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AMANDA LUÍSA SALES

ELABORAÇÃO, CARACTERIZAÇÃO E AVALIAÇÃO DA BIOATIVIDADE DE BEBIDAS FERMENTADAS (KOMBUCHA) A PARTIR DE SUBPRODUTOS DA CADEIA PRODUTIVA DO CAFÉ

Rio de Janeiro
2023

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Tese apresentada para o Programa de Pós-Graduação em Nutrição como pré-requisito para obtenção do título de Doutora em Ciências Nutricionais

Orientadores: Prof. Dra. Adriana Farah e Prof. Dr. Marco Miguel

Rio de Janeiro

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Tese de doutorado submetida ao Programa de Pós-Graduação em Nutrição do Instituto de Nutrição Josué de Castro da Universidade Federal do Rio de Janeiro – UFRJ, como parte dos requisitos necessários à obtenção do título de Doutora em Ciências Nutricionais.

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Dedico esta tese à minha vó, Ana Borges Tigrinho (*in memorian*), meu maior exemplo de vida.

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"*Guarda y graba en tu corazón las palabras de las personas que te ayudan a crecer*"

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LISTA DE ABREVIATURAS

SCOBY: Symbiotic culture of bacteria and yeasts – cultura simbiótica de bactérias e leveduras

ATCC: American Type Culture Collection

CC: cascas de café

FC: folha de café

FCM: folha de café com mate

BT: black tea

CCB: coffee cascara from Brazil

CCN: coffee cascara from Nicaragua

CL: coffee leaves

CLM: coffee-mate leaves

Inf: infusion

K: kombucha;

D0. 3. 6 and 9: days 0. 3. 6 and 9 of fermentation, respectively

n.d. values below limit of detection

EGC: epigallocatechin gallate

GC: gallocatechin

EGCG: epigallocatechin gallate

GCG: gallocatechin gallate

EC: epicatechin

CG: catechin gallate

GA: gallic acid

CA: caffeic acid

QUER: quercetin

KAEMP: kaempferol

3-CQA: 3-caffeoylquinic acid

4-CQA: 4- caffeoylquinic acid

5-CQA: 5- caffeoylquinic acid

3-FQA: 3-feruloylquinic acid

4-FQA: 4- feruloylquinic acid

5-FQA: 5- feruloylquinic acid

3,4-diCQA: 3.4-dicaffeoylquinic acid

3,5-diCQA: 3.5- dicaffeoylquinic acid

4,5-diCQA: 4.5- dicaffeoylquinic acid

MG: mangiferin

ISOMG: isomangiferin

TPC: total phenolic compounds

CA: caffeic acid

GA: gallic acid

FA: Ferulic acid

pCA: p-coumaric acid

SA: sinapic acid

BA: benzoic acid

3.4-diOHbenzoic acid: 3.4-dihidroxybenzoic acid

HA: hippuric acid

3,4-diOHyphenylacetic acid: 3,4-dihidroxyphenylacetic acid

4-OHphenylacetic acid: 4-hidroxyphenylacetic acid

VA: vanillic acid

DihydroCA: dihydrocaffeic acid

RATA: Rate All That Apply

IS: indoxyl sulphate

G: glucose

UA: uric acid

LPS: lipopolysaccharide

NO: nitric oxide

ANEXOS

Anexo 1: Aprovação no Comitê de Ética em Pesquisa

Anexo 2: Artigo intitulado "Potential intracellular antioxidant and anti-inflammatory effects and bioactive profiles of coffee cascara and black tea kombucha beverages",, referente ao estudo 4, publicado na revista Foods

Anexo 3: Artigo intitulado “Volatile, microbial and sensory profiles and consumer acceptance of coffee cascara kombuchas”, referente ao estudo 2, publicado na revista Foods

RESUMO

Elevadas quantidades de subprodutos são geradas durante a colheita e processamento do café para a obtenção dos grãos. Quando descartados inadequadamente, podem causar impacto ambiental desfavorável. Recentemente, a polpa e a folha vêm sendo estudadas devido ao elevado teor de compostos bioativos. Uma das alternativas de reaproveitamento é o preparo de bebidas. O objetivo da presente tese foi elaborar bebidas fermentadas tipo kombucha por uma cultura simbiótica de leveduras e bactérias, a partir dos subprodutos da cadeia produtiva do café, caracterizá-las química, físico-química, microbiológica e sensorialmente, e avaliar sua bioatividade. Na análise microbiológica, foi observada maior proporção de bactérias acéticas pertencentes ao gênero *Komagateibacter* e de leveduras pertencentes ao gênero *Pichia*. No processo de fermentação, foi observada redução nos teores médios de sólidos solúveis (12,2-9,0 °Brix), sacarose (70%, em média) e no pH (4,0-3,4), associado ao aumento da acidez titulável (0,04-0,9 mEq/L). Houve redução no valor total de compostos fenólicos identificados em KCP durante a fermentação (38%, em média). Nos kombuchas elaborados com cascas dos frutos do cafeeiro (KCC), o teor de compostos fenólicos em KCC e KFC aumentaram durante a fermentação (98% e 33%, em média, respectivamente). Com relação à atividade antimicrobiana, KCP, KCC e KFC apresentaram maior halo de inibição contra *E. coli e S. enteritidis*. Ao analisar o perfil de compostos voláteis nos kombuchas, foi identificado aumento no número de ácidos e ésteres, e redução no número de álcoois e aldeídos durante a fermentação. Em KCC, 1-heptanol, hexadecanol, isopulegol, cis e trans-óxido de linalool, butirato de etila, hexanoato de etila, acetato de isoamila, e γ-nonalactona foram formados a partir das infusões, enquanto nos KFC, observados também nas bebidas de KFC com erva mate foram observados 2,4-heptadienal, tetradecanal, tridecanal, undecanal, (S)-2-heptanol, (z)-3-hexanoato de etila, dihidrojasmonato de metila e 3,5-octadien-2-one. Os kombuchas que obtiveram as melhores médias de aceitação foram aqueles elaborados com as cascas da Nicaragua (7,0 ± 0,15) e com folhas de café com erva mate (6,6 ± 2,0) fermentadas durante 3 dias. Houve também associação entre os atributos sensoriais e os compostos voláteis. Com relação à bioatividade, as infusões e KCP e KCC foram capazes de reduzir as espécies reativas de oxigênio em células tubulares proximais (41%, em média) com redução de ácido úrico (10%-55%) no sobrenadante celular, e exerceram atividade anti-inflamatória, reduzindo os teores de óxido nítrico (81%-90%).

A viabilidade celular não foi alterada. Pode-se concluir que as cascas dos frutos e as folhas do cafeeiro são matérias-primas viáveis para a produção de kombuchas com boa aceitação entre consumidores, com potenciais benefícios à saúde. Portanto, é uma alternativa sustentável para o reaproveitamento destes subprodutos, melhorando a produtividade do setor cafeeiro.

Palavras-chaves: Café; subprodutos da indústria; cascas de café; folhas de café; kombucha; bebida fermentada; fermentação; compostos bioativos; atividade antioxidante; atividade anti-inflamatória

ABSTRACT

High amounts of byproducts are generated during coffee harvesting and processing to obtain green coffee beans. These byproducts, when improperly disposed of, can cause undesirable environmental impacts. Recently, coffee cascara and leaves have been studied due to their high content of bioactive compounds. One of the alternatives for reusing is to develop beverages. This study aimed to elaborate fermented beverages like kombucha through a symbiotic culture of bacteria and yeasts from by-products of the coffee processing, evaluating its chemical, physicochemical, microbiological, and sensory characteristics and evaluate its bioactivity. The results were presented in four studies. In the microbiological analysis, was observed a high proportion of acetic acid bacteria belonging to the Komagateibacter genera and yeasts from Pichia genera. During the fermentation process, were observed a decrease in soluble solids content (12.2-9.0 °Brix), sucrose (70%, on average), and pH (4.0-3.4), associated with the increase in titrable acidity (0.04-0.9 mEq/L). Fermentation caused the release of bound phenolic compounds from the infusions, especially total chlorogenic acids, with an average increase 98%, 30%, 47% and 26% in coffee cascara (CCK), black tea (BT), coffee leaves (CLK) and coffee-mate leaves kombuchas, respectively, up to day 9. Concerning antibacterial activity, BTK, CCK, and CLK exhibited higher inhibition zone against E.coli and S.enteritidis. In the volatile compounds profile analyzed, were identified an increase in acids and esters, and a decrease in alcohols and aldehydes along fermentation. In CCK, 1-dodecanol, 1-heptanol, hexadecanol, isopulegol, (E)-linalool oxide, (Z)-linalool oxide, ethyl butyrate, ethyl hexanoate, isoamyl acetate, and γ-nonalactone were formed from the infusions, while in CLK and also in coffee leaf with yerba mate kombuchas were observed 4-heptadienal, tetradecanal, tridecanal, undecanal (S)-2-heptanol, ethyl (Z)-3-hexenoate, methyl dihydrojasmonate and 3,2-octadien-2-one. The kombuchas with high acceptance means were those made with coffee cascara from Nicaragua (7.0 ± 0.15) and coffee leaves with yerba mate (6.6 ± 2.0) fermented for 3 days. There was also an association between volatile compounds and sensory attributes. In relation to bioactivity, black tea and coffee cascara infusions and kombuchas were able to decrease reactive oxygen species in proximal tubular cells (41%, on average) and decreased uric acid in cell supernatant (10%-55%). Infusions and kombuchas exerted anti-inflammatory activity, decreasing oxide nitric content (81%-90%). The cell viability was not affected. It can be concluded that coffee cascara and coffee leaves are promising raw materials for kombucha production with good

acceptance, with potential health benefits. Therefore, it is a sustainable alternative for the reusing these byproducts, improving the coffee chain sustainability.

Keywords: coffee; industry byproducts; coffee husk; coffee leaf; kombucha; fermented beverage; fermentation; bioactive compounds; antioxidant activity; anti-inflammatory activity

SUMÁRIO

1. INTRODUÇÃO

O café pertence à família Rubiaceae e ao gênero *Coffea*, sendo as espécies *C. arabica* e *C. canephora* de maior importância comercial. Os grãos estão localizados em frutos denominados polpas, sendo seu grau de maturação importante contribuinte para a qualidade da bebida final (Farah e dos Santos, 2015). Durante o processamento desses frutos para obtenção das sementes pelos métodos seco e semi-seco, são originados subprodutos, tais como, casca e polpa, que representam percentual significativo do peso seco do fruto (Pereira et. al., 2020; Esquivel e Jiménez, 2012). Quando o método úmido é aplicado, ocorre fermentação natural das cerejas, degradando a polpa e restando a casca como subproduto (Duarte et al., 2010).

O fruto de café, também conhecido como cereja, consiste em casca, polpa, mucilagem, pergaminho e os grãos de café verde. A casca, também conhecida como pericarpo, atinge a coloração vermelha durante o amadurecimento dos frutos (Heeger et al., 2016). Dependendo do processamento dos frutos, seco ou úmido, a polpa e casca de café são os primeiros subprodutos, e correspondem a 29% e 12% de toda a cereja (peso seco). Estima-se que para cada um milhão de sacas de 60kg de sementes secas de café, cerca de 218.400 toneladas de cascas e polpas secas de café são produzidas (DePaula et al., 2022). A casca possui alto teor de compostos bioativos, como cafeína, polifenóis e taninos, e, por isso, quando descartada de forma inadequada, pode ocasionar problemas ambientais em países produtores de café (Heeger et al., 2016).

As folhas de café oriundas da colheita dos frutos com as sementes de café verde podem não ser consideradas resíduos, mas o volume obtido durante e após as colheitas pode dificultar a manipulação da colheita (Pandey et al., 2000). Os estudos envolvendo as folhas de café vêm crescendo devido ao alto teor de compostos bioativos (de Almeida et al., 2018), aumentando a exploração do potencial de utilização das folhas de café pela medicina e indústrias de suplementos alimentares (Trevisan et al., 2016; Chen et al., 2019). Tendo em vista que este subproduto pode ser uma fonte barata e de fácil aquisição, o potencial comercial das folhas de café é grande, porém seu consumo não é global (Trevisan et al., 2016).

