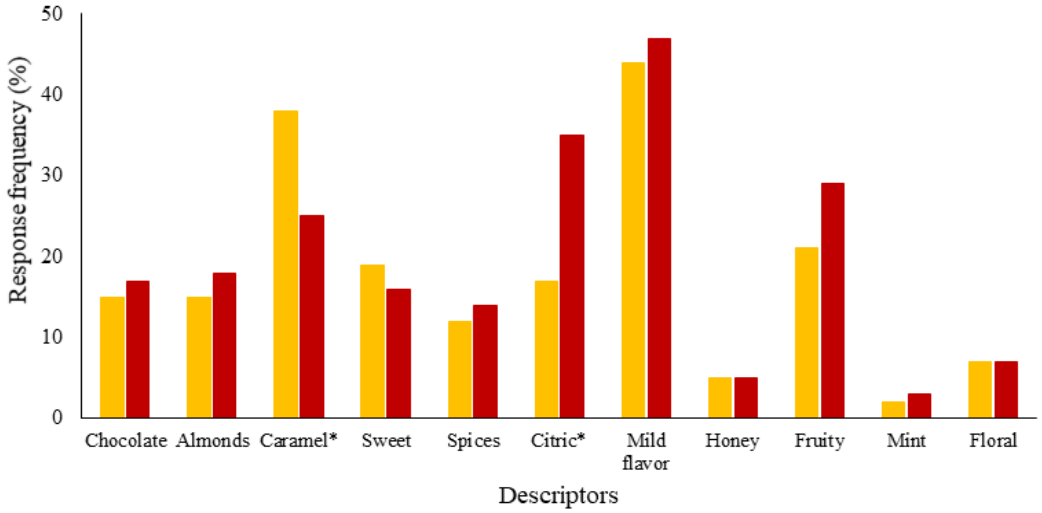

Figure 4. The frequency response of the CATA test with consumers before and after information provided to tasters. The sensory descriptors with * show statistical difference at $p < 0.05$ by the Cochran test from the same sample before and after the information.

4 Discussion

The survey aimed to understand consumers' knowledge and perspectives about commodity, specialty, and fermented coffees. Brazil is still the largest producer and exporter of coffee in the world. According to the Empresa Brasileira de Pesquisa Agropecuária (2019), every year, the beverage's world consumption grows around 1.5%. In particular, Brazilians are

drinking more coffee (the second-largest consumer of the beverage), and consumption is projected to increase by 3.5%, confirming an optimistic projection for coffee growers. Therefore, consumers' expectations and desires must be considered, reaching a broader market (Wang & Yu, 2016).

The third wave of coffee is based on the differentiation and experience of consuming this complex beverage (Boaventura et al., 2018; Guimarães et al., 2019). Participants' interest in trying specialty coffees indicates a growth trend for this market, with higher availability and approximation of consumers. More than quality, consumers want to service and pleasant experiences (Sousa et al., 2016; Wang & Yu, 2016; Ufer et al., 2019). Despite consumers' interest, price is one of the factors that influence their purchase decision, and promotions can stimulate increased sales (Samoggia & Riedel, 2018).

Coffee aroma and flavor are the most important properties determining consumers' preference and acceptance (Bressanello et al., 2018; Wang et al., 2019). Customers place significant value on coffee shops that provide sensory experiences, unique coffee flavors, and consistent quality (Kim & Lee, 2017). And as consumers become more demanding and aware (Ratton & Spers, 2020), they are more concerned with knowing the characteristics and information of the products they consume. Consequently, producers tend to change their postharvest processes to meet the demands. Thus, the controlled fermentation process aims to improve the quality of specialty coffees, diversifying the flavor and aroma of the beverage (Evangelista et al., 2014; Bressani et al., 2018, 2020; Haile & Kang, 2019; Wang et al., 2019).

There are no studies that meet consumer expectations regarding flavor descriptors. In this work, it was found that chocolate is the flavor that consumers most expect to find in specialty coffees, followed by caramel, fruit, and nuts, and almonds. However, many consumers still have the habit of coffee with excessive roasting (commodity), which may explain why 28.6% of the participants expected the coffee to have a roasted flavor. According to Monteiro

(Monteiro et al., 2010), consumers preferred the most darkly roasted coffees, regardless of the type of beverage. On the other hand, Giacalone et al. (2019) showed that consumers preferred coffees with regular roasting. According to the degree of roasting, some chemical compounds' composition can be variable because there is partial degradation of phenolic compounds and the development of aromatic and bioactive compounds (Cruz et al., 2017).

The human being has always looked for sweet taste, which is significantly related to pleasure and reward (Almeida, 2017). As expected, the sweet taste is what consumers most expect to find in coffee. Despite being considered an attribute negative in some foods (Varela et al., 2014), many consumers appreciate a certain amount of bitterness in products such as coffee, beer, or dark chocolate. In a study by Giacalone et al. (2019), the consumers strongly correlate bitterness with darker roasting.

Research has shown that consumers are more demanding and willing to try different products. Even though they can recognize the beverage's quality, they still have limited perception to describe the sensory characteristics. Therefore, the sensory evaluation performed by trained Q-Grader tasters is essential because it helps establish the purchase and sale value of specialty coffees through their score based on SCA protocol and sensory descriptors' perceptions (Conley & Wilson, 2020). However, it is also important to know the behavior regarding preferences, intentions, and desires.

The average scores of flavor and overall impression in S1 and S2 did not differ significantly. However, a slight increase can be seen after adding the information, showing an influence on the coffee's acceptability and perception of sensory characteristics. Overall, the coffee had reasonable rates of sensory acceptance for both flavor and overall impression. The information influences the sensory perceptions and responses because it creates expectations (Spence, 2015; Spence & Carvalho, 2020). Consumers who drink coffee more often showed high expectations for sensory analysis. In the same sense, the perception of the sensory

descriptors was different for the two sessions. Despite having been served with the same coffee in both sessions, most tasters correlate the specialty/fermented coffee with citrus and fruity descriptors. These results indicate that untrained consumers perceived some attributes described by the Q-Graders. However, they could be influenced by the information provided due to expectations about specialty/fermented coffees.

5 Conclusions

Descriptive-analytical research clearly showed how consumers understand specialty coffees and their perspectives on the beverage. In general, the participants knew little about specialty/fermented coffee but were willing to try different coffees. Chocolate is the flavor that consumers most expect to find in specialty coffee. These results are important, as they can help practitioners better understand the trend of the market and adjust the position of products or services more assertively.

Sensorially, the coffee had a good acceptance. The information mainly positively influenced the group who drink coffee 3-5 times a week. The caramel and citric descriptor showed a significant difference between S1 and S2, the citric descriptor being the most related to fermented coffees (after information). Consumers' expectations can be influenced by information, modifying their sensory perception.

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ANEXO A - Material complementar referente ao artigo 1

Supplementary material.

Table 1. Volatile compounds found in green coffee samples (BF= Before fermentation; □ = 72 h of fermentation in closed biorreactor; ■ = final drying) by GC-MS and their sensorial perception.

		Treatments (%)								
Compound	Sensory descriptors**	BF	Sc	SC	Cd	CT	Td	ST	SCT	SF
Acids										
Acetic acid	Sour, pungent, vinager	2.60	-	2.39	10.77	6.18	10.34	5.93	5.22	8.94
Acetic acid	Sour, pungent, vinager	-	11.84	6.39	6.85	2.38	13.92	3.03	2.96	10.31
2-methyl-propanoic acid	Burnt, cheese, rancid	-	-	1.22	1.50	1.85	3.11	1.75	1.97	2.31
2-methyl-propanoic acid	Burnt, cheese, rancid	-	2.89	2.07	5.01	1.81	2.95	2.09	2.73	2.44
Butanoic acid	Butter, cheese, rancid	-	0.23	0.30	0.31	0.24	-	0.30	0.28	-
Butanoic acid	Butter, cheese, rancid	-	0.31	-	0.53	0.31	0.40	0.35	0.45	0.38
2-methylbutanoic acid	Fruity-sour, sweaty, fermented	-	2.81	2.69	-	-	-	-	-	-
2-methylbutanoic acid	Fruity-sour, sweaty, fermented	-	0.35	0.51	0.41	0.45	-	0.34	0.38	-
Hexanoic acid	Acid, pungent, rancid	-	0.96	1.29	0.96	0.89	0.65	0.99	0.84	0.55
Hexanoic acid	Acid, pungent, rancid	-	1.71	2.99	3.31	2.89	4.04	5.14	3.69	2.98
Heptanoic acid	Rancid, sour, sweat	-	-	-	-	0.07	0.05	0.03	0.10	0.03
Heptanoic acid	Rancid, sour, sweat	-	0.16	0.17	0.14	0.35	0.18	0.24	0.21	0.17
Octanoic acid	Acid, fat, rancid, sweat	-	0.11	0.05	0.04	0.07	0.22	0.09	0.11	0.09
Octanoic acid	Acid, fat, rancid, sweat	-	0.83	0.72	0.64	1.42	0.61	0.72	0.97	0.53
Decanoic acid	Dust, fat, rancid, sweat	-	0.52	0.39	0.36	0.24	0.23	0.40	0.49	0.14
Decanoic acid	Dust, fat, rancid, sweat	-	0.29	0.16	0.25	0.56	0.33	0.28	0.41	0.11
9-decenoic acid	-	-	-	-	0.11	-	-	-	0.10	0.19
Benzoic acid	Pungent, sour	-	-	-	-	-	-	0.04	0.11	0.03
Benzoic acid	Pungent, sour	-	-	0.04	0.05	0.05	0.06	0.04	0.05	0.03
Nonanoic acid	Mild nut-like, fat, green, sour	-	0.13	0.20	0.12	0.33	0.15	0.17	0.28	0.24
Dodecanoic acid	Fat, fruit, metal, wax	-	-	-	-	0.03	0.03	0.04	0.31	-
Alcohols										
(Z)-2-penten-1-ol	Citrus-like flavor	0.91	-	-	-	-	-	-	-	-
2-heptanol	Fresh, lemon-like, grassy-herbaceous,Sweet-floral odor. Green, fatty, nutty, sweet	15.42	-	1.98	2.50	4.56	5.92	2.61	2.30	5.68

3-methyl-1-pentanol	Nutty, sweet, and sugared flavor, caramel-like odor	5.66	-	1.15	1.58	2.47	0.72	1.30	1.51	2.53
3-methyl-1-pentanol	Nutty, sweet and sugared flavor, caramel-like odor	-	0.50	1.22	1.93	1.34	2.66	1.65	1.00	0.94
(Z)-3-hexen-1-ol	Bell pepper, grass, herb	2.15	-	-	-	-	-	-	-	-
1-hexanol	Green, grassy, fatty, leafy	1.40	-	0.11	-	0.98	2.30	1.07	-	1.44
3-octenol	Mushroom	1.45	-	0.22	-	-	-	-	-	-
2-ethyl-1-hexanol	Citrus, green, oil, rose	5.46	-	5.83	4.70	5.96	11.92	4.62	3.68	7.92
(S)-3-ethyl-4-methylpentanol		12.97	2.45	1.90	2.64	3.32	4.84	2.24	2.07	3.47
3,7-dimethyl-1,6-octadien-3-ol	Sweet orange-like flavor, flowery, fruity	8.46	2.60	2.90	3.43	4.98	7.53	3.53	2.47	5.75
3,7-dimethyl-1,6-octadien-3-ol	Sweet orange-like flavor, flowery, fruity	-	3.32	2.50	1.93	2.03	1.58	3.15	2.27	3.22
Benzyl alcohol	Floral, roasted bread	3.96	1.73	1.63	1.86	2.08	2.89	1.94	1.22	2.17
Benzyl alcohol	Floral, roasted bread	-	4.11	4.06	3.33	3.76	3.49	3.09	4.23	3.42
Phenylethyl Alcohol	Fruit, rose, sweet apple	1.84	4.32	4.83	3.76	3.27	3.56	5.70	6.15	8.08
Phenylethyl Alcohol	Fruit, rose, sweet apple	-	5.76	5.15	4.59	2.81	5.02	3.21	7.44	9.65
3-ethyl-4-heptanol	-	-	7.61	-	0.14	-	0.47	0.34	0.05	-
3-ethyl-4-heptanol	-	-	0.10	0.30	-	0.23	0.34	0.31	0.26	0.32
2,3-butanediol	Natural odor of cocoa butter, sweet	-	-	-	1.05	0.84	1.24	1.47	2.28	0.57
2,3-butanediol	Natural odor of cocoa butter, sweet	-	8.08	6.32	7.74	5.84	7.59	7.26	7.07	6.89
L-.alpha.-terpineol	Fresh, mint, oil, sweet	-	-	-	0.06	0.04	0.10	0.05	0.04	0.13
L-.alpha.-terpineol	Fresh, mint, oil, sweet	-	0.05	0.12	-	-	0.26	0.35	0.13	0.13
2,6-dimethyl-1,7-octadiene-3,6-diol	-	-	-	-	1.87	-	-	-	-	-
4-nonanol	-	-	7.61	4.42	5.67	4.64	4.82	6.95	4.95	3.90
1-tetradecanol	Weak oily fatty	-	0.05	0.08	-	0.17	0.06	0.12	0.08	0.11
2,6-methyl-2,7-octadiene-1,6-diol	-	-	-	-	-	0.03	-	-	-	0.02
Aldehydes										
Hexanal	Green	11.05	-	-	-	-	-	-	-	-
Hexanal	Green	-	-	-	8.03	8.08	6.97	7.08	-	-
(E,E)-2,4-heptadienal	-	0.31	0.23	-	0.16	0.80	0.97	0.50	0.08	0.99
(E,E)-2,4-heptadienal	-	-	1.42	1.47	1.68	1.46	1.65	2.41	1.93	1.87
Benzaldehyde	Burnt sugar, roasted, pepper	2.73	-	-	-	-	-	-	-	-
Benzaldehyde	Burnt sugar, roasted, pepper	-	2.49	2.22	2.28	2.08	2.54	2.30	2.24	2.13
(E,Z)-2,6-nonadienal	Fatty-like flavor qualities	6.71	-	1.01	-	-	-	-	-	-
(E,Z)-2,6-nonadienal	Fatty-like flavor qualities	-	1.12	1.54	-	0.82	-	-	-	-
Benzeneacetaldehyde	Floral, sweet, caramel odor	1.23	0.11	0.02	0.03	0.12	0.02	0.04	0.41	0.01
Benzeneacetaldehyde	Floral, sweet, caramel odor	-	0.20	0.20	0.07	0.27	0.05	0.24	0.13	0.13
4-methyl-benzaldehyde	-	0.95	1.00	0.81	0.79	1.19	1.51	0.99	1.25	1.72
2-ethenyl-2-butenal	-	-	-	0.17	-	-	-	-	-	-
2-ethenyl-2-butenal	-	-	6.05	10.02	6.83	7.20	6.21	8.67	-	6.28