O desenvolvimento de produtos inovadores a partir de subprodutos do café tornou-se prioridade na pesquisa da área de nutrição e química de alimentos (Iriondo-DeHond et al., 2019). Infusões de cascas exibiram atividade antioxidante (Duangjai et

al., 2016; Heeger et al., 2016; Magoni et al., 2018), antimicrobiana (Duangjai et al., 2016; Moreira et al., 2018), anti-inflamatória e atóxico (Magoni et al., 2018). Estas apresentaram também efeitos antiadipogênico e lipolítico (Duangjai et al., 2018), e atividade protetora contra dano celular induzido pela geração de radicais livres (Iriondo-DeHond et al., 2019). Já as infusões elaboradas a partir das folhas de café possui potencial antioxidante (de Almeida et al., 2018), pode reduzir a ocorrência de infecções oportunistas em indivíduos portadores do vírus HIV (Lamorde et al., 2010), diarreia, dores intestinais, cefaleia, enxaqueca, dor estomacal, melhora de tosse e febre (Patay et al., 2016), anti-hipertensivo, imunomodulador, antiinflamatório, entre outros benefícios (Chen et al., 2019).

Entre as possibilidades para elaboração de bebidas, está a produção de Kombucha, bebida fermentada originalmente preparada a partir da infusão das folhas de *Camellia sinensis* com açúcar, utilizando uma cultura simbiótica de bactérias e leveduras (SCOBY, do inglês, *Symbiotic culture of bacteria and yeast*) (Murphy et al., 2018; BRASIL., 2019). Atualmente, kombuchas elaborados a partir de novas matérias-primas, como outros tipos de chás (diferentes de *C. sinensis*), frutas, ervas, leite e subprodutos da indústria de alimentos vêm ganhando atenção. As principais razões para o aumento da utilização de novas matérias-primas são, principalmente, para reutilização de subprodutos da agroindústria de alimentos para redução de resíduos, uso de matérias-primas regionais para atrair os consumidores, fonte de fitonutrientes e para produção de bebidas com características sensoriais diferenciadas (Leonarski et al., 2022). Vale ressaltar que não há trabalhos desenvolvendo Kombucha a partir de folhas ou polpa de *C. arabica*.

2. REVISÃO BIBLIOGRÁFICA

2.1. Planta de café: caracterização e métodos de colheita dos grãos

A planta de café pertence à família Rubiaceae, que compreende alguns dos 500 gêneros e mais de 600 espécies. De todas as espécies catalogadas no gênero *Coffea*, apenas três possuem importância comercial: *Coffea arabica, C. canephora* e *C. liberica* (Ferreira et al., 2019). Em 2021, a produção mundial de café foi de, aproximadamente, 10 milhões de toneladas, sendo 28% correspondente à produção brasileira. Em 2022, o consumo de café no Brasil foi de 21 milhões de sacas, com consumo per capita de 6kg (EMBRAPA Café, 2023).

O cafeeiro é uma planta perene e normalmente vive cerca de 10 a 15 anos na natureza após a secagem, mas a produção de frutos diminui consideravelmente mais cedo. Além disso, em plantações comerciais, as árvores devem ser renovadas regularmente. A configuração do cafeeiro varia dependendo das espécies e variedade. De forma geral, o cafeeiro possui um tronco principal com ramificações laterais primárias, secundárias e terciárias (Farah e dos Santos, 2014) (Figura 1). O desenvolvimento e o crescimento da planta são dependentes da espécie, variedade e condições ambientais da plantação (Ferreira et al., 2019).

A superfície foliar do cafeeiro adulto varia de acordo com a espécie, preservação da planta, níveis de irradiação, entre outros. As folhas das principais variedades comerciais, *C. arabica* e *C. canephora* são geralmente finas, brilhantes e enceradas, elípticas e veias visíveis. Normalmente crescem em pares que são opostos a cada um na ramificação (Ferreira et al., 2019).

Figura 1. Foto de cafeeiro da espécie *C. arabica* (Ferreira et al., 2019)

É uma planta de dia curto, por isso o início da sua floração ocorre em condições de 8 a 11 horas de luminosidade diária. A polinização ocorre dentro de 6h após a

floração e o processo de fertilização é finalizado entre 24 a 48h após polinização. Após esta etapa o fruto se desenvolve em uma "cereja" contendo dois grãos de café, composto pela casca, polpa, pergaminho e mucilagem (Figura 2) (Farah e dos Santos, 2014).

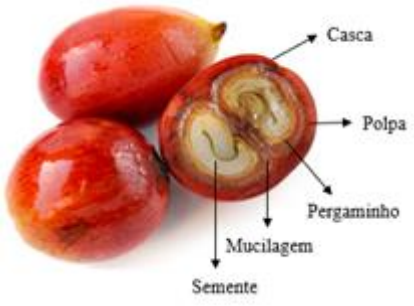


Figura 2. Corte transversal do fruto do cafeeiro, com suas partes.

A indústria cafeeira é uma das maiores indústrias alimentícias do mundo. É dividida em dois principais setores: o primeiro inclui a separação da casca e da mucilagem das cerejas de café para obtenção dos grãos de café; a segunda parte é responsável pela torrefação e transformação da bebida, os quais ocorrem após a exportação e distribuição dos grãos aos países consumidores (Oliveira et al., 2021). As principais técnicas existentes para a colheita dos frutos de café que podem ser empregadas são a manual ou mecânica. As escolhas dos melhores métodos dependem de diversos fatores, como o terreno da plantação, inclinação, variedade de café, mão de obra, custo, tamanho da fazenda e grau de maturação dos frutos (Sanz-Uribe et al., 2017).

Na colheita manual, apenas frutos maduros são colhidos e colocados em um cesto ou telas. Esta técnica é comum em países próximos à Linha do Equador, onde os produtores de café realizam oito a nove colheitas por ano, removendo apenas os frutos maduros a cada vez devido à falta de uniformidade na maturação dos frutos. Isto aumenta o custo de produção, pois grande número de mão de obra é utilizada (Guimarães et al., 2019). Quando é possível realizar a colheita apenas uma vez ,por causa da maturação uniforme, os frutos podem ser retirados simultaneamente do cafeeiro, podendo ser manual ou mecânico. Dessa forma, quando o início da colheita é tardio e apenas uma colheita pode ser feita, alguns frutos da primeira floração caem no chão, protegido por uma tela, antes da colheita iniciar. Estes frutos devem ser coletados e processados separadamente dos outros de maneira que a qualidade do produto final

não seja perdida, assim como frutos que não atingiram a maturação completa, maduros e que secaram no cafeeiro (Guimarães et al., 2019).

A colheita mecanizada, permite que grandes fazendas em áreas com alto custo de mão de obra se tornem mais competitivas para atendimento da demanda do mercado mundial de café. As máquinas utilizadas podem ser de mãos mecanizadas a tratores. Dependendo do método utilizado e de quando o café é colhido, os frutos resultantes podem constituir diferentes graus de maturação, como parcialmente maduros, imaturos e muito maduros, além de outros defeitos (Guimarães et al., 2019).

2.2. Processamento dos frutos de café

O processamento dos frutos de café é a principal atividade da indústria cafeeira, convertendo o fruto do café em bebida (Figura 3). Dois métodos são frequentemente utilizados para obtenção dos grãos de café verdes: processamento seco e úmido. Esses dois métodos diferem entre si em complexidade e produto final. Além disso, os subprodutos obtidos também dependem do tipo de processamento empregado (Gemechu et al., 2020). De uma forma ou outra, a grande demanda pelos grãos de café produz uma quantidade expressiva de subprodutos durante os processos para a obtenção dos grãos crus e torrados (Oliveira et al., 2021).

No processamento seco, os frutos colhidos são espalhados em um local seco e limpo, e normalmente alcançam a umidade ideal (10-12%) em 12 a 15 dias, sob condições climáticas ensolaradas. Estes são então descascados e consequentemente são removidas a mucilagem, o pergaminho e a polpa (Murthy e Naidu, 2012; Heeger et al., 2016). No Brasil, as cerejas de café geralmente são processadas pelo método seco, resultando nas cascas de café, fontes de matéria orgânica e nutrientes (Pandey et al., 2000).

Existe um número considerável de variações do processamento semi-seco. Este método é intermediário ao método seco e úmido e começou a ser utilizado no Brasil no início dos anos 1990. Originalmente, os grãos eram apenas lavados antes da secagem. Depois a polpa do fruto que cobre os grãos passou a ser removida por despolpadores antes da secagem, ressaltando-se a importância da utilização de equipamento adequado e água limpa. Assim como no método seco, as sementes secas permanecem envolvidas na mucilagem e não ocorre o processo de fermentação para sua remoção (Duarte et al., 2010). Uma variação deste método resulta no processo também conhecido como *honey*

process (Heeger et al., 2010), no qual os frutos são integralmente e lentamente secos permitindo algum tipo de fermentação e desenvolvimento de sabor antes da separação da polpa.

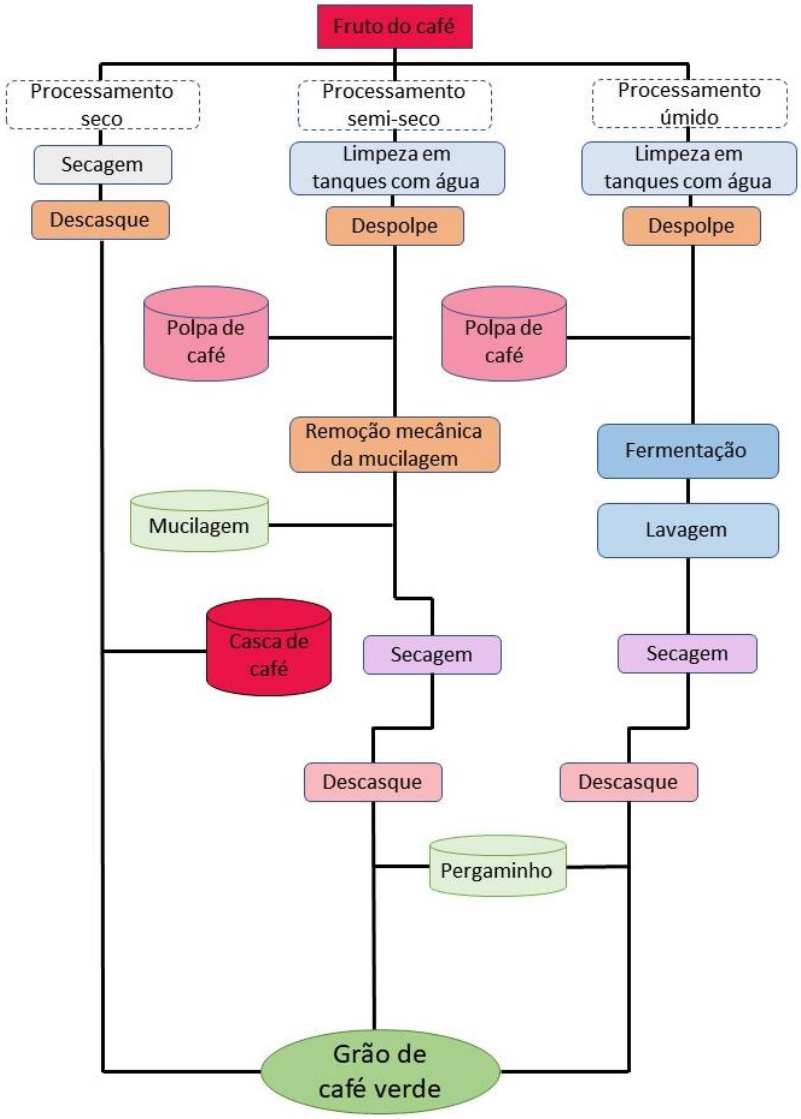


Figura 3. Esquema dos métodos de beneficiamento dos frutos de café para obtenção dos grãos de café verde (Adaptado de Gemechu et al., 2020)

No processamento úmido e no semi-seco ocorre separação dos frutos maduros e imaturos na água. Os frutos maduros afundam e os imaturos permanecem na superfície. Após esta separação são removidas a polpa e a casca com despolpador, seguido por fermentação de 12 a 24 horas e então os frutos são lavados. No processo de fermentação (que pode ser substituídos pelo uso de enzimas) a mucilagem e os componentes remanescentes são removidas (Sanz-Uribe et al., 2017; Heeger et al., 2016).