(E)-SS-2-octenal	Fatty-nutty, fruity, green	-	10.92	16.41	0.27	0.87	2.62	1.15	0.69	1.43
(E)-SS-2-octenal	Fatty-nutty, fruity, green	-	-	6.24	0.82	1.00	1.33	1.06	1.09	0.94
(E,E)-2,4-nonadienal	Fruity (citrus), fatty	-	-	-	0.09	0.04	0.17	0.06	0.04	0.16
SS-pentadecanal	-	-	0.37	0.12	0.19	0.36	0.38	0.49	1.37	0.35
SS-pentadecanal	-	-	0.66	0.18	0.13	0.51	0.26	0.32	0.41	0.21
2,6-dimethyl-10-methylene-2,6,11-dodecatrienal	-	-	0.13	0.06	0.04	0.03	-	0.04	0.08	-
Dodecanal	Fatty odor, woody taste	-	0.10	-	-	0.11	0.02	-	0.02	-
Esters										
Octanoic acid, ethyl ester	Fruity	1.74	7.61	-	16.40	11.82	8.38	17.57	10.43	3.67
Octanoic acid, ethyl ester	Fruity	-	2.86	6.77	2.13	3.63	0.45	2.31	2.71	1.32
3-nonenoic acid, ethyl ester	-	0.08	0.25	-	0.51	0.44	0.48	0.67	0.86	0.33
3-nonenoic acid, ethyl ester		-	1.20	0.80	3.20	0.77	2.02	1.09	1.17	2.25
Benzeneacetic acid, ethyl ester	Powerful and quite diffusive honey-musky odor with traces of jasmine-floral notes	0.26	0.58	0.94	0.61	0.65	0.73	0.70	0.90	0.81
Benzeneacetic acid, ethyl ester	Powerful and quite diffusive honey-musky odor with traces of jasmine-floral notes	-	0.28	0.78	0.26	0.29	0.19	0.32	0.31	0.29
2-hydroxy-benzoic acid, ethyl ester	-	0.15	1.23	0.84	0.82	0.85	0.9	1.08	0.76	0.58
2-hydroxy-benzoic acid, ethyl ester	-	-	1.24	1.26	0.68	1.08	0.84	0.88	1.21	1.29
Pentanoic acid, ethyl ester	Juicy, blueberry, apple	-	-	-	12.68	18.09	6.06	7.61	14.70	15.32
Ethyl hexanoate	Green apple	-	-	1.55	-	-	-	-	-	-
Acetic acid, 2-ethylhexyl ester	-	-	2.06	-	-	-	-	-	-	-
Propanoic acid, 2-hydroxy-, ethyl ester	-	-	-	2.10	-	-	-	-	-	-
Formic acid, heptyl ester	-	-	-	-	0.09	0.09	0.10	0.43	0.06	0.51
Formic acid, heptyl ester	-	-	-	0.05	-	0.51	0.61	0.10	0.51	0.03
Octyl acetate	-	-	6.70	3.63	3.44	3.87	4.75	1.89	2.13	6.52
Formic acid, octyl ester	-	-	0.73	-	-	-	-	-	-	-
Formic acid, octyl ester	-	-	2.03	1.67	3.27	1.23	2.18	1.78	2.41	2.37
Decanoic acid, methyl ester	-	-	0.51	0.97	-	-	-	-	-	-
Decanoic acid, ethyl ester	Fruity, fatty, pleasant	-	-	2.17	10.66	6.27	4.19	10.12	10.67	2.59
Decanoic acid, ethyl ester	Fruity, fatty, pleasant	-	2.65	2.47	1.69	2.61	1.30	1.82	2.09	1.15
Benzoic acid, ethyl ester	-	-	0.60	0.93	0.86	1.02	1.15	1.21	1.26	1.12
Benzoic acid, ethyl ester	-	-	0.80	0.83	0.51	0.57	-	0.58	0.51	0.52
Undecanoic acid, ethyl ester	-	-	10.83	10.77	-	-	-	-	-	-
Ethyl 9-decenoate	-	-	-	0.69	3.68	2.16	1.18	4.43	3.80	0.74
3-mercaptohexyl acetate	-	-	-	0.09	0.05	0.13	0.15	0.05	0.06	0.16

3-mercaptohexyl acetate	-	-	0.49	0.55	0.60	0.58	0.59	0.64	0.58	0.47
Methyl salicylate	Caramel, peppermint	-	4.42	2.62	3.31	3.03	3.89	3.14	2.34	2.66
Methyl salicylate	Caramel, peppermint	-	6.57	6.61	3.69	5.9	7.08	5.29	7.28	6.71
Dodecanoic acid, methyl ester	-	-	-	-	-	-	-	-	0.15	-
Dodecanoic acid, methyl ester	-	-	-	0.27	0.26	-	0.49	-	-	-
2-phenylethyl acetate	Fruity, honeyed, floral	-	0.93	0.84	0.81	0.73	0.25	1.17	1.16	0.25
Pentadecanoic acid, 3-methylbutyl ester	-	-	-	-	-	-	-	-	0.07	-
Cyclobutane carboxylic acid, 4-pentadecyl ester	-	-	-	-	0.05	0.04	0.07	0.04	0.15	0.02
Isopropyl myristate	Cherry, cinnamon, waxy	-	-	-	-	0.07	-	-	-	-
Tetradecanoic acid, ethyl ester	-	-	0.37	0.22	0.24	0.42	0.18	0.48	0.56	0.27
Tetradecanoic acid, ethyl ester	-	-	0.71	2.17	0.3	1.18	0.15	0.38	0.59	0.71
Pentadecanoic acid, ethyl ester	-	-	-	-	0.03	0.05	0.04	0.04	0.11	0.04
Pentadecanoic acid, ethyl ester	-	-	0.10	0.10	0.07	0.19	0.07	0.08	0.21	0.17
Hexadecanoic acid, methyl ester	Sweet, pineapple	-	0.26	0.15	0.16	0.19	0.34	0.28	0.34	0.23
Hexadecanoic acid, methyl ester	Sweet, pineapple	-	0.26	0.18	0.11	0.38	0.13	0.19	0.27	0.33
Heptadecanoic acid, ethyl ester	-	-	2.16	1.72	-	1.50	1.05	2.39	3.60	1.23
Heptadecanoic acid, ethyl ester	-	-	4.89	5.59	1.36	7.51	1.10	2.26	7.12	7.16
2-ethylhexyl salicylate	-	-	-	-	-	-	-	-	0.14	0.06
2-ethylhexyl salicylate	-	-	-	0.05	0.06	0.10	0.11	0.09	0.05	0.02
Homosalate	-	-	0.13	0.03	0.02	0.16	0.03	0.04	-	0.08
Homosalate	-	-	0.04	0.06	0.10	0.10	0.06	0.09	0.05	0.03
(Z,Z)-9,12-octadecadienoic acid, methyl ester	-	-	0.96	0.79	0.98	0.79	0.84	1.25	1.69	0.71
(Z,Z)-9,12-octadecadienoic acid, methyl ester	-	-	0.74	0.78	0.24	1.39	0.37	0.43	0.68	1.53
1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	-	-	-	-	-	0.03	0.03	0.02	0.02	0.08
1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	-	-	0.11	0.17	0.14	0.35	0.10	0.17	0.18	0.31
(Z,Z,Z)-9,12,15-octadecatrienoic acid, ethyl ester	-	-	0.34	0.18	0.25	0.25	0.23	0.29	0.32	0.13
(Z,Z,Z)-9,12,15-octadecatrienoic acid, ethyl ester	-	-	0.21	0.20	0.08	0.51	0.20	0.09	0.32	0.45
Ethyl 9-hexadecenoate	-	-	0.33	0.02	0.03	0.21	0.16	0.28	0.44	0.18
Ethyl 9-hexadecenoate	-	-	-	0.11	-	-	-	-	0.03	-
Ethyl hydrogen succinate	-	-	-	-	-	-	0.09	-	-	-
Hexanoic acid, ethyl ester	Pineapple, banana, fruity	-	-	3.05	-	-	-	-	-	-

Acetic acid, 3-methylpentyl ester	-		-	-	-	-	-	-	-	1.30
Furans										
2,2,6-trimethyl-2H-pyran-3-ol, 6-ethenyltetrahydro	-	-	-	-	-	0.23	0.31	0.31	0.21	0.27
2,2,6-trimethyl-2H-pyran-3-ol, 6-ethenyltetrahydro	-	-	0.58	0.42	0.61	0.50	0.75	0.65	0.59	0.58
5-methyl-2-furanmethanol	-	-	0.18	-	0.16	0.15	0.17	0.18	0.22	0.27
Hydrocarbons										
Tetradecane	Mild, waxy tasting	5.43	1.07	0.06	-	-	-	-	-	0.28
Tetradecane	Mild, waxy tasting	-	1.97	0.57	0.58	0.53	0.54	0.62	0.74	0.49
2,6,10,14-tetramethyl-pentadecane	-	0.26	-	-	-	-	-	-	-	-
2,6,10,14-tetramethyl-pentadecane	-	-	0.47	0.54	-	0.45	0.42	0.56	0.49	0.48
3-ethyl-1,4-hexadiene	-	0.60	-	-	-	-	-	-	-	-
8-methyl-1-undecene	-	-	0.64	-	1.63	1.91	-	1.83	1.41	0.80
8-methyl-1-undecene	-	-	3.68	1.01	2.37	1.28	1.41	1.99	1.98	1.02
Hexadecane	-	-	0.71	-	-	0.20	0.33	0.15	0.16	0.27
Hexadecane	-	-	1.64	1.30	1.36	1.30	1.48	1.29	1.28	1.47
Heptadecane	-	-	0.09	-	0.19	0.29	0.33	0.15	0.11	0.29
Heptadecane	-	-	0.11	-	0.17	0.27	0.17	0.17	0.16	0.21
Eicosane	-	-	-	-	-	0.05	-	0.10	0.17	0.11
6,10,14-trimethyl-2-pentadecanone	Mild waxy, fresh oily, jasmine	-	-	-	-	0.22	0.10	0.21	0.26	0.13
6,10,14-trimethyl-2-pentadecanone	Mild waxy, fresh oily, jasmine	-	0.52	0.26	0.21	0.51	0.28	0.28	0.49	0.47
Eneicosane	-	-	-	0.05	0.04	0.05	0.03	0.07	0.09	0.05
Eneicosane	-	-	-	0.03	-	0.07	0.08	-	0.07	0.06
2-octene	-	-	1.17	5.26	2.49	2.32	1.89	3.78	2.47	2.40
Ketones										
Acetoin	Green pepper, rancid	2.44	-	-	-	-	-	-	-	-
2,3-pentanedione	Oily-buttery odor, caramel- like’	-	13.96	-	-	-	-	-	-	-
2,3-pentanedione	Oily-buttery odor, caramel- like’	-	-	-	0.71	0.65	1.21	-	-	-
Cyclopentanone	-	-	-	12.71	-	-	-	-	-	-
1-hydroxy-2-propanone	Mushroom and sweet taste	-	-	0.22	-	-	-	-	-	-
Lactones										
Dihydro-5-pentyl-2(3H)-furanone	Strong, fatty, coconut odor	-	0.12	0.07	0.08	0.08	0.06	0.10	0.24	-
Dihydro-5-pentyl-2(3H)-furanone	Strong, fatty, coconut odor	-	0.29	0.13	0.16	0.34	0.37	0.22	0.23	0.13
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	Caramel, fruity (strawberry)	-	1.29	2.56	1.72	1.77	0.74	2.04	1.04	0.29
Phenols										
4-ethyl-2-methoxy-phenol	Medicine, phenol, smoke	-	-	-	-	-	-	0.01	-	-
4-ethyl-2-methoxy-phenol	Medicine, phenol, smoke	-	0.04	0.04	-	-	0.01	0.11	0.02	0.05

4-ethyl-phenol, 4-ethyl-	Medicine, phenol, stable	-	0.10	-	-	0.06	-	0.03	0.08	0.04
Pyrazines										
2-methoxy-3-(2-methylpropyl)-pyrazine	Green earth	0.63	-	0.33	-	-	-	-	-	-
2-methoxy-3-(2-methylpropyl)-pyrazine	Green earth	-	2.96	3.00	2.80	2.74	3.19	3.05	2.86	2.39
Pyrazine	Sweet odor	-	-	-	-	-	-	-	1.32	0.86
2-methylpyrazine	Nutty, roasted, sweet, green, biter, almond, weak flavor	-	1.93	-	-	-	-	-	-	-
2,6-dimethylpyrazine	Cocoa, coffee, roasted nut	-	2.01	-	-	-	-	-	-	-
2-ethyl-5-methylpyrazine	Fruit, roast, sweet	-	3.87	-	-	-	-	-	-	0.65
2-ethyl-5-methylpyrazine	Fruit, roast, sweet	-	-	-	6.27	4.24	-	6.44	5.39	-
Acethylpyrazine	-	-	-	-	-	-	-	-	0.19	-
Acethylpyrazine	-	-	0.20	0.05	0.26	0.24	0.23	0.28	0.41	0.14
Pyridines/Pyrroles										
3-pyridinol	-	-	0.03	0.04	-	0.03	-	-	-	0.04
Terpenes										
Linalool	Bergamot, lavender, rose, floral, citrus, orange, lemon	-	-	-	0.64	-	-	0.94	0.78	0.23
Kaur-16-ene	-	-	-	-	-	-	-	0.04	-	-
Kaur-16-ene	-		0.08	0.10	0.11	0.22	0.09	0.10	0.32	0.11
Others										
Furanodienone	-	-	0.18	0.18	0.15	0.18	0.12	0.29	0.59	0.20
Furanodienone			0.13	0.17	0.07	0.13	0.08	0.10	0.14	0.20
Caffeine	-	-	0.52	0.12	0.28	0.28	-	0.06	0.16	0.33
Caffeine	-		-	0.22	0.25	0.14	0.17	-	-	0.22
3,5,5-trimethyl-2-cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)	-	-	0.25	0.18	0.20	0.11	0.09	0.11	0.19	0.04
1-ethenyl-1-methyl-2,4-bis(1-methyl-ethenyl)- yclohexane,	-	-	0.29	0.29	0.26	0.14	0.19	0.14	-	-
3-buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	-	-	0.06	0.20	0.03	0.19	0.02	0.02	0.03	0.15
Not identified*										
RT:18.071	-	-	-	-	-	0.15	0.09	0.09	0.19	0.08
RT:27.282	-	-	-	0.04	-	-	-	-	-	-
RT:6.615	-	-	-	-	-	-	-	-	-	0.47
RT:12.066	-	-	-	-	0.94	1.07	1.00	1.20	1.16	1.11
RT:16.991	-	-	-	-	-	0.11	-	0.01	0.01	-
RT:19.171	-	-	-	0.03	-	-	-	-	-	0.03

RT:20.861	-	-	-	0.09	-	-	-	-	-	0.12

(%) = Relative percentage of volatile compounds. The compounds were identified by mass spectrum agreed with Nist and/or Kovats Index agreed with literature data. *Compound not identified by the NIST library or the Kovats Index. -: compound not detected. ** Sensory attributes are taken from: Flament, & Bessière-Thomas, (2002); Martinez et al. (2017); Bressani et al. (2018); Hu et al. (2018); Li et al. (2019). Treatments: BF= before fermentation; Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Spontaneous fementation (SF) = without inoculation.

Table 2. Volatile compounds found in roasted coffee samples by GC-MS and their sensorial perception.

		Treatments (%)							
Compound	Sensory descriptors	Sc	SC	Cd	CT	Td	ST	SCT	SF
Acids									
Acetic acid	Sour, pungent, vinager	22.84	16.70	15.33	16.37	18.62	14.07	15.11	15.2
Propanoic acid	Sour, acid	1.21	0.79	0.67	0.85	0.86	0.79	0.73	0.90
Butanoic acid	Butter, cheese, rancid	-	-	0.13	-	-	-	-	0.19
3-methyl-2-butenoic acid	-	0.42	0.26	0.28	0.25	0.18	0.23	0.32	0.37
Hexanoic acid	Acid, pungente, rancid	-	-	0.22	0.21	0.15	0.18	0.23	0.26
Octanoic acid	Oily-rancid, sweat-like odor	0.55	0.41	0.34	0.27	0.18	0.28	0.31	0.35
Dodecanoic acid	Sour-fatty, rancid odor	-	-	-	-	0.12	-	-	-
n-hexadecanoic acid	Virtualy odorless, bland taste	-	0.24	0.14	-	0.21	0.12	0.08	0.21
Alcohols									
1,2-ethanediol, dipropanoate	-	-	-	-	-	0.74	-	-	-
2,3-butanediol	Natural odor of cocoa butter, sweet	-	1.08	0.17	0.25	-	-	0.19	-
Benzyl alcohol	Floral, roasted bread	0.21	0.15	0.11	0.11	0.06	0.08	0.11	0.15
Phenylethyl alcohol	Fruit, rose, sweet apple	0.15	0.09	0.08	0.06	0.05	0.07	0.07	0.15
2-thiophenemethanol	-	0.22	0.17	0.14	0.14	0.08	0.09	0.12	0.15
1-hexadecanol	Faint waxy, nearly odorless	-	0.13	0.09	0.12	0.11	0.07	0.11	0.09
Aldehydes									
4-methyl-benzaldehyde	-	1.65	1.12	1.05	1.04	0.88	0.69	1.26	1.35
1H-pyrrole-2-carboxaldehyde	Vegetable (mushroom)	-	1.14	1.11	0.88	0.78	0.83	1.01	1.11
Esters									
1-(acetyloxy)-2-butanone	-	1.19	0.76	0.86	0.93	0.64	0.81	0.83	0.92
Methyl salicylate	Caramel, peppermint	0.41	0.27	0.28	0.30	0.21	0.20	0.28	0.38
2-hydroxy-benzoic acid, ethyl ester	-	-	0.12	0.09	0.11	0.08	0.07	0.10	0.16
Pentadecanoic acid, ethyl ester	-	-	0.15	0.08	0.11	0.05	0.09	0.08	0.10
Pentadecanoic acid, methyl ester	-	-	-	-	-	0.08	-	-	-
Heptadecanoic acid, ethyl ester	-	0.25	0.17	0.18	0.14	0.16	0.12	0.16	0.21
Homosalate	-	0.15	-	-	0.08	0.09	0.05	0.11	-
Furans									
1-(2-furanyl)-ethanone	Balsamic-sweet odor	1.32	0.88	1.01	1.02	1.08	0.90	1.32	1.50
5-methyl-2-furancarboxaldehyde	Caramel	7.91	4.79	5.48	5.56	5.70	5.01	6.40	7.14
2-furanmethanol	Mild, slightly caramelized flavor	3.14	36.02	35.43	31.71	29.65	41.12	31.08	30.07
Furfural	Caramel, toasted odor	5.31	-	3.92	3.81	5.08	3.03	5.43	5.79
5-hydroxymethylfurfural	Sweet, herbaceus	-	0.43	0.57	0.42	0.62	0.42	0.59	0.74
Furaldehyde									