2.3. Cascas dos frutos de café

Os principais subprodutos desses métodos de processamento, a polpa e a casca de café, representam 29% e 12% do peso seco da cereja, respectivamente (figura 2) (Janissen e Huynh, 2018). Toneladas desse subproduto são geradas a partir do processamento dos frutos de café e descartadas anualmente. Foi estimado que para cada milhão de sacas de 60kg de sementes secas de café, cerca de 218.400 toneladas de cascas e polpas de café secas são produzidos (DePaula et al., 2022).

Figura 4. Frutos secos do café antes da separação mecânica das sementes (A); casca e polpa secas (método de processamento seco ou semi-seco) (B) e casca e polpa de café (método de processamento úmido) (C)(Ref. Del Castillo et al., 2019a).

As cascas e polpas, que formam um conjunto único, já que a polpa é delgada, possuem uma série de compostos bioativos, a maioria presente também nas sementes. Em sua composição pode-se encontrar ácidos clorogênicos, flavonóides (Iriondo-DeHond et al., 2019), antocianinas (Prata et al., 2007), entre outros (Tabela 1). Apesar de uma gama de compostos bioativos promotores da saúde serem encontrados na cereja de café, 90% do material das cerejas, diferente da semente, são descartados durante o processamento como resíduos agrícolas ou subprodutos (Del Castillo et al., 2019a). No entanto, esses resíduos constituem fontes de contaminação e problemas ambientais, pois o descarte inadequado das cascas de café durante seu processamento pode ocasionar a poluição da água, solo e desequilíbrio do meio ambiente, devido à elevada concentração de cafeína, fenólicos livres e taninos (Murthy e Naidu, 2012).

Tabela 1. Composição química das cascas de café

Composto	Teor (mg/g)	Referência
Ácidos clorogênicos	0,07 - 1,7	Heeger et al., 2016; Iriondo De-Hond et al., 2019; Silva et al., 2021
Ácido cafeico	0,001 – 0,006	Silva et al., 2021
Ácido gálico	0,002 – 0,06	Silva et al., 2021
Cafeína	0,98 - 13,9	Iriondo De-Hond et a., 2019; Rebollo-Hernanz et al., 2019; Heeger et al., 2016
Flavonoides totais	0,006 – 15,69	Heeger et al., 2016; Iriondo De-Hond et a., 2019; Silva et al., 2021
Aminoácidos	25,05	Iriondo De-Hond et al., 2020
Antocianinas	0,17-0,20	Prata et al., 2007
Fibras totais	4,74	Rios et al., 2020
Fibras solúveis	1,61	Rios et al., 2020
Fibras insolúveis	1,61	Rios et al., 2020
Proteínas totais	0,95	Rios et al., 2020
Lipídeos totais	0,02 – 2,0	Rios et al., 2020; Iriondo De-Hond et al., 2020
Carotenóides totais	0,02	Moreira et al., 2018
Taninos condensados (Equivalente de catequina)	12,73 – 79,71	Silva et al., 2021

Devido à elevada concentração de compostos fenólicos, alternativas vêm sendo estudadas para contribuir com a sustentabilidade no manejo deste resíduo em regiões produtoras de café. Dentre as alternativas estão a obtenção de um extrato rico em ácidos clorogênicos e reduzido em cafeína, que poderia ser utilizado para alimentação animal, fertilizante ou compostagem (Silveira et al., 2019) e isolamento dos carotenóides produzidos por levedura, que poderiam ser utilizados pela indústria de alimentos e cosméticos (Moreira et al., 2018).

É importante ressaltar que a agência reguladora de segurança de alimentos na Europa reconhece as cascas de café como alimento (European Food Safety Authority, 2021), podendo ser utilizada no preparo de infusões (Heeger et al., 2016; Iriondo-DeHond et al., 2019), incorporada em iogurtes (Iriondo De-Hond et al., 2020) e utilizada na forma de farinha para elaboração de pães (Rios et al., 2020). Em relação aos benefícios à saúde, foram encontrados *in vitro* atividade antioxidante, antimicrobiana,

antiadipogênica e lipolítica (Duangjai et al., 2016; Duangjai et al., 2018; Khochapong et al., 2021).

2.4. As folhas de café

A poda periódica do cafeeiro produz as folhas de café (Figura 3) como subprodutos, porém são consideradas de baixo valor comercial quando comparado com as sementes de café. Estima-se que 3,3 toneladas de folhas por hectare são descartadas durante a produção de café no Brasil (Matiello et al., 2010). As folhas de café, principalmente as folhas secas (Figura 4), são preparadas de forma similar ao chá verde de *Camellia sinensis* (Trevisan et al., 2019). São utilizadas para produção de chás, sendo comum o consumo no continente africano e asiático para fins medicinais (Pandey et al., 2000; Campa et al., 2012; Novita et al., 2018; Monteiro et al., 2020). Levando em consideração que o Brasil é um dos maiores produtores de café, a exploração das folhas de café é uma alternativa para produtores de café para sua reutilização após a colheita, sendo economicamente viável a utilização para o processamento e preparo de infusões (Acidiri et al., 2020).

Entre os principais compostos fenólicos presentes na folha de café (tabela 2), estão os xantonóides mangiferina (1,3,6,7-tetrahidroxixantona-C2-β-glicosídeo) e isomangiferina (Trevisan et al., 2016; Talamond et al., 2008; Monteiro et al., 2020; Matkowski et al., 2013). Além destes compostos, também estão presentes os ácidos clorogênicos, cafeína, trigonelina e teobromina (Monteiro et al., 2020). Ressalta-se que os valores de compostos bioativos podem variar entre folhas verdes e oxidadas (Acidri et al., 2020). Benefícios *in vitro* encontrados são relacionados aos compostos bioativos, como atividade antioxidante e antiinflamatória (Chen et al., 2018; Segheto et al., 2018), além da prevenção do desenvolvimento de síndrome metabólica em ratos (Patil et al., 2022).

Tabela 2. Composição química das folhas de café

Composto	Quantidade (mg/g)	Referência
Sacarose	0,82 – 2,63	Acidiri et al., 2020
Ácidos clorogênicos	0,02- 48	Monteiro et al., 2020; Chen et al., 2018; Rodrígues-Gómez et al., 2018; Acidri et al., 2020
Cafeína	0,85–14,9	Chen et al., 2018; Monteiro et al., 2020; Acidri et al., 2020
Teobromina	0,7 - 1,7 mg/g	Monteiro et al., 2020
Trigonelina	0,47– 11,7	Chen et al., 2018; Monteiro et al., 2020; Acidri et al., 2020
Mangiferina	0,35–1,1	Campa et al., 2012; Trevisan et al., 2016; Chen et al., 2018; Talamond et al.,2008; Monteiro et al., 2020, Matkowski et al., 2013; Acidri et al., 2020
Isomangiferina	0,05 - 3,6	Trevisan et al., 2016; Chen et al., 2018; Talamond et al.,2008; Monteiro et al., 2020, Matkowski et al., 2013
Rutina	0,37 – 2,48	Chen et al., 2018;
Quercetina	1,4 – 11,3	De Almeida et al., 2018
Kaempferol	0,5 – 2,9	De Almeida et al., 2018
Carotenóides	0,24 – 0,37	Acidiri et al., 2020
Flavonóides totais	0,001 – 0,006	Trevisan et al., 2019

Figura 5. Folhas de café *C. arabica* jovens e verdes (ref: Ferreira et al., 2019)

Figura 6. Folhas de café *C. arabica* secas (Ref. Novita et al., 2018)

Diversas iniciativas sustentáveis podem ser implementadas em todas as etapas da obtenção e produção do café, entre elas, a elaboração de novos produtos à base de café. O consumo de chás elaborados a partir de folhas e cascas de *C. arabica* como fonte natural de compostos fenólicos e outros compostos bioativos, pode contribuir significativamente para elevar a ingestão de compostos que promovem a saúde e não são encontrados em outras fontes alimentares (Heeger et al., 2016; Trevisan et al., 2019).

2.5. Kombucha: caracterização, composição química e benefícios

Segundo a Instrução Normativa n° 41, de 17 de setembro de 2019 (BRASIL, 2019), Kombucha é a bebida fermentada obtida através da respiração aeróbia e fermentação anaeróbia do mosto obtido pela infusão ou extrato de *Camellia sinensis* e açúcares por cultura simbiótica de bactérias e leveduras (SCOBY, do inglês, *Symbiotic Culture of Bacteria and Yeast*) microbiológicamente ativas, normalmente entre uma membrana de celulose (Rezac et al., 2018).

É uma bebida tradicional originada na China. Relatos de seu primeiro consumo surgiram em 220 anos Antes de Cristo (AC), sendo consumida primeiramente na Ásia devido aos seus benefícios à saúde. Durante o império japonês, a bebida era utilizada por médicos Kombu para tratar distúrbios relacionados ao trato gastrointestinal. Com a expansão comercial, seu consumo foi disseminado para países como Rússia e Alemanha, e nos anos 1950 tornou-se muito popular na França (Jayabalan et al., 2014). Quando recém fermentada, a bebida possui sabor similar ao de cidra de maçã adocicada e gaseificada. A fermentação prolongada pode resultar em sabor e odor ácido e de vinagre (Dutta et al., 2019).

Kombucha e produtos baseados em kombucha são vendidos comercialmente em boticários europeus há, pelo menos, 60 anos. A primeira marca comercial criada nos

Estados Unidos, ativa até os dias de hoje, e também a atual líder de mercado, foi estabelecida em 1995 por GT Dave em sua cozinha familiar (Kombucha Brewers International, 2022). O mercado global de kombuchas foi avaliado em USD 2.64 bilhões em 2021 e é esperado que seja expandido em uma taxa anual de 15,6% de 2022 até 2030 (Grand View Reasearch, 2021). A curiosidade de consumidores e a popularidade da bebida são as principais razões por trás da tendência atual de mercado e de pesquisas sobre a bebida (Kim e Adhikari, 2020).

A cultura SCOBY é a responsável pela simbiose presente na elaboração do Kombucha, apesar dos microorganismos estarem presentes tanto no líquido fermentado quanto no biofilme de celulose. O biofilme forma inicialmente uma fina camada na superfície do líquido, e as bactérias produtoras de celulose se encaminham para esta camada e se agregam, tornando-se maior à medida que a fermentação avança e com as fermentações subsequentes, formando múltiplas camadas conectadas por filamentos (May et al., 2019).

Os principais microorganismos presentes na SCOBY pertencem a gêneros de bactérias lácticas, acéticas e leveduras, sendo os principais gêneros e espécies já identificados apresentados na tabela 3. Para que a fermentação ocorra, a SCOBY mantém as bactérias acéticas na sua superfície, permitindo a sua multiplicação na presença de oxigênio e projetando as leveduras para a parte inferior, o que viabiliza a fermentação anaeróbia (Coelho et al., 2020).

É importante ressaltar que, na maioria das vezes, não é possível conhecer a composição microbiana exata do kombucha popular, pois depende da fonte do inóculo para fermentação do chá (Jayabalan et al., 2014) e da localidade em que a SCOBY foi adquirida (Laavanya et al., 2021). Além disso, a razão entre leveduras e bactérias acéticas na produção de kombucha é importante para a produção de seus metabólitos. Uma vez que estas estirpes podem utilizar a sacarose adicionada, o aumento da população inicial de um desses grupos de microorganismos pode colonizar rapidamente os nichos, dominando a outra estirpe e influenciando a composição final dos metabólitos, devido à competição entre os microorganismos (Nguyen et al., 2015). Em kombuchas comercializados no continente europeu e nos Estados Unidos, foram identificadas a presença de microorganismos probióticos (Andresen et al., 2022; Yang et al., 2022). Pela legislação brasileira, não é permitida a adição de microorganismos com alegação probiótica ao kombucha (BRASIL, 2019).

Os microorganismos do gênero *Acetobacter* foram identificados como os formadores da rede de celulose, a qual forma a base física para o desenvolvimento da simbiose. A fermentação prolongada dos açúcares pelas leveduras e estirpes bacterianas resulta na formação de ácidos, dióxido de carbono, e etanol, que são conservantes naturais e responsáveis por manter a qualidade do kombucha durante a fermentação prolongada (Dutta et al., 2019). As enzimas produzidas pelas leveduras convertem sacarose em glicose e frutose, produzindo etanol e dióxido de carbono. Além disso, as enzimas presentes nas bactérias oxidam o etanol, produzindo ácido acético, reduzindo o pH do chá, e convertem glicose a ácido glicurônico e frutose em ácido acético (Dufresne e Farnworth, 2000; May et al., 2019). Além disso, a cafeína e as xantinas presentes nos chás estimulam a síntese de celulose pelas bactérias (Dufresne e Farnworth, 2000).