5-acetoxymethyl-2-furaldehyde	Sweet	0.41	0.33	-	-	0.21	0.23	-	-
Furanones									
Dihydro-2-methyl-3(2H)-furanone	Sweet, bread, buttery, nutty	0.68	0.36	-	0.57	-	0.40	0.31	0.12
2,5-dimethyl-4-hydroxy-3(2H)-furanone	Sweet, candy, caramel, strawberry, sugar	0.61	0.50	0.30	0.31	0.23	0.32	0.32	0.34
Hydrocarbon									
Tetradecane	-	0.26	-	-	-	-	-	-	-
Ketones									
1-hydroxy-2-propanone	Pungent, sweet-caramellic, somewhat choking-ethereal odor, sweet taste	6.47	4.26	2.76	5.25	4.42	3.49	3.34	1.05
2,5-octanedione	Creamy-cheesy taste, mildy buttery	0.37	0.18	0.21	0.26	0.22	0.25	-	-
3-methyl-1,2-cyclopentanedione	-	0.55	0.44	0.30	0.36	0.26	0.33	0.35	0.29
3-ethyl-2-hydroxy-2-cyclopenten-1-one	Caramell-like odor	0.20	0.17	0.12	0.12	0.08	0.11	0.11	0.11
1-(1H-pyrrol-2-yl)- ethanone	-	1.24	0.84	0.79	0.62	0.50	0.61	0.71	0.78
6,10,14-trimethyl-2-pentadecanone	Mild waxy, fresh oily, jasmine	0.13	-	0.06	0.06	-	-	-	-
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	-	-	-	-	-	-	-	0.14	-
Lactones									
Butyrolactone	Caramel, fruit, roasted nut	2.70	1.80	1.54	1.90	1.63	1.60	1.34	1.33
3-methyl-2(5H)-furanone	-	0.14	0.12	0.09	0.11	0.07	0.04	0.09	0.09
2(5H)-furanone	Buttery	0.08	0.53	0.40	0.55	0.47	0.38	0.34	0.38
4-methyl-5H-furan-2-one	-	0.11	0.08	0.05	0.08	0.04	0.05	0.06	0.08
2-pyrrolidinone	-	0.28	0.23	0.17	0.10	0.12	0.17	0.21	0.31
Phenols									
p-cresol	-	-	-	-	-	-	0.17	0.10	0.23
2-methoxy-4-vinylphenol	-	1.60	1.21	1.39	0.95	0.63	0.87	1.25	1.71
Pyrazines									
Methylpyrazine	Nutty, roasted, sweet, green, biter, almond, weak flavor	2.10	1.70	2.54	0.92	-	1.67	2.03	2.53
2,6-dimethylpyrazine	Cocoa, coffee, roasted nut	2.17	1.37	1.66	1.80	1.41	0.65	1.65	2.60
Trimethylpyrazine	Nutty and roasted, roasty odor	0.20	0.12	0.11	-	0.07	0.18	-	-
2-ethyl-5-methylpyrazine	Fruit, roast, sweet	19.10	11.56	11.86	12.93	12.92	10.79	10.62	9.40
1-(6-Methyl-2-pyrazinyl)-1-ethanone	Popcorn flavor	0.35	0.22	0.24	0.23	0.16	0.12	0.19	0.25
Pyridines/Pyrroles									
Pyrrole	Toasted odor, sweet, nutty, ethereal	0.38	0.25	0.30	0.30	0.28	0.23	0.87	1.05
2-Pyridinecarboxaldehyde	-	0.98	-	-	-	-	-	-	-
N-acetyl-4(H)-Pyridine	Fatty, dusty, nutty	0.53	2.94	0.35	0.36	0.27	0.23	0.34	0.40

5H-1-Pyridine	-	0.27	0.17	0.18	0.12	0.13	0.14	0.16	0.29
3-Pyridinol	-	0.54	0.37	0.45	0.37	0.35	0.33	0.50	0.84
Others									
Furfuryl formate	Floral odor	0.22	0.13	0.16	0.17	0.16	0.16	1.13	1.18
1-methyl-1H-pyrrole-2-carboxaldehyde	Burnt	-	0.68	0.55	0.61	0.31	0.53	0.62	0.71
2-thiophenecarboxaldehyde	-	0.26	-	0.16	0.15	0.11	0.04	0.19	0.24
3-ethyl-2-hydroxy-2-cyclopenten-1-one	Sweet flavor	-	-	-	-	-	-	-	0.15
1-(2-furanylmethyl)-	-	0.44	0.27	0.29	0.24	0.19	0.20	0.31	0.42
trans-furfuryllideneacetone-1H-pyrrole	-	0.13	0.08	0.08	0.07	0.04	-	-	0.12
Furan, 2,2'-[oxybis(methylene)]bis-	-	0.22	0.18	0.14	0.14	0.09	0.11	0.16	0.18
Maltol	Roasted, nuts odor, nutty	2.38	2.06	1.58	1.16	0.94	1.15	1.19	1.18
4-hydroxy-3-methylacetophenone	-	-	-	0.06	0.06	0.04	0.04	0.14	-
1,1,2-triacetoxyethane	-	4.43	2.39	1.53	2.43	3.39	1.39	1.69	1.22
Benzene, (ethenyloxy)-	-	0.25	0.20	0.19	0.15	0.13	0.14	0.15	0.26
5-hydroxymethyldihydrofuran-2-one	-	-	0.07	0.10	0.06	0.08	0.06	0.10	0.11
Bis(2-furfuryl)disulfide	-	-	-	-	-	-	-	0.08	-
Caffeine	-	-	0.36	0.62	0.38	0.31	0.46	0.34	-
Not identified*									
RT:8.654	-	0.94	-	0.31	0.87	0.51	0.59	-	0.42
RT:8.795	-	-	0.52	-	-	-	-	-	-
RT:20.820	-	-	0.26	0.17	0.18	0.13	0.17	0.22	0.25
RT:24.870	-	0.19	0.11	0.26	0.09	0.13	0.11	0.11	0.19

(%) = Relative percentage of volatile compounds. The compounds were identified by mass spectrum agreed with Nist and/or Kovats Index agreed with literature data. *Compound not identified by the NIST library or the Kovats Index. -: compound not detected. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation.

Table 3. Pearson's Correlation Matrix between yeasts populations and sensory attributes.

Variables	*S. cerevisiae*	*C. parapsilosis*	*T. delbrueckii*	Fragance/Aroma	Flavor	Aftertaste	Acidity	Body	Balance	Overall
S. cerevisiae	**1**									
C. parapsilosis	-0.107	**1**								
T. delbrueckii	-0.109	-0.109	**1**							
Fragance/Aroma	-0.176	0.670	0.068	**1**						
Flavor	0.040	**0.792**	0.035	**0.790**	**1**					
Aftertaste	0.053	**0.738**	0.159	**0.843**	**0.979**	**1**				
Acidity	-0.286	0.674	0.015	**0.843**	**0.763**	**0.742**	**1**			
Body	-0.285	**0.924**	0.139	**0.781**	**0.840**	**0.838**	**0.754**	**1**		
Balance	-0.109	**0.786**	0.048	**0.963**	**0.888**	**0.916**	**0.888**	**0.877**	**1**	
Overall	-0.295	**0.793**	-0.231	**0.756**	**0.815**	**0.781**	**0.810**	**0.863**	**0.861**	**1**

Values in bold are different from 0 with a significance level alpha=0.05.