Tabela 3. Microorganismos identificados no líquido fermentado do kombucha e SCOBY

Classificação	**Estirpes**	**Referência**
Bactérias ácido lácticas	*Oenococcus oeni, Lactobacillus nagelii, Lactobacillus satsumensis, Lactococcus* spp., *Leuconostoc* spp., *Enterococcus* spp.	Coton et al. 2017; Marsh et al., 2014
Bactérias ácido acéticas	*Acetobacter lovaniensis, Acetobacter okinawensis, Acetobacter peroxydans, Acetobacter syzygii, Acetobacter tropicalis, Gluconacetobacter eurapaeus, Gluconacetobacter hansenii, Gluconacetobacter intermedius, Gluconacetobacter liquefaciens, Gluconacetobacter xylinus, Gluconobacter cerinus, Gluconobacter oxydans, Gluconacetobacter entanii, Tanticharoemia sakaeratensis, Komagataeibacter rhaeticus, K. europaeus, K. intermedius*	Sievers et al., 1995; Coton et al. 2017; Marsh et al., 2014; Gaggia et al., 2019; Arikan et al., 2020; Harrison and Curtis, 2021; Villarreal-Soto et al., 2020; Andresen et al., 2022; Yang et al., 2022

Tabela 3. continuação

Leveduras	*Candida boidinii, Dekkera anomala, Dekkera bruxellensis, Hanseniaspora valbyensis, Wickerhamomyces anomalusPichia membranifaciens, Saccharomyces cerevisiae, Saccharomyces uvarum, Torulaspora microellipsoides, Zygosaccharomyces bailii, Zygotorulaspora florentina, Zygosaccharomyces bailii, Schizosaccharomyces pombe, Torulaspora delbrueckii, Rhodotorula mucilaginosa, Candida stellata, Brettanomyces bruxellensis, Davidiella* spp.*, Wallemia* spp.*, Lachancea* spp.*, Leucosporidiella* spp.*, Kazachstania* spp.*, Kluyveromyces* spp.*, Naumovozyma* spp.*, Meyerozyma* spp.*, Saccharomyces* spp.*, Hanseniaspora* spp..	Teoh et al., 2004; Coton et al. 2017; Marsh et al., 2014; Chakravorty et al., 2016; Harrison and Curtis, 2021; Fabricio et al., 2022; Teoh et al., 2014; Gaggia et al., 2019; Tran et al., 2020; Villarreal-Soto et al., 2020; Harrison and Curtis, 2021; Torán-Pereg et al., 2021; Fabricio et al., 2022; Landis et al., 2022
Outras	*Bifidobacterium* spp. *Propionibacterium* spp., *Thermus* spp., *Allobaculum* spp., *Ruminococcaceae incertae sedis*	Marsh et al., 2014

O substrato mais utilizados para a preparação de Kombucha é o chá preto, apesar de chá verde também ser utilizado. Uma das formas de preparo é, utilizando Kombucha previamente fermentado, adicionar chá preto adoçado e frio. Após 8 a 12 dias, a primeira fração de Kombucha está pronta: uma parte é retirada para consumo, enquanto na outra adiciona-se mais chá preto. As películas formadas na SCOBY podem ser retiradas para iniciar novas culturas. Outro método de produção é cobrir o recipiente com papel toalha ou filtros amarrados com elástico e deixar a bebida fermentar por 7 a 8 dias. Parte da bebida é refrigerada para consumo e a outra parte para inicia uma nova fermentação. O processo de fermentação do kombucha é realizado em local fechado a 22-30 °C e ocorre após cerca de 7 a 10 dias. A cada fermentação, uma nova película é formada na superfície da cultura anterior. (Chadrakala et al., 2019). A figura 7 resume as etapas de elaboração da bebida.

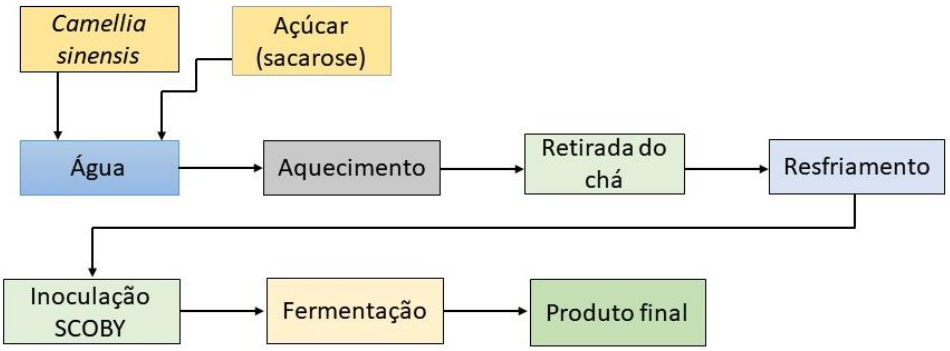


Figura 7. Etapas de elaboração de Kombucha (Adaptado de Mo et al., 2008). Nota: SCOBY – *Symbiotic Culture of Bacteria and Yeasts*

No início do processo de fermentação do kombucha, as leveduras produzem a enzima invertase, a qual quebra a sacarose em seus monossacarídeos, glicose e frutose. A partir desta quebra ocorre a simbiose entre os microorganismos (May et al., 2019). A partir da glicólise, ocorre formação de etanol. Por outro lado, as bactérias acéticas utilizam a glicose para produzir ácido glicônico e etanol. Este ácido estimula as leveduras a produzir etanol, enquanto as bactérias acéticas utilizam para produção de ácido acético (Coelho et al., 2020).

A composição da bebida do kombucha depende da matéria-prima utilizada como fonte de nutrientes, a concentração do chá, composição microbiana da SCOBY, tempo e temperatura da fermentação e pH do processo. Mudanças em alguns destes parâmetros podem impactar a qualidade final do produto e as propriedades nutricionais, biológicas e sensoriais (Emiljanowicz e Malinowska-Pańczyk, 2019). A composição química do kombucha apresenta diversos ácidos orgânicos como, acético, glicônico, glicurônico, cítrico, láctico, málico, tartárico, malônico, oxálico, succínico, pirúvico, úsnico; açúcares como sacarose, glicose e frutose, vitaminas do complexo B e vitamina C, aminoácidos, aminas biogênicas, purinas, pigmentos, lipídeos, proteínas, etanol, gás carbônico, fenóis, polifenóis, minerais, ânions, ácido sacárico-D-lactona e produtos finais provenientes do metabolismo de leveduras e bactérias (Jayabalan et al., 2014).

De forma geral, a fermentação é um processo que utiliza a capacidade de multiplicação e atividade metabólica de microorganismos para a estabilização e transformação de material biológico. Durante a fermentação, metabólitos que promovem a sobrevivência e a multiplicação de microorganismos são produzidos, enquanto inibe a multiplicação e proliferação de outros (Terefe e Augustin, 2019). Estas

características são tempo-dependentes e determinadas pela microbiota, assim como pelos parâmetros físico-químicos, incluindo temperatura, pH, atividade de água, potencial de óxido-redução e substrato disponível Estes parâmetros estão associados aos efeitos nas propriedades finais e caracterização dos produtos fermentados (Terefe e Auguntin, 2019; Marco et al., 2021).

2.5.1. Cultura simbiótica de bactérias e leveduras - SCOBY

O nome SCOBY foi criado por Len Porzio na década de 1990 para diferenciar a nova cultura formada no Kombucha a partir da "cultura mãe". O biofilme deve ter entre 6 e 12 mm de espessura. Se muito fino, pode sofrer contaminação, porém, se estiver muito espesso, pode impedir a entrada de oxigênio. Sua cor varia de branco a marrom claro. SCOBYs saudáveis sempre produzirão novas culturas e são resistentes ao rasgo quando pressionados entre o polegar e o dedo indicador (Soares et al., 2021) (Figura 8).

Figura 8. imagens da SCOBY utilizada para a fermentação de Kombucha (Adaptado de Soares et al., 2021). Nota: SCOBY – cultura simbiótica de bactérias e leveduras, do inglês, *symbiotic culture of bacteria and yeasts*

A SCOBY vem ganhando atenção por produzir como subproduto celulose de produção bacteriana, proveniente da fermentação das bactérias acéticas. Sua composição química é similar à proveniente das plantas, com significativa diferença na nanoestrutura das fibras, conferindo melhores propriedades físico-químicas (Song et al., 2017). Esta fonte de celulose bacteriana passou por um processo de transição tecnológica, saindo da posição de resíduo sem valor agregado para uma fonte promissora no desenvolvimento de novos materiais e produtos. Alguns exemplos são o couro vegano que vem sendo utilizado na indústria da moda para fabricação de roupas e

sapatos (Soares et al., 2021) e a aplicação da SCOBY em preparações culinárias (Torán-Pereg et al., 2021).

Durante a fermentação do kombucha, a atividade metabólica de leveduras osmofílicas e tolerantes ao meio ácido (*Zygosaccharomyces*, *Pichia*, *Brettanomyces*, *Saccharomyces*, *Saccharomycodes* e *Candida*) em simbiose com as bactérias acéticas, o principal grupo de bactérias presente na SCOBY, ativam sua multiplicação para a produção de celulose utilizando glicose e frutose como precursores, que são provenientes do metabolismo das leveduras (Cottet et al., 2020). Com relação às bactérias acéticas, além do gênero *Acetobacter*, *Gluconobacter* também possui resultados satisfatórios na formação da celulose (Fernandes et al., 2020).

Bioquimicamente, a simbiose entre as leveduras e as bactérias acéticas acontece da seguinte forma: a sacarose adicionada para o preparo da bebida será absorvida pelas leveduras através de transportadores de açúcares presentes na superfície de suas células, e por ação da enzima invertase a sacarose é quebrada em seus monossacarídeos glicose e frutose. A glicose será destinada a glicólise, formando piruvato e posteriormente acetato, que entrará no Ciclo de Krebs. O piruvato, por ação da piruvato descarboxilase, formará acetaldeído, que ao ser transformado por aldeído desidrogenase, ocorrerá a produção de etanol. Através de enzimas presentes na membrana das bactérias acéticas, o etanol será convertido em acetaldeído pela enzima álcool desidrogenase, que então será transformado em ácido acético pela enzima aldeído desidrogenase. Estas vias estimularão a multiplicação das leveduras (Laavanya et al., 2021).

Outras vias bioquímicas também são ativadas nas bactérias acéticas. A frutose proveniente da quebra da sacarose pelas leveduras também será metabolizada em ácido acético. Além disso, a glicose será transportada para o citoplasma das células, e pela ação da glicose oxidase formará os ácidos orgânicos. Além disso, a glicose passará por outras reações bioquímicas. Será convertido em glicose-6-fosfato pela glicoquinase e, posteriormente, em glicose-1-fosfato pela fosfoglicomutase, que por ação da uridina difosfato glicose-pirofosforilase será transformada em uridina difosfoglicose. Este último composto é o precursor de celulose das bactérias acéticas, que então será destinado para a síntese de celulose (Figura 9) (Laavanya et al., 2021).

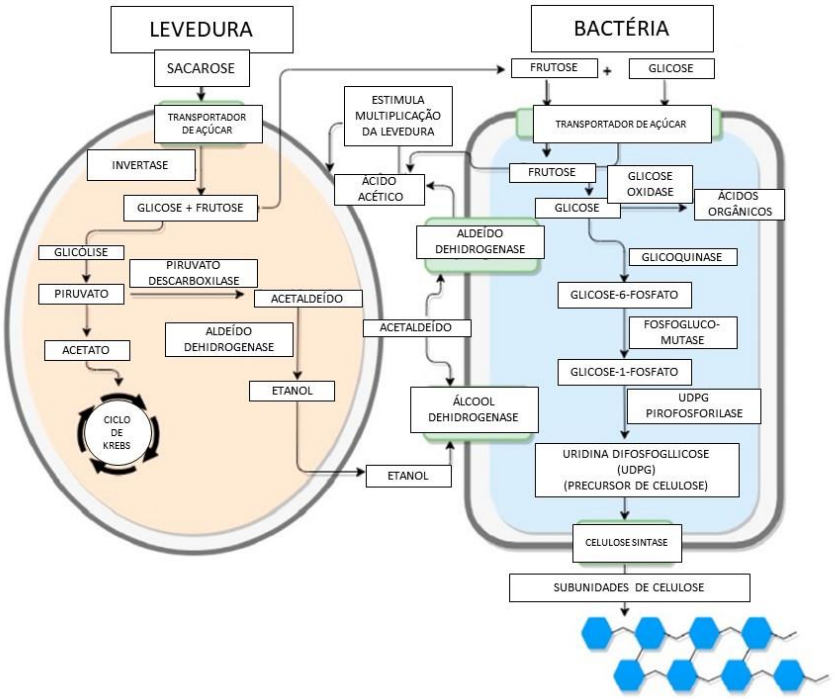


Figura 9. Exemplo de metabolismo de substratos pela cultura simbiótica de bactérias e leveduras utilizada para produção de kombucha (Adaptado de Laavanya et al. (2021).

2.5.1.1. Bactérias ácido acéticas

As bactérias ácido acéticas foram primeiramente descritas como "bactéria vinagre" por Louis Pasteur há mais de 150 anos atrás. São um grupo de bactérias importantes e diversas que estão envolvidas na produção de ácido acético durante a elaboração de vinagre (Lynch et al., 2019). São microorganismos Gram-negativos, aeróbios, não formadores de esporos. A partir da sua presença em alimentos, o seu metabolismo promove a oxidação incompleta de açúcares e álcoois, levando ao acúmulo de ácidos orgânicos como produto final (Guillamón e Mas, 2011). Outro produto proveniente do seu metabolismo é a celulose (Dourado et al., 2016). São classificadas como bactérias acéticas devido a sua capacidade de oxidar etanol em ácido acético (Dourado et al., 2016). São conhecidas pela produção de determinados alimentos e bebidas, como vinagre, kombucha e pela fermentação de sementes de cacau. Também podem ser contaminantes de vinho, cerveja, refrigerante e frutas.

Por mais de 100 anos, apenas o gênero *Acetobacter* pertenceu à classificação de bactéria acética, com apenas uma espécie, *Acetobacter aceti*. Após 37 anos de descoberta do gênero *Acetobacter*, foi proposto o gênero *Gluconobacter*, o qual possui a característica de oxidar glicose em ácido glicônico e transformar etanol em ácido

acético, diferindo desta forma do gênero *Acetobacter* (Yamada e Yukphan, 2008). Atualmente, as bactérias acéticas englobam doze gêneros (Sengun e Karabiyikli, 2011).

São conhecidas por terem uma habilidade única de fermentação, chamada de fermentação oxidativa, a qual é um processo de oxidação incompleta onde os substratos são oxidados por desidrogenases ligados a membrana e os produtos oxidados são liberados para o meio de cultura. A disponibilidade de oxigênio deve ser constante, visto que pode afetar a fermentação e a produtividade das bactérias se não disponível em concentrações adequadas. A fermentação oxidativa é uma característica importante das bactérias acéticas como a liberação dos produtos correspondentes à oxidação. O principal exemplo é a produção de ácido acético através do etanol (Saichana et al., 2015).

No kombucha, as bactérias acéticas estão presentes em maior número. Apesar da composição microbiana do kombucha depender das condições de cultivo, como disponibilidade de nutrientes, temperatura e presença de oxigênio, as bactérias acéticas fazem parte de uma comunidade bacteriana relativamente estável e responsável pela produção de ácido acético através do etanol, ácido glicônico, glicurônico e ácido D-sacárico-1,4-lactona através da glicose (De Roos e De Vuyst, 2018) e pela membrana de celulose (Nguyen et al., 2015).

2.5.1.2. Leveduras

Leveduras são microorganismos eucarióticos, que vivem em uma ampla variedade de nichos ecológicos, principalmente na água, solo, ar e na superfície de plantas e frutas (Maicas, 2020). As espécies mais conhecidas pertencem ao subfilo Saccharomycotina. Este grupo diversificado inclui o principal sistema de modelo eucariótico, *Saccharomyces cerevisiae*, e a levedura comensal humana e patógeno oportunista *Candida albicans*, além de outras espécies (Hittinger et al., 2015). Algumas espécies de leveduras podem ser consideradas probióticas por resistirem a passagem pelo trato gastrointestinal ou pela inibição de patógenos entéricos, como *Debaryomyces hansenii, Torulaspora delbrueckii, Kluyveromyces lactis, Kluyveromyces marxianus, Kluyveromyces lodderae*. Porém, apenas *Saccharomyces boulardii* apresentou efeitos clínicos benéficos em estudos (Moslehi-Jenabian et al., 2010).

As leveduras são organismos heterotróficos nos quais o metabolismo energético e de carbono estão interconectados e o anabolismo é associado ao catabolismo.

Metabolizam preferencialmente açúcares, que são convertidos principalmente em etanol e gás carbônico, mas podem utilizar outros substratos, como aminoácidos e ácidos orgânicos, polióis, álcoois, ácidos graxos, entre outros, dependendo das espécies. De acordo com a via utilizada para a produção de energia, as leveduras podem ser classificadas como não fermentativas, com apenas metabolismo respiratório; fermentativas obrigatórias, apenas capazes de metabolizar glicose através de fermentação alcoólica; e fermentadoras-facultativas, exercendo metabolismo respiratório ou fermentativo, ou ambos, em um metabolismo misto dependendo das condições de multiplicação, tipo e concentração das fontes de carbono, e/ou disponibilidade de oxigênio (Tofalo e Suzzi, 2016).

As leveduras são conhecidas por serem microorganismos produtores de etanol, característica explorada para a produção de bebidas fermentadas. Em condições anaeróbicas, a levedura utiliza ácido pirúvico gerado pelo catabolismo de açúcar como forma de redução da coenzima NADH. O ácido pirúvico é convertido em acetaldeído e então, em etanol, regenerando NAD+ para permitir a glicólise e a produção de ATP. A reação que leva a produção de etanol é importante e é a base para as principais indústrias de fermentação. Além disso, a descarboxilação de piruvato pelas leveduras libera gás carbônico, propriedade explorada na fabricação de pães por fermento biológico. O motivo das leveduras produzirem álcool é devido ao fato das suas células necessitarem de energia e para manter o balanço de oxi-redução ao consumirem açúcares em condições de anaerobiose. Durante a fermentação de açúcar pelas leveduras, a produção de etanol, gás carbônico, ATP, balanço de oxi-redução e a multiplicação das leveduras estão relacionados, e a taxa de fermentação está diretamente ligada com a reprodução celular (Walker e Walker, 2018).

O papel das leveduras na fermentação de alimentos inclui a produção de álcool, melhora da textura de alimentos devido a fermentação, preservação do alimento devido a acidificação e produção de toxinas, melhora dos valores nutricionais e remoção de fatores anti-nutricionais, desenvolvimento de *flavor*, aroma, textura e de peptídeos bioativos e produção de vitaminas (Rai e Kumaraswamy, 2017). Possuem potencial biotecnológico na indústria de alimentos e são responsáveis pela produção de grande parte de produtos fermentados, desde bebidas alcoólicas e não alcoólicas, produtos lácteos como os queijos, fabricação de pães e condimentos (Rai et al., 2017; 2019).

Com relação a composição de leveduras no Kombucha, até o momento não há estirpes associadas de forma universal com a produção da bebida, sendo as espécies mais comuns osmotolerantes, fermentativas e produtoras de ácido. As interações das diversas culturas são complexas e, portanto, é difícil determinar a contribuição exata das leveduras na composição química da bebida fermentada (Teoh et al., 2004). As leveduras não apenas contribuem para o *flavor* do kombucha, mas também estimulam as bactérias acéticas a produzir ácido glicurônico (Nguyen et al., 2015).

2.5.2. Benefícios do Kombucha à saúde

Sabe-se que o chá de *Camellia sinensis* pode exercer diversos benefícios à saúde, como atividades antioxidante, anti-inflamatória, antimicrobiana, antiviral, reduz a ocorrência de câncer, é antimutagênico e vermífugo, propriedades antidiabética e antialérgica, melhora de peso corporal, cardio, neuro e hepatoprotetor (Aboulwafa et al., 2019). Estudos *in vitro* e em modelos animais mostram que o kombucha de *C. sinensis* pode exercer atividades similares ao extrato não fermentado de *C. sinensis*, (Ramani et al., 2019; Villareal-Soto et al., 2019; Kaewkod et al., 2019; Cardoso et al., 2020; Srihari et al., 2013a; Jayabalan et al., 2008; Bhattacharya et al., 2011; Greenwalt et al., 1998; Wang et al., 2013; Srihari et al., 2013b).

Outro benefício do Kombucha é a inativação de micotoxinas. Taheur et al. (2020) observou a biodegração de Aflatoxina B1 por microorganismos isolados do Kombucha, além da redução da sua letalidade em células hepáticas. Ismaiel et al. (2015) observou a inativação de patulina produzida por fungos em maçãs. Até o momento, não há estudos clínicos que mostrem benefícios do consumo de kombucha (Kapp et al., 2019). Apesar do apelo de alimento funcional, o kombucha não possui aprovação de órgãos reguladores sobre potenciais efeitos funcionais na saúde, devido à falta de estudos clínicos que mostrem os efeitos desta bebida na saúde humana (Morales, 2020).

Alguns estudos relacionaram o consumo de kombucha com determinados efeitos tóxicos à saúde humana. Até o momento, poucos estudos foram publicados. Os casos relatados na literatura são de um jovem de 22 anos, portador do vírus da imunodeficiência humana, apresentando hipertermia severa, acidose láctica e insuficiência renal aguda 15 horas após a ingestão de 1L de kombucha não pasteurizado. Após tratamentos com antibióticos, o paciente apresentou melhora. O kombucha consumido passou por análise microbiológica e foi encontrada multiplicação de

Candida sp. após 33 dias. Uma pessoa que compartilhou a mesma bebida não apresentou efeitos adversos. Concluiu-se que o kombucha pode apresentar efeitos colaterais em pacientes imunodeprimidos (Kole et al., 2009).

Outros casos de toxicidade envolveram uma mulher com 55 anos de idade com histórico de alcoolismo, apresentou icterícia por 6 semanas após consumir 2 copos de kombucha por dia durante 2 meses. Também apresentou aumento nas transaminases hepáticas, que normalizaram 7 semanas depois. Outra paciente de 51 anos apresentou xerostomia, tontura, náuseas, vômitos, dor de cabeça e dor no pescoço, em uso de hormônio tireoidiano e reposição de estrogênio. Ela relatou o consumo de meio copo por dia de kombucha por meses. Por fim, outros casos foram de pacientes apresentando tremores, falta de ar, agitação, hipotensão, taquicardia e taquipnéia. Foi encontrado aumento nos leucócitos e tratados como reação alérgica. Em nenhum dos casos os efeitos colaterais foram relacionados ao consumo de kombucha, e portanto, sem evidências de efeitos colaterais (Srinivasan et al., 1997).

2.5.2. Kombucha e atividade antioxidante

Segundo Halliwell e Gutteridge (2007), antioxidante é uma substância que, quando presente em baixas concentrações comparadas com um substrato oxidante, inibe a oxidação deste substrato. A partir desta classificação, compostos fenólicos, os quais são derivados do metabolismo secundário das plantas, protegem diversos organismos de oxidação, portanto, compostos fenólicos são considerados como antioxidantes naturais (Granato et al., 2017).

Radicais livres são átomos, moléculas ou íons com elétrons desemparelhados altamente instáveis e ativos através de reações químicas com outras moléculas. São derivados de três elementos: oxigênio, nitrogênio e enxofre. Os radicais livres com o oxigênio como elemento central são denominadas como espécies reativas de oxigênio (ERO) e incluem os radicais superóxido (O_2^-), hidroxila (OH·), peroxil (ROO·), alcóxi (RO·) e óxido nítrico (NO·) (Gulcin, 2020).

Organismos vivos são expostos constantemente a espécies reativas de oxigênio, os quais correm como subprodutos do metabolismo, processo respiratório e autooxidação de xenobióticos, ou como resultados de estresse desencadeado por diversas doenças. O estresse oxidativo e resultados da desproporção entre ERO e defesa antioxidante. O estresse oxidativo desregula diversas funções celulares e causa várias

condições patológicas nas quais o excesso de ERO causa uma sobrecarga nas defesas antioxidantes do organismo, levando a injúria tecidual e morte celular acelerada como base de muitas doenças (Gulcin, 2020).