ANEXO B - Material complementar referente ao artigo 2

Table 1**.** Spearman correlation matrix of antioxidant activity (ABTS, DPPH, FRAP), TPC and individual compounds (caffeine, chlorogenic acids and trigonelline) presented in coffee.

	Phases*	TPC	DPPH	ABTS	FRAP	Chlorogenic acid	Caffeine	Trigonelline	Amount of yeast
Phases*	1.000								
TPC	**0.702**	1.000							
DPPH	**0.869**	**0.661**	1.000						
ABTS	**0.853**	**0.596**	**0.905**	1.000					
FRAP	**0.711**	**0.609**	**0.777**	**0.817**	1.000				
Chlorogenic acid	-0.420	-0.470	-0.314	-0.306	-0.280	1.000			
Caffeine	-0.219	-0.077	-0.226	-0.225	-0.094	0.279	1.000		
Trigonelline	**-0.503**	-0.260	**-0.498**	-0.432	-0.274	0.341	**0.729**	1.000	
Amount of yeast	0.000	0.016	0.091	0.050	0.020	0.263	0,123	0.054	1.000

***** Fermentation time (0 and 72 h) and roasted bean. Correlations is significant at the 0.05 level; the moderate to strong correlations are highlighted.

Table 2. Volatile compounds found in green (BF= before fermentation; □ = 72 h of fermentation in closed biorreactor; ■ = final drying) and roasted coffee samples (■ after roasted) by GC-MS.

	Treatments (%)								
Compound	BF	1Sc	1Cd	1Td	2SC	2CT	2ST	3SCT	Control
Acids									
Acetic acid	-	8.12	9.67	21.01	7.37	4.86	3.36	11.38	15.72
Acetic acid	-	-	19.12	23.73	16.24	6.44	21.64	8.50	31.16
Acetic acid	-	20.49	20.05	20.58	18.75	19.28	23.37	22.96	21.29
Hexanoic acid	-	0.69	0.44	0.42	1.21	0.43	0.68	0.76	0.42
Hexanoic acid	-	5.07	1.35	1.81	2.22	0.57	2.18	1.51	1.82
Octanoic acid	-	0.60	0.43	0.35	1.38	0.46	0.80	0.86	0.19
Octanoic acid	-	2.64	0.69	0.62	1.78	0.61	0.91	0.87	0.29
Decanoic acid	-	-	-	0.04	0.20	0.08	0.11	0.12	0.25
Decanoic acid	-	0.53	0.17	0.13	0.36	0.11	0.24	0.21	0.28
Butanoic acid	-	0.35	-	-	-	-	-	-	-
3-methyl-butanoic acid	-	0.24	0.16	0.54	-	0.21	0.48	0.30	1.04
3-methyl-butanoic acid	-	2.12	-	-	0.31	-	-	-	-
Heptanoic acid	-	0.25	0.28	0.28	0.28	-	0.38	0.21	0.18
Nonanoic acid	-	-	0.15	0.11	-	-	0.19	-	0.22
3-methyl-2-butenoic acid	-	0.19	0.16	0.17	0.21	0.18	0.16	0.16	0.17
Propanoic acid	-	0.80	0.81	0.73	0.82	0.75	0.83	0.78	0.83
Alcohols									
(+-)-5-methyl-2-hexanol	22.88	3.51	4.97	2.33	3.09	3.80	2.14	1.34	12.95
(+-)-5-methyl-2-hexanol	-	-	11.07	23.52	9.80	5.04	9.81	24.48	7.74
3-methyl-1pentanol	7.42	-	-	-	-	-	-	-	-
3-hexen-1-ol, (Z)-	0.61	-	-	-	-	-	-	6.55	-
3-hexen-1-ol, (Z)-	-	0.84	-	-	-	-	-	-	-
5-methyl-3-heptanol	2.00	0.29	0.38	0.43	0.18	0.40	-	-	-
5-methyl-3-heptanol	-	7.17	-	-	-	-	-	-	-
5-methyl-3-heptanol	0.86	-	-	-	-	-	-	-	-
(S)-3-ethyl-4-methylpentanol	14.09	2.14	2.16	2.66	2.39	1.38	1.61	0.18	4.25
(S)-3-ethyl-4-methylpentanol	-	-	1.84	2.78	2.65	1.83	2.39	2.56	2.50
3,7-dimethyl-1,6-octadien-3-ol	6.52	2.51	2.60	3.28	2.23	1.72	1.28	0.16	4.07
3,7-dimethyl-1,6-octadien-3-ol	-	-	0.96	1.30	1.37	2.28	1.15	2.59	1.26
Benzyl alcohol	4.50	1.54	1.79	1.66	2.47	1.27	1.08	1.22	2.81
Benzyl alcohol	-	4.81	2.44	2.37	1.89	1.68	3.28	2.57	2.36
Benzyl alcohol	-	0.06	0.05	0.05	0.05	0.06	0.04	0.04	0.05
Phenylethyl alcohol	4.25	4.41	5.90	5.28	11.05	4.26	5.89	6.69	4.41
Phenylethyl alcohol	-	12.43	4.62	5.03	5.49	5.65	11.46	5.43	7.67
Phenylethyl alcohol	-	0.09	0.09	0.09	0.10	0.11	0.08	0.07	0.09
2-propyl-1-pentanol	-	0.73	2.02	5.03	2.92	0.84	2.26	1.62	5.59
2-propyl-1-pentanol	-	1.00	-	-	-	-	-	-	-
3-methyl-1-butanol	-	12.12	-	3.68	-	-	-	20.92	-
3-methyl-1-butanol	-	0.37	-	-	-	-	-	-	-
7-methyl-4-octanol	-	-	-	8.54	-	4.56	8.57	-	15.10
7-methyl-4-octanol	-	-	7.47	6.85	11.63	6.05	8.40	8.40	10.15
1-dodecanol	-	-	-	-	-	-	0.04	-	0.10
1-dodecanol	-	-	0.03	0.04	0.04	-	-	0.03	0.06
2-methyl-1-undecanol	-	-	0.06	-	0.04	-	0.06	-	0.08
2-thiophenemethanol	-	0.08	0.09	0.09	0.09	0.08	0.07	0.07	0.08
2-methyl-1-undecanol	-	0.10	0.09	0.12	0.10	0.11	0.12	0.11	0.12
2-furanmethanol	-	24.79	25.00	23.62	24.69	24.68	22.36	22.53	22.37
5-methyl-2-furanmethanol	-	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03
Aldehydes									
2-hexenal, (E)-	3.73	2.73	6.47	1.40	10.64	6.01	5.19	-	2.68