Considerando a avaliação da atividade antioxidante em modelo celular através da indução de estresse oxidativo, alguns estudos foram conduzidos utilizando kombuchas. Bhattacharya et al. (2011) induziu o estresse oxidativo em células hepáticas com hidroperóxido de terc-butila, para avaliar a produção intracelular de ERO. vou observado que kombucha de chá preto reduziu a produção de ERO nas células hepáticas, quando comparado com células tratadas apenas com hidroperóxido de terc-butila. Em outro estudo, foi avaliado o nível de ERO tecidual em diferentes tecidos de murinos (fígado, rim, pâncreas e coração) com diabetes induzida por aloxana, após tratamento com kombucha de chá preto. Foi observado que o kombucha inibiu significativamente a produção de ERO (Bhattacharya et al., 2013).

Com relação a kombuchas elaborados a partir de novos substratos, foi observado que kombucha elaborado com hortelã reduziu a produção intracelular de ERO induzida por H_2O_2 em células embrionárias renais com efeito similar ao ácido ascórbico (potente antioxidante). Após a administração de kombuchas elaborados com chá oolong e chá oolong combinado com hortelã, foi observado uma tendência de inibição na produção de ERO, independente doa dias de fermentação (dias 0 - antes da fermentação e após 14 dias) (Tanticharakunsiri et al., 2020).

2.5.3. Kombucha e atividade anti-inflamatória

Inflamação é quando determinada parte do organismo responde a uma injúria ou infecção. Isso ocorre quando o organismo libera citocinas que levam a uma resposta imune para lutar contra determinada infecção ou injúria tecidual. Uma vez que a injúria ou infecção é tratada, o processo inflamatório termina. Porém, a inflamação crônica é uma resposta imune anormal, na qual o processo inflamatório não termina ou inicia quando não presença de injúria ou inflamação. Com o decorrer do tempo, a inflamação crônica pode danificar células saudáveis, tecidos e órgãos, podendo desenvolver doenças como câncer, doenças cardiovasculares, diabetes, asma, Alzheimer e doenças autoimunes (National Cancer Institute, 2023).

A resposta imunológica pode ser dividida nas seguintes fases distintas: homeostase e vigilância; detectar e iniciar a resposta imune (resposta pró-inflamatória);

restauração da homeostase imunológica e sob certas condições fisiológicas e patológicas, a formação de memória imune inata (Austermann et al., 2022).

Os macrófagos possuem papel chave na manutenção da homeostase e regeneração tecidual após uma injúria (Viola et al., 2019). Na resposta inflamatória, os macrófagos ativados produzem grandes quantidades de fatores inflamatórios, como interleucina-6 (IL-6), fator de necrose tumoral-alfa (TNF-α) e óxido nítrico (NO) para induzir lesão tecidual no local inflamatório (Zhu et al., 2021). Embora os macrófagos sejam essenciais para o controle e eliminação eficazes de infecções, remoção de fragmentos e células mortas, promovendo o reparo tecidual e a cicatrização de feridas, eles também contribuem para o dano tecidual e patologia durante infecções e doenças inflamatórias (Moghaddam et al., 2018), resultando em uma associação causal de macrófagos com estados de doença, como fibrose, obesidade e câncer (Wynn et al., 2013).

Compostos bioativos como catequinas, ácidos clorogênicos e cafeína já foram estudados anteriormente como potenciais substâncias anti-inflamatórias (Hwang et al., 2016; Farah and Lima, 2019; Musial et al., 2020). Levando em consideração que estes compostos são comumente identificados em kombuchas (Villarreal-Soto et al., 2019; 2020), torna-se essa bebida como um potencial agente anti-inflamatório. Villarreal-Soto et al. (2019) avaliaram a atividade anti-inflamatória do chá não fermentado e do kombucha de chá preto contra a enzima 5-LOX e indicam uma melhora na atividade anti-inflamatória após a administração de kombucha.

2.5.5. Benefícios de Kombucha produzidos a partir de novos substratos

Recentemente, estudos vêm sendo conduzidos para a elaboração de bebidas fermentadas por novos substratos para a obtenção de kombucha com potenciais benefícios à saúde (Battikh et al., 2012; Ayed et al., 2016; Vitas et al., 2018; Rahmani et al., 2019; Gaggia et al., 2019; Tu et al., 2019; Aung e Eun, 2021) e boa aceitação sensorial, considerando diferentes dias de fermentação (Ayed et al., 2016; Vitas et al., 2016; Tu et al., 2019). Os novos substratos apresentaram composição microbiológica próxima ao kombucha tradicional e, em alguns casos, redução do teor de etanol e ácido acético (Velincanski et al., 2014; Ayed et al., 2016; Gaggia et al., 2019; Aung e Eun, 2021), boa adaptação da SCOBY, aumento de compostos fenólicos, atividade antioxidante e antihipertensiva (Rahmani et al., 2019; Tu et al., 2021), atividade

antimicrobiana e antifúngica (Velincanski et al., 2014; Battikh et al., 2012; Ayed et al., 2016). Aung e Eun, (2021) encontraram aumento nos teores de compostos bioativos após 14 dias de fermentação em kombuchas elaborados com *Porphyra dentata*.

Além dos benefícios já listados, estudos em murinos indicam que o Kombucha tradicional e de outras fontes alimentares pode exercer benefícios no manejo da Diabetes Mellitus, promovendo melhora do estresse oxidativo e perfil lipídico (Bhattacharya et al., 2013; Zubaidah et al., 2018) e efeito hipoglicêmico (Srihari et al., 2013b).

2.5.5. Kombucha a partir do café e seus subprodutos

Além dos novos ingredientes utilizados para a elaboração da bebida, alguns estudos já foram conduzidos com o intuito de elaborar Kombucha utilizando a bebida de café como principal substrato, pois é uma das bebidas mais consumidas mundialmente junto com os chás (Statista, 2022). Watawana et al. (2015) elaborou Kombuchas a partir de café moído e solúvel, e observaram aumento atividade inibitória de α-amilase e da atividade antioxidante da bebida após a fermentação, estando relacionado com o aumento nas concentrações de ácido clorogênico e ácido cafeico durante 7 dias de fermentação.

Zofia et al. (2020) avaliaram Kombuchas elaborados a partir de café verde. Baixas concentrações de polifenóis e flavonóides foram encontradas aos 7 dias de fermentação. Porém, com 14 e 28 dias de fermentação, os valores aumentaram, bem como a atividade antioxidante da bebida, inclusive a nível celular. Os autores também avaliaram as propriedades antienvelhecimento dos extratos fermentados de café verde, e observaram a inibição *in vitro* de colagenase e elastase aos 14 dias de fermentação.

Bueno et al. (2021) elaboraram Kombucha utilizando a bebida de café extraída de grãos de torrefação média-escura e adicionou probióticos (*Lactobacillus casei* e *L. rhamnosus*). O kombucha com probiótico manteve a viabilidade destes microorganismos sob simulação de fluidos gástricos e intestinais. O número de células viáveis também ficou acima do mínimo recomendado para um produto ser considerado probiótico. Até o momento, não há estudos sobre a elaboração de Kombucha utilizando subprodutos provenientes da colheita do café (frutos e folhas do cafeeiro).

3. JUSTIFICATIVA

Considerando que o Brasil é o maior país produtor de café e que desde o seu cultivo até a produção da bebida a quantidade de subprodutos descartados é expressiva, tornam-se necessários estudos que avaliem a possibilidade de reaproveitamento destes resíduos na alimentação. Além disso, alguns destes subprodutos, como a casca do fruto e a folha do cafeeiro, possuem compostos bioativos que podem exercer diversos benefícios à saúde. Neste contexto, a utilização de tecnologias naturais para fabricação de bebidas potencialmente saudáveis, como o Kombucha pode indicar uma alternativa sustentável e saudável para consumo.

4. OBJETIVO GERAL

Elaborar bebidas fermentadas de subprodutos da cadeia de produção do café a partir de uma cultura simbiótica de bactérias e leveduras (Kombucha), caracterizá-las química, físico-química, sensorial e microbiologicamente e avaliar seus efeitos em modelo *in vitro* de estresse oxidativo e atividade anti-inflamatória.

5. OBJETIVOS ESPECÍFICOS

- Produzir kombuchas a partir de infusões de cascas de frutos e folhas do cafeeiro;
- Analisar a composição química físico-química e de compostos orgânicos voláteis das infusões e após a fermentação;
- Analisar sensorialmente as bebidas
- Analisar a composição microbiológica da cultura simbiótica de bactérias e leveduras
- Avaliar a atividade antimicrobiana dos kombuchas frente a microorganismos patogênicos de origem alimentar
- Avaliar *in vitro* a atividade antioxidante do kombucha de cascas do cafeeiro e chá preto e em modelo de nefropatia diabética induzida;
- Avaliar *in vitro* a atividade anti-inflamatória de kombuchas elaborados com cascas dos frutos do cafeeiro e chá preto.

Os objetivos do presente trabalho de tese serão apresentados sob a forma de quatro estudos.

ESTUDO 1: Kombuchas de cascas de frutos e folhas do cafeeiro: composição química e atividade antibacteriana

Diversas etapas estão envolvidas na produção de café. A poda e a colheita dos grãos de café produzem folhas de café com subprodutos. O processamento dos frutos produz as cascas. Estes subprodutos são grande fonte de compostos bioativos, portanto, a conversão dos resíduos do café em ingredientes alimentares pode ser importante para sustentabilidade e saúde. Kombucha é uma bebida fermentada elaborada de chá de *Camellia sinensis* adoçado, no qual uma cultura simbiótica de bactérias e leveduras é adicionada. Recentemente, ingredientes alternativos, principalmente subprodutos, vem sendo utilizados para o preparo de kombucha. O objetivo deste trabalho foi elaborar kombuchas de infusões de cascas dos frutos e de folhas do cafeeiro e avaliar sua composição química, físico-química e atividade antibacteriana. Folhas de café comerciais e amostras de cascas de café foram utilizadas para preparar as infusões e bebidas fermentadas. Kombucha elaborado com chá preto comercial (10%), sacarose (10%) e SCOBY (2,5%) foram adicionados às infusões e fermentadas por 0, 3, 6 e 9 dias. Kombucha de chá preto foi utilizado como controle. O perfil microbiológico foi avaliado, e compostos fenólicos, açúcares simples foram analizados por CLAE-DAD. Acidez e concentração de sólidos solúveis foram também avaliados. A atividade anbacteriana *contra Escherichia coli* American Type Culture Collection 25922, *Salmonella enteritidis* ATCC 13076, *Listeria monocytogenes* ATCC 11229 and *Lactiplantibacillus plantarum* ATCC 8414 foi avaliada através do diâmetro do halo de inibição. Na análise microbiológica, em todas as bebidas foi observada maior proporção de bactérias acéticas pertencentes ao gênero Komagateibacter e de leveduras pertencentes ao gênero Pichia. Foram observados aumento na acidez titulável e redução de pH, sólidos solúveis e sacarose (10g/100mL - 7g/100mL). Nos kombuchas de chá preto, foram observados redução de catequinas (44%, em média) e aumento de ácidos clorogênicos (19%) durante a fermentação. Nas infusões e kombuchas de cascas de frutos e folhas do cafeeiro, os principais compostos fenólicos foram os ácidos clorogênicos, com um aumento, em média, 102% e 30%, respectivamente. Outros compostos identificados foram rutina, quercetina, ácidos fenólicos livres, cafeína, trigonelina, porém mangiferina foi identificada somente nas bebidas de folha de café. Em relação a atividade antibacteriana, infusões de chá preto, cascas de café e folha de

café exibiram maior inibição contra *L.monocytogenes*. Após a incubação com kombuchas, a atividade inibitória de *E.coli* e *S.enteritidis* aumentou. Não foi encontrado inibição de *L.plantarum*. Os resultados mostram que as folhas e as cascas dos frutos do cafeeiro são subprodutos promissores para o preparo de kombucha, com elevadas quantidades de compostos bioativos e potenciais benefícios à saúde.