2-hexenal, (E)-	-	-	1.90	2.00	3.03	7.98	2.38	4.75	1.68
2-octenal, (E)-	3.93	42.53	16.25	14.41	0.70	20.50	0.52	2.21	4.26
2-octenal, (E)-	-	0.60	0.33	-	0.30	0.39	-	0.54	-
2,4-heptadienal, (E,E)-	0.44	-	-	-	-	-	-	-	-
Pentadecanal- SS	0.11	-	-	-	-	-	-	-	-
Benzaldehyde	-	0.45	0.93	0.98	1.18	0.95	0.56	-	1.94
Benzaldehyde	-	-	1.49	1.62	1.62	1.26	2.13	-	2.08
2-nonenal, (E)-	-	-	0.12	0.19	0.16	0.07	0.26	-	0.26
2-nonenal, (E)-	-	-	-	-	0.28	-	-	0.50	0.23
2,4-nonadienal, (E,E)-	-	-	-	-	-	-	-	-	0.19
Benzeneacetaldehyde, .alpha.-ethylidene-	-	-	-	-	-	0.05	-	-	-
Benzeneacetaldehyde, .alpha.-ethylidene-	-	0.18	0.10	0.08	0.04	0.06	0.11	0.08	0.08
1-methyl-1H-pyrrole-2-carboxaldehyde	-	0.40	0.41	0.36	0.42	0.40	0.31	0.34	0.34
Furfural	-	0.15	2.69	2.07	5.28	1.39	2.21	6.79	0.54
Furfural	-	8.07	6.97	7.22	9.15	8.84	8.88	9.00	8.85
Esters									
Methyl salicylate	6.36	6.12	4.66	7.72	6.08	3.49	3.96	13.31	7.95
Methyl salicylate	-	16.91	20.98	9.14	8.87	4.64	12.22	13.17	9.90
Methyl salicylate	-	0.35	0.32	0.32	0.02	0.36	0.02	0.02	0.25
Homosalate	-	-	-	-	0.02	-	-	0.02	-
Furans/Furanone									
2-propyl-furan	-	-	-	0.36	-	-	0.30	0.14	1.58
2-propyl-furan	-	-	0.29	-	0.35	0.69	0.34	0.64	0.98
Dihydro-5-pentyl-2(3H)-furanone	-	0.61	0.49	0.49	0.55	-	0.54	0.41	0.41
1-(2-furanyl)-ethanone	-	1.40	1.33	1.18	1.50	1.36	1.24	1.28	1.36
2-furanmethanol, acetate	-	1.59	1.71	1.34	1.56	1.59	1.11	1.33	1.37
1-(acetyloxy)-2-butanone	-	1.15	1.09	0.92	1.09	1.06	0.92	0.94	0.99
5-methyl-2-furancarboxaldehyde	-	5.59	4.91	4.73	5.81	5.40	4.82	5.06	5.14
4-(2-furanyl)- 3-buten-2-one	-	0.03	0.03	0.03	0.04	0.04	0.02	0.02	0.03
5-hydroxymethylfurfural	-	0.18	0.16	0.34	0.28	0.48	0.30	0.33	0.44
3-methyl-2(5H)-furanone	-	-	0.02	0.05	-	0.05	0.03	0.00	0.00
2(5H)-Furanone	-	0.55	0.54	0.62	0.54	0.52	0.63	0.55	0.61
Hydrocarbons									
8-methyl-4-decene	1.47	-	20.80	2.96	12.05	28.36	34.93	-	3.69
8-methyl-4-decene	-	-	0.41	0.37	0.76	6.23	0.38	1.05	-
Tetradecane	0.41	0.16	-	0.12	-	-	-	-	-
Tetradecane	-	0.23	-	-	-	-	-	-	-
cis-1,3-dimethyl-cyclopentane	-	-	0.08	-	-	0.08	0.15	-	-
cis-1,3-dimethyl-cyclopentane	-	-	-	-	0.19	0.11	-	-	-
Ethyl 9-decenoate	-	1.58	1.35	0.62	4.05	1.78	3.86	0.13	0.14
Ethyl 9-decenoate	-	0.16	0.21	-	0.21	2.32	-	0.19	-
Eicosane	-	-	0.06	0.03	0.05	0.03	0.04	-	0.11
Eicosane	-	-	0.06	-	0.03	0.04	-	-	-
9-methyl-nonadecane	-	-	-	-	-	-	0.03	-	0.05
9-methyl-nonadecane	-	-	0.08	0.05	-	-	0.06	0.06	0.08
3,5,5-trimethyl-2-hexene	-	-	-	-	-	-	0.13	-	0.13
Pentyl-cyclopropane	-	0.07	0.32	0.55	0.59	0.23	0.40	-	0.42
Pentyl-cyclopropane	-	-	-	-	-	-	0.32	0.27	0.41
6,9-dimethyl-tetradecane	-	0.18	-	-	-	-	-	-	-
6,10,14-trimethyl-2-pentadecanone	-	0.20	0.21	0.14	0.21	-	0.18	0.10	0.16
Heptadecane	-	0.09	0.07	0.07	0.11	0.04	0.11	-	0.16
Heptadecane	-	0.13	0.22	0.11	0.23	0.06	0.10	0.09	0.16

2,5-octanedione	-	0.14	0.14	0.13	0.14	0.14	0.13	0.13	0.15
Ketones									
Acetoin	2.54	0.56	-	0.68	-	-	-	0.55	-
1-(acetyloxy)-2-propanone	-	23.92	23.19	23.57	21.85	22.27	22.97	23.97	22.93
3-methyl-1,2-cyclopentanedione	-	0.23	0.28	0.31	0.26	0.22	0.24	0.20	0.29
Lactones									
Dihydro-3,5-dimethyl-2(3H)-furanone	-	-	-	1.19	-	-	-	2.35	-
Dihydro-3,5-dimethyl-2(3H)-furanone	-	-	0.27	0.41	0.26	0.26	0.38	0.28	0.40
4-methyl-5H-furan-2-one	-	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.05
2-pyrrolidinone	-	0.16	0.09	0.26	0.16	0.19	0.19	0.16	0.21
Phenols									
4-ethyl-2-methoxy-phenol	-	0.18	-	-	0.09	-	0.13	0.05	-
4-ethyl-2-methoxy-phenol	-	-	-	0.03	0.04	-	-	0.04	-
2-methyl-phenol	-	0.07	0.07	0.08	0.09	0.07	0.05	0.05	0.07
Pyrazines									
1-(6-methyl-2-pyrazinyl)-1-ethanone	-	0.11	0.11	0.11	0.11	0.10	0.09	0.09	0.10
Pyridines/Pyrroles									
N-acetyl-4(H)-pyridine	-	0.45	0.43	0.38	0.44	0.45	0.34	0.36	0.39
4-pyridinol	-	0.44	0.57	0.97	0.74	0.87	0.59	0.53	0.78
1H-pyrrole-2-carboxaldehyde	-	0.07	1.45	1.53	1.52	1.44	1.24	1.15	1.40
Indole	-	0.16	0.19	0.19	0.21	0.23	0.13	0.13	0.18
1-(1H-pyrrol-2-yl)-	-	0.74	0.86	0.82	0.85	0.79	0.59	0.56	0.73
Terpenes									
trans-.beta.-Ionone	-	-	-	-	-	-	-	-	0.05
Others									
Benzeneacetic acid, ethyl ester	1.61	1.87	4.27	2.51	4.14	2.94	3.12	2.59	1.47
Hexadecanoic acid, methyl ester	0.25	0.09	0.22	0.13	0.39	0.15	0.20	0.22	0.61
Hexadecanoic acid, methyl ester	-	0.53	0.53	0.15	0.23	0.20	0.25	0.16	0.24
Heptadecanoic acid, ethyl ester	0.14	0.81	2.15	0.88	4.22	1.64	1.75	2.35	1.08
Heptadecanoic acid, ethyl ester	-	10.22	8.76	1.92	3.19	2.18	3.16	3.11	2.42
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	-	0.10	0.24	0.34	-	0.21	0.06	-	0.30
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	-	-	0.87	1.63	1.22	0.27	1.12	0.47	1.27
n-Caprylic acid isobutyl ester	-	-	-	-	-	-	0.11	0.35	-
Decanoic acid, methyl ester	-	0.96	0.48	0.36	1.21	0.45	0.93	0.79	0.32
Decanoic acid, methyl ester	-	2.98	-	-	-	0.60	-	-	-
Undecanoic acid, ethyl ester	-	-	-	-	-	-	-	7.97	-
Undecanoic acid, ethyl ester	-	4.52	-	-	-	-	-	-	-
Benzoic acid, ethyl ester	-	0.05	0.67	1.17	10.00	0.46	0.55	-	0.48
Benzoic acid, ethyl ester	-	-	0.61	0.36	0.23	0.61	0.35	0.55	-
4-Decenoic acid, ethyl ester, (Z)-	-	-	-	-	-	-	-	0.85	-
4-Decenoic acid, ethyl ester, (Z)-	-	0.15	0.22	0.06	0.13	0.04	0.16	0.11	-
Benzoic acid, 2-hydroxy-, ethyl ester	-	0.10	1.63	2.35	1.81	0.98	1.08	3.95	1.83

Benzoic acid, 2-hydroxy-, ethyl ester	-	2.16	4.37	1.10	0.92	1.30	1.33	1.48	0.93
Acetic acid, 2-phenylethyl ester	-	-	0.35	1.54	-	0.12	0.29	-	0.81
Acetic acid, 2-phenylethyl ester	-	0.73	0.51	0.31	0.23	0.16	0.44	0.15	1.11
3-Buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	-	-	-	-	-	-	0.02	-	0.05
3-Buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-	-	-	0.04	0.04	0.03	-	0.04	-	0.03
Pentadecanoic acid, 3-methylbutyl ester	-	-	-	-	-	-	0.05	-	-
Pentadecanoic acid, 3-methylbutyl ester	-	-	0.02	-	0.11	-	-	0.03	-
Pentadecanoic acid, ethyl ester	-	0.06	-	0.05	0.03	-	0.03	-	-
Pentadecanoic acid, ethyl ester	-	0.20	0.23	0.05	0.07	0.04	0.06	0.09	0.04
9-Octadecenoic acid (Z)-, ethyl ester	-	-	0.06	0.03	0.13	0.05	0.06	0.08	0.03
9-Octadecenoic acid (Z)-, ethyl ester	-	0.17	0.06	-	0.03	0.06	0.03	0.02	-
9,12-Octadecadienoic acid, ethyl ester	-	0.17	0.87	0.25	1.02	0.47	0.46	0.74	0.46
9,12-Octadecadienoic acid, ethyl ester	-	0.83	0.61	0.10	0.27	0.63	0.23	0.18	0.19
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	-	0.06	0.37	0.09	0.39	0.19	0.18	0.28	0.06
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	-	0.19	0.21	0.03	0.08	0.25	0.07	0.06	0.07
Propanoic acid, 2-hydroxy-, ethyl ester	-	5.97	-	-	-	-	-	-	-
Octanoic acid, ethyl ester	-	3.16	-	-	-	-	-	-	-
Dibutyl phthalate	-	-	0.17	0.66	7.36	0.06	0.55	1.14	6.14
Dibutyl phthalate	-	0.08	0.04	0.06	0.07	0.06	0.08	0.07	0.06
Furfuryl formate	-	0.58	0.61	0.48	0.60	0.57	0.47	-	0.49
3-thiophenecarboxaldehyde	-	0.10	-	0.08	-	0.09	-	-	0.08
1H-pyrrole, 1-(2-furanylmethyl)-	-	0.32	0.35	0.31	0.37	0.33	0.22	0.23	0.27
Furan, 2,2'-[oxybis(methylene)]bis-	-	0.12	0.16	0.12	0.17	0.14	0.07	0.08	0.13
Maltol	-	1.12	1.72	2.58	2.15	1.39	2.23	1.60	2.10
Not identified*									
RT:14.215	2.23	-	-	-	-	-	-	-	-
RT:6.545	-	-	-	-	-	-	-	3.62	-
RT:7.575	-	-	-	-	-	-	-	1.39	-
RT:7.575	-	3.71	-	-	-	-	-	-	-
RT:7.885	-	-	-	0.78	-	-	-	1.76	-
RT:8.880	-	1.49	1.36	1.49	1.30	0.98	0.98	0.83	1.56
RT:8.880	-	-	0.98	2.18	1.70	1.29	1.96	1.88	1.50
RT:13.775	-	1.46	4.86	0.73	12.35	4.89	9.84	0.18	-
RT:13.775	-	4.53	-	-	3.34	6.49	-	2.27	0.15
RT:14.215	-	-	-	4.61	1.78	-	4.54	-	2.71
RT:14.215	-	1.52	1.45	1.24	1.64	1.45	1.29	1.43	1.36
RT:14.875	0.24	-	-	-	-	-	-	-	-
RT:15.170	-	-	-	0.18	-	-	0.24	-	-
RT:15.170	-	0.15	0.12	0.20	0.13	-	0.16	0.12	0.13
RT:16.285	-	-	-	-	-	-	-	-	0.12

RT:16.285	-	-	-	-	0.05	-	-	-	-
RT:16.680	-	1.18	0.24	0.4	1.07	0.28	0.90	0.50	0.06
RT:16.680	-	1.12	0.25	-	1.04	-	-	0.30	-
RT:17.260	-	-	0.02	-	0.1	-	0.03	-	-
RT:19.315	-	0.27	0.55	0.23	0.6	0.3	0.38	0.35	0.19
RT:19.315	-	1.74	1.49	0.38	0.63	0.40	0.42	1.01	0.22
RT:21.020	-	-	-	-	-	-	-	0.25	0.17
RT:12.815	-	-	0.14	0.21	0.10	-	0.15	-	0.14
RT:13.405	-	-	-	1.27	0.78	-	1.69	0.66	-
RT:18.690	-	-	0.05	0.08	0.20	-	0.09	0.11	0.08
RT:18.690	-	0.37	0.44	-	-	0.42	0.27	0.26	-
RT:21.160	-	-	-	-	-	-	-	0.04	-
RT:21.160	-	0.04	0.06	0.10	0.12	0.09	0.06	0.06	0.10
RT:22.145	-	-	0.04	-	0.04	-	-	0.03	-
RT:11.030	-	0.73	0.73	0.90	0.73	0.78	0.71	0.75	0.90
RT:13.405	-	2.37	2.55	2.51	2.07	2.09	2.12	2.07	2.22
RT:13.505	-	-	0.19	-	-	-	-	-	-
RT:20.650	-	0.12	0.13	0.21	0.13	0.13	0.16	0.13	0.17

(%) = Relative percentage of volatile compounds. The compounds were identified by mass spectrum agreed with Nist and/or Kovats Index agreed with literature data. *Compound not identified by the NIST library or the Kovats Index. -: compound not detected. Treatments: Sc = *S. cerevisiae*; Cp = *C. parapsilosis*; Td = *T. delbrueckii*; SC = *S. cerevisiae* + *C. parapsilosis*; ST = *S. cerevisiae* + *T. delbrueckii*; CT = *C. parapsilosis* + *T. delbrueckii*; SCT = *S. cerevisiae* + *C. parapsilosis* + *T. delbrueckii*; Control = without inoculation. Spontaneous fementation (SF) = without inoculation;

ANEXO C - Material complementar referente ao artigo 3

Supplementary material.

1. Questionnaire: Study on consumer perception of commodity, specialty, and fermented coffees.

Part 1
E-mail
What is your gender?
What is your age?
In what region do you currently live?
What is your level of education?

Part 2
How often do you drink coffee?
In what kind of place (s) do you usually drink coffee?
Which characteristic (s) do you observe when buying coffee?
Do you know what specialty coffee is?
If you don't know, would you like to know?
“Special coffees are beans free of impurities and defects that have different sensory attributes, including clean and sweet, balanced body, acidity, and scores 80 or higher by a certified Q-Grader on the Specialty Coffee Association’s quality scale. In addition to intrinsic quality, specialty coffees must have certified traceability and environmental, economic and social sustainability criteria at all stages of production."
From this statement, do you consume/ have you consumed specialty coffee?
If you haven't consumed, would you like to consume?
For what reason?
What is the reason for not consuming?

Part 3
What is or are the reason (s) you consume specialty coffees?
As a consumer of specialty coffees, would you like to purchase coffees with traceability?
How much would you be willing to pay for a special coffee package (250 grams)?

Which descriptor (s) matter the most when you are consuming specialty coffee?
What basic taste (s) do you expect to find in a specialty coffee?
What flavor (s) do you expect to find in a specialty coffee?

Part 4
Did you know that coffee can undergo controlled fermentation?
Have you ever consumed fermented coffee?
Would you consume fermented coffee?
Explain the reason for the answer above (avoid writing because yes/no).
Did you know that a controlled coffee fermentation helps to generate different flavors and aroma of the beverage?
What flavors/aromas would you like to find in the coffee?
Do you know what is coffee inoculated with microorganisms?
Have you ever consumed fermented coffee inoculated with microorganisms?
If not, would you consume a coffee that has been inoculated (knowing that the microorganisms used come from the coffee itself and assist in the production with desirable volatile compounds)?
Would you consume a coffee that has been inoculated (not knowing which microorganism was used)?
Why?
What did you like most about drinking a fermented coffee?
Would you consume a coffee that has been inoculated (knowing that the microorganisms used come from the coffee itself and assist in the production of desirable volatile compounds)?
Would you consume a coffee that has been inoculated (without knowing which microorganism was used)?

2. Sensory Assessment Sheet with consumers.

Sensory Assessment Sheet

Name: _____Sex: ( ) Female ( ) Male

Age range (years): ( ) 18 a 30; ( ) 31 a 40; ( ) 41 a 50; ( ) 51 a 60; ( ) 61 years or more
Profession: _____
Coffee Consumption: ( ) Daily ( ) 5 to 3 times a week ( ) 1 to 2 times a week ( ) Rarely
Do you consume coffee: ( ) with sugar ( ) without sugar

Please drink the sample and pick the OPTION (s) that you consider appropriate to describe the sample. Then indicate how much you liked or disliked the sample using the hedonic scale.

Sample number: _____ Note flavor: _____ Note overall impression: _____

( ) Chocolate ( ) Caramel ( ) Spice ( ) Mild flavor ( ) Milk ( ) Mint
( ) Almonds ( ) Sweet ( ) Citric ( ) Honey ( ) Fruity ( ) Floral

Other (s):

_____

_____