Palavras-chave: subprodutos de café; planta de café; fermentação; bebidas fermentadas; casca de cafe; colheita do café

1. Introduction

Coffee is among the most consumed foods globally. In 2020, the coffee world production was, approximately, 10.5 tons (ICO, 2021). Several steps are involved in coffee production. Pruning and harvesting generates coffee leaves as by-products that are generally considered of no or low value compared with the highly valuable coffee seeds, especially in organic crops. After the fruits are harvested, they may undergo different types of processing to release the seeds traditionally roasted and ground for the beverage extraction (DePaula et al., 2022). The first and main by-product generated during coffee processing is the cascara. The definition and composition of this by-product depends on the type of processing employed: the wet or dry method (Iriondo-DeHond et al., 2020). Along the years, an increase in these wastes has directly related to the rise of coffee consumption in the world (Alves et al., 2017).

These by-products are a great source of bioactive compounds and considering that the world population is also growing their conversion into food ingredients can be of great important for human fed; For centuries, both ingredients have been used for medicinal infusion preparation in Africa, where the coffee plant originated, and more recently, in Asia. These materials were proven to be non-toxic and rich in bioactive compounds, such as polyphenols and caffeine (Campa et al., 2012; Trevisan et al., 2016; de Almeida et al., 2018; Acidiri et al., 2020;) Moreover, coffee husk is an officially authorized food in the Europe (EFSA, 2021).

Kombucha is a fermented beverage made from sweetened black or green *Camellia sinensis* tea, to which a specialized culture is added. The latter is comprised of a symbiotic culture of bacteria and yeast (SCOBY), normally within a cellulose-type membrane (Leal et al., 2018; Rezac et al., 2019). The SCOBY is composed of yeast, lactic and acetic bacteria (Emiljanowicz and Malinowska-Pańczyk, 2019). Benefits such as antioxidant and antiinflamatory activity, colon, breast prostate and lung cells antiproliferative activity, antibacterial activities, liver protection, hypoglicemiant effect and weight loss in diabetic rats of kombucha were found *in vitro* and in animal studies, (Jayabalan et al., 2008; Bhattacharya et al., 2011; Srihari et al., 2013). The growing interest in kombucha has attracted the use of new raw materials for fermentation, such as different types of infusions , milk, and a wide range of agro-industrial materials, mainly by-products of fruit industries (Leonarski et al., 2022). The global kombucha market size was valued at USD 2.64 billion in 2021 and is expected to expand at an

annual growth rate (CAGR) of 15.6% from 2022 to 2030 (Grand View Research, 2021). Consumer awareness and popularity are the main reasons behind the current trend of the flourishing market and active research on kombucha (Kim and Adhikari, 2020).

Some studies previously evaluated the antibacterial activity of *C. sinensis* kombuchas against pathogenic bacteria and yeasts (Battikh et al., 2011; Bhattacharya et al., 2016; Kaewkod et al., 2019) and kombucha made with new raw materials (Vitas et al., 2018; Velicanski et al., 2014; Ahmed et al., 2020). Studies about the antibacterial activity of coffee cascara and coffee leaf are scarce, and at this moment, there is no studies evaluating the antimicrobial activity of coffee cascara and coffee leaf kombuchas. It is known that coffee has the ability to inhibit the growth of pathogenic and food spoilage microorganisms (Gloria et al., 2019) and share many bioactive compounds with coffee leaf and coffee cascara, so these byproducts are promising candidates for natural antibacterial agents.

The aim of this study was to elaborate Kombucha beverages made of coffee cascara leaves infusions and characterize its main chemical composition and antibacterial activity.

2. Material and Methods

The experimental design of the study is shown in Figure 1.

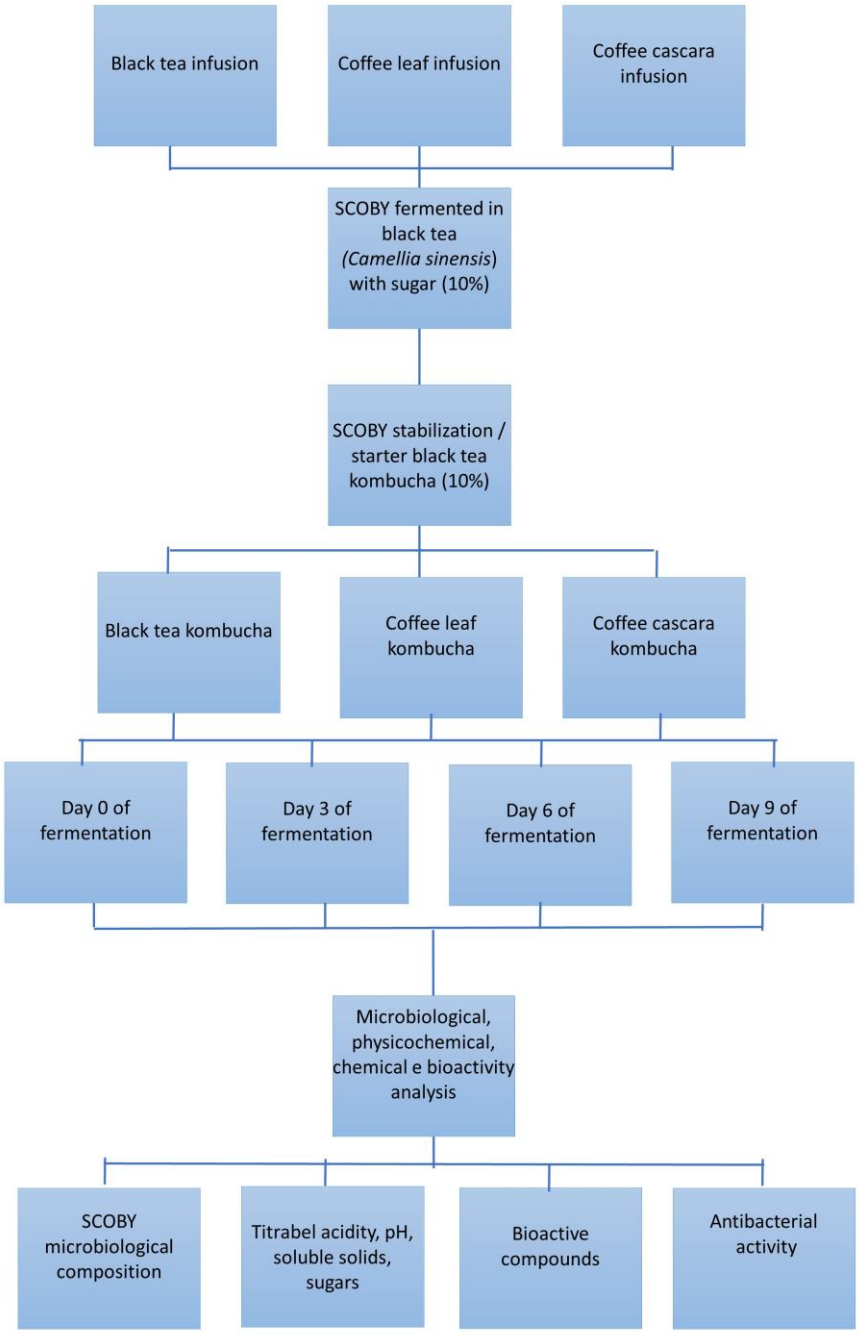


Figure 1. Experimental design of study 1

2.1. Reagents

Catechins, including (-)-catechin (>98%), (-)-epicatechin (>98%), (-)-gallocatechin (>98%), (-)-epigallocatechin, (-)-catechin gallate (>98%), (-)-gallocatechin gallate (>98%), (-)-epicatechin gallate (>98%) and (-)-epigallocatechin gallate (>98%), gallic acid (≥ 99%), 5-caffeoylquinic acid (≥ 95%) rutin (hydrate, ≥ 94%), quercetin (hydrate, ≥ 95%), kaempferol (≥ 97%), caffeic acid (≥ 98%), ferulic acid (≥ 99%), p-coumaric acid (≥ 98%), sinapic acid (≥ 98%), benzoic acid (≥ 99.5%), 3,4-dihydroxybenzoic acid (≥ 97%), hippuric acid (≥ 98%), 3,4-dihydroxyphenylacetic acid (≥ 98%), 4-hydroxyphenylacetic acid (≥ 98%), vanillic acid (≥ 97%), dihydrocaffeic acid (≥ 98%), caffeine (≥ 99%) and trigonelline (trigonelline hydrochloride, ≥ 98.5%) for HPLC references were provided by Sigma Chemical Co (St. Louis, Missouri, United States).

For dicaffeoylquinic acids (diCQA), a mixture of 3,4- diCQA; 3,5- diCQA; and 4,5-diCQA from Carl Roth (Karlsruhe, Germany) was used. Feruloylquinic acids (FQA), were synthesized from 3-feruloylquinide and 4- feruloylquinide (FQL) by hydrolysis in 50% aqueous tetrahydrofuran (Huynh-Ba, 1995). Mangiferin standard was isolated and purificated in the Organic and Inorganic Chemistry Laboratory at Federal University of Ceará (Lima, 2020); sucrose was provided by PROQUIMIOS Produtos Científicos, Rio de Janeiro, Brazil; glucose, galactose and fructose were provided by VETEC Química Fina, Rio de Janeiro, Brazil.

2.2. Samples

A leading commercial (Chinese) black tea brand was purchased in a Brazilian food market; organic arabica coffee (*Coffea arabica*) cascara samples were acquired directly from producers (in Espírito Santo, Brazil - dry processed, and in Nicaragua - wet processed). A leading commercial *C. arabica* leaf tea from Nicaragua was purchased from a reliable trader in Canada. These samples were used to elaborate the infusions for Kombucha preparation.

2.3. Infusions preparation, kombucha consortium, and fermentation

Kombucha consortium and fermentation - The Kombucha consortium was part of the collection of the Microbiology Institute of the Federal University of Rio de Janeiro, Brazil. Previously cultivated in green tea, the consortium was separately fermented

three times in black tea, coffee cascara and coffee leaf infusion prior to experimental use to stabilize the microbial consortium in these matrixes (Villarreal-Soto et al., 2020). All kombucha beverages were prepared according to the protocol described by Nummer (2013).

Infusions- Black tea and coffee cascara infusions at 3% (weight/volume-w/v) coffee leaves at 2.5% (w/v) were prepared pouring water at 95 °C over the raw material and letting it steep for 10 min. *Black tea kombucha* - Black tea kombucha was prepared mixing 10% (v/v) of black tea infusion, 10% (w/v) sugar and 2.5% (v/v) of a symbiotic culture of bacteria and yeast (SCOBY) and letting the mixture ferment at 23 °C. Samples were collected before (day 0) and after 3; 6 and 9 days of fermentation. An extra sample with pH 2.8 ± 0.05 was collected after 14 days of fermentation to be used as a starter for the coffee cascara kombucha production.

Coffee cascara kombucha – Coffee cascara kombuchas were prepared using 80% (v/v) of the coffee cascara infusion, 10% (v/v) of the black tea kombucha starter, 10% (w/v) sugar, and 2.5% (w/v) of SCOBY. The mixture was let ferment at 23 °C. Samples were collected before fermentation (day 0) and after 3; 6 and 9 days of fermentation.

Coffee leaf kombuchas - Coffee leaf kombuchas were prepared using 2.5% (w/v) of the coffee leaves, 10% (w/v) sugar, 10% (v/v) of the black tea kombucha starter and 2.5% (w/v) of SCOBY. The coffee leaf tea infusion was prepared and after cooling to room temperature, the sugar, the starter and SCOBY were added. The mixture was let ferment at 23 °C. Samples were collected before fermentation (day 0) and after 3; 6 and 9 days of fermentation for physicochemical and sensory analyses.

2.4 Analysis of bioactive compounds

2.4.1. Analysis of catechins, chlorogenic acids and free phenolic acids

The analyses of catechins were carried out using a High Performance Liquid Chromatography–Diode Array Detector (HPLC-DAD) system, according to Liang et al. (2007), composed of two Jasco PU-2080 HPLC pumps, a column heater – Model 7981 – Jones Chromatography, a MD-2010 Plus multiwavelength diode array detector and a Jasco As-950 intelligent sampler. The column was a reversed-phase Waters Spherisorb® C18 5 µm ODS2. The Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was used. A gradient elution was carried out with a mixture of two solvents containing acetonitrile/acetic acid/water at 1 mL/min. Detection was performed

at 280 nm. An external standard curve containing seven catechins (catechin, catechin gallate, epicatechin, gallocatechin gallate, epigallocatechin gallate, epigallocatechin and gallocatechin) was prepared in de-ionized water for calibration and quantification of catechins.

The analyses of nine chlorogenic acids (three caffeoylquinic acids, three feruloylquinic acids and three dicaffeoylquinic acids) and free phenolic acids were performed using a HPLC-DAD system, according to Farah et al. (2006a) and Duarte and Farah (2011), with adaptations, using a reverse-phase column (Magic C30, 150 × 2 mm × 5 μm, 100 Å, Michrom Bioresources Inc, Auburn, CA, United States). The two-phase LC mobile system consisted of 0.3% formic acid (eluent A) and methanol (eluent B). The gradient was programmed to operate with a flow rate of 1.0 mL/min, and DAD was set at 325 nm for chlorogenic acids and at 280 nm for phenolic acids. Rutin, quercetin and mangiferin identification and quantification were performed using external standard curves and molar extinction coefficients. Peaks identities were confirmed by LC-MS and their UV spectra.

2.4.2. Analysis of caffeine and trigonelline

The analyses of caffeine and trigonelline were performed using a HPLC-DAD system, according to Farah et al. (2006a; 2006b) with adaptations, using a reverse-phase column (Magic C30, 150 × 2 mm × 5 μm, 100 Å, Michrom Bioresources Inc, Auburn, CA, United States). The HPLC-DAD was set to 272 nm for caffeine and 264 nm for trigonelline. 40% methanol was used as mobile phase, running at 1.0 mL/min.

2.5. pH, total titratable acidity, total soluble solids determination

Triplicate samples were prepared for analysis. pH was measured using a pH meter (Kasvi K39-0014PA, São José dos Pinhais, Paraná, Brazil). Total titratable acidity was determined by titration with 0.1 N NaOH and phenolphtalein as indicator, according to Adolfo Lutz Institute (2008) and the results expressed in mEq/L. Total soluble solids were evaluated using a handheld refractometer (Pocket Refractometer Pal – 1, ATAGO, Japan). Results were expressed in °Brix.

2.6. Analysis of sucrose and other sugars

Sucrose was analyzed by a HPLC Refractive Index Detector (RID) system (mod.# 2414, Waters, Milford-MA**,** USA), according to Wischral et al (2018), using a Hi-Plex column H 8 μm (300 × 7.7 mm) (Agilent, Santa Clara-Ca, USA) at 30 °C with 20 μL of injection volume and $H_2SO_4$0.005 mol/L as mobile phase at 0.4 mL/min. For complementary sugars (glucose, fructose, galactose, xylose, arabinose, cellobiose and glycerol), the column temperature was 60 °C, and the mobile phase flow was 0.6 mL/min.

2.7. DNA extraction, amplicon sequencing data analysis and library preparation

DNA was extracted from the liquid and the biofilm samples after 14 days fermentation (starter culture) for black tea kombucha, and after 9 days fermentation for all other beverages, following the protocol described by Neoprospecta Microbiomes Technologies (Yamanaka et al., 2018). The identification of bacteria was performed using a high-performance sequence of the V3/V4 region of the 16S rRNA gene. The libraries' preparation followed a proprietary protocol (Neoprospecta Microbiome Technologies, Brasil). Amplification with primers to the V3-V4 region of the rRNA 16S gene, 341F with sequence (CCTACGGGRSGCAGCAG), and 806R with sequence (GGACTACHVGGGTWTCTAAT was performed. For yeasts, a high-performance sequence of the ITS1 region, primer ITS1 (GAACCWGCGGARGGATCA), and primer ITS2 (GCTGCGTTCTTCATCGATGC) were used. The libraries were sequenced using the MiSeq Sequencing System (Illumina Inc., USA). If the sequencing was paired-end, the kits V3 with 600 cycles or V2 with 500 cycles were used. For a single-end, the kit V2 with 300 cycles was used. The sequences were analyzed through pipeline Sentinel. In pipeline Sentinel, the archives fastQ were evaluated for quality Phred (QP) using the software FastQC v.0.11.8 (Andrews, 2010). Next, the archives fastQ were submitted to primers trimming and low-quality sequences (Phred < 20) (Cock et al., 2009). For paired-end data, before the trimming step, two pairs of archives (R1 and R2) were merged into a single file using PANDAseq v.2.11 (Masella et al., 2012).

Clusters with an abundance lower than 5 were removed from the analysis because those structures were related to chimera sequences (Smyth et al., 2010). The taxonomic identifications were carried out with BLASTN v.2.6.0+ (Altschul et al., 1990), using a propriety or public database as reference. As for the species definition,

within the 20 hits returned for each cluster, a Python instruction evaluated whether one of the three requirements would be met by the hits: 1) highest bit-score; 2) lowest e-value; and 3) taxonomies with greater representation. The bacteria species were defined using 99% of identity. For yeasts, the species were defined using 97% of identity. The hits that fit one of the previous items were chosen as representative species. Analyses were performed in triplicate samples.

2.8. Antibacterial activity of Kombucha beverage

Antimicrobial activity was evaluated as described in Vukmanovic et al. (2022) with modifications. The foodborne pathogens used for antimicrobial activity test were the Gram-negative bacteria *Escherichia coli* American Type Culture Collection (ATCC) 25922 and *Salmonella enteritidis* ATCC 13076 and the Gram-positive bacteria *Listeria monocytogenes* ATCC 19117 previously cultivated in Brain Heart Infusion (BHI) broth (BD, Difco, New Jersey, USA) at 37 °C for 24h. Then, an inoculum was made for each bacteria in BHI agar and incubated at 37 °C for 24h, in aerobiosis. After incubation, suspensions in saline solution were made in order to obtain a cell concentration of 10^8 CFU/mL. After, an aliquot of cells representing 1 x 10^6 to 1 x 10^7 CFU/mL was homogenized in 35 mL of melted (45 ◦C) Mueller-Hinton agar (Kasvi, São José dos Pinhais, Paraná, Brazil) and poured into Petri dishes. Wells of 5 mm diameter were made with a sterile metal tube utilizing a vacuum pump. Samples sterilized (100 μL) with a polyethersulfone membrane (PES), 0.22 μm pore size (Syringe Driven Filters, Jet Bio-Filtration, Guangzhou, China), were then transferred into the wells of agar plates inoculated with test microorganisms. As controls, acetic acid at 2% (v/v) and sterile water were used. Plates were incubated at 37 °C for 24 h, in aerobiosis. After the incubation, the diameters of the inhibition zones were measured with a caliper.

As control of antimicrobial activity, the same method was used for the strain *Lactiplantibacillus plantarum* ATCC 8414, cultivated in De Man, Rogosa and Sharp broth (BD, Difco, New Jersey, USA) at 37 ºC for 48h, in anaerobiosis (GasPak™ anaerobic). Then, an inoculum was made in MRS agar and incubated at 37 °C for 48h, in anaerobiosis. 100 μL of the suspensions was homogenized in 35 mL of melted (45 °C) MRS semi-solid agar and poured into Petri dishes. Plates were incubated at 37 °C for 48 h, in anaerobiosis.

Kombuchas were also adjusted to neutral pH with NaOH 5M solution for antibacterial activity to exclude the effect of natural low pH of kombuchas. The evaluation of antimicrobial activity was carried out in triplicate, and results are recorded as the diameter of the halo zone (mm).

3. Statistics

All data are reported as mean ± standard deviation. Analysis of variance (ANOVA), followed by Tukey's test, were performed using GraphPad Prism (Version 8.4.2, Informer Technologies, Los Angeles, CA, USA) to determine significant differences between samples at $p < 0.05$.

4. Results

4.1. Microbial composition

The microbial community of the starter culture (biofilm and liquid of black tea kombucha after 14 days of fermentation) and the final liquid and biofilm composition of coffee cascara and coffee leaves were analyzed (Figure 2 and 3). Data analysis of 16S rRNA gene sequence revealed two bacterial phyla in all samples, Proteobacteria and Firmicutes. Proteobacteria was the most abundant phyla, especially in coffee cascara and coffee leaves kombuchas, with percentage higher than 90%.

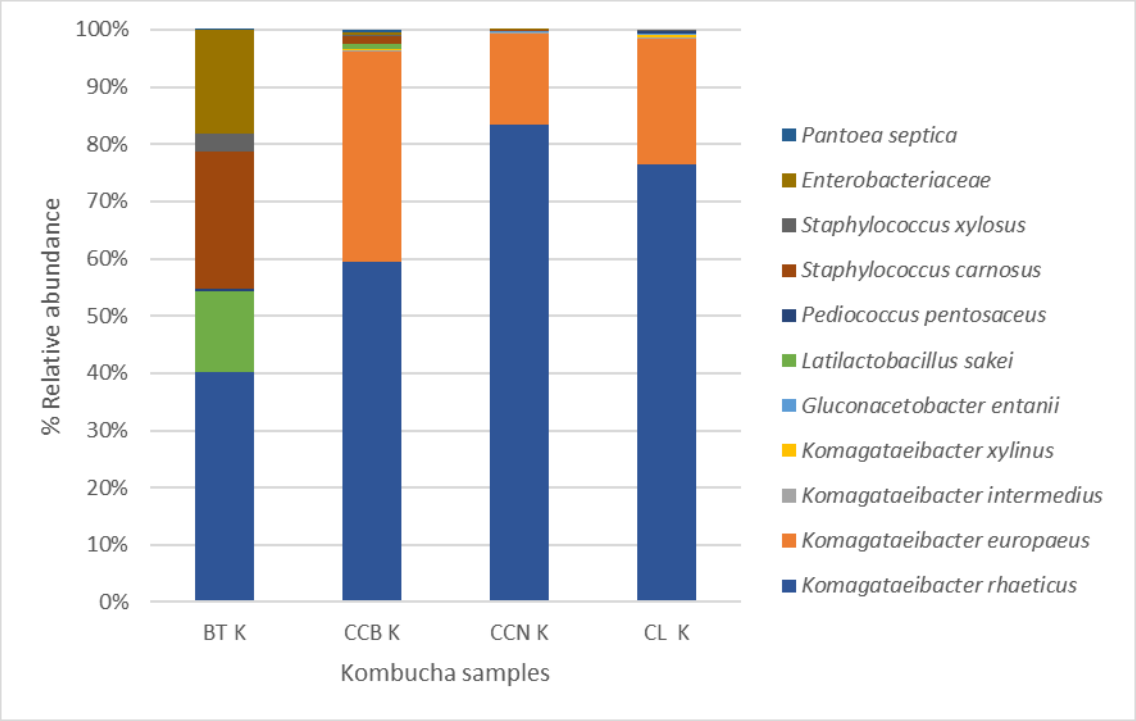


Figure 2. Bacterial composition of the solid and liquid phases of the black tea coffee cascara and coffee leaves kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha; CCB K. coffee cascara kombucha from Brazil; CCN K. Nicaragua coffee cascara kombucha; CL K. Coffee leaves kombucha

In all kombucha beverages, *Komagataeibacter*, an acetic acid bacteria was the most abundant genus observed in the liquid and in the biofilm. In coffee cascara and coffee leaves kombuchas, characterized for the first time, *K. rhaeticus* comprised > 80% of CCN kombuchas microorganisms and 60% and 77% of CCB and CL kombuchas microorganisms contained in the liquid and solid cultures, respectively. In addition to a high percentage of *K. rhaeticus* (60% - 77%), *K. europaeus* (0.01% - 37*%), K. intermedius* (0.2 – 0.3%) and *Gluconacetobacter entanii* (0.03 – 0.06%) were also identified in coffee cascara, coffee leaves kombuchas. In the present study, low percentages of *Lactobacillus* (0.08% - 0.9%), Enterobacteriaceae (0.06% - 0.8%) and *Staphylococcus* (0.4 - 1.3%) were additionally observed in coffee cascara and coffee leaves kombuchas.

Staphylococcus carnosus and *Staphylococcus xylosus* were identified in black tea, coffee cascara and coffee leaves kombuchas. Two lactic acid bacteria were identified in black tea kombucha and in BCC kombucha, *Latilactobacillus sakei* and *Pediococcus pentosaceus*, while in CL kombucha only *P. pentosaceus* was identified and in CCN kombucha, only *L. sakei*.

Regarding yeasts, data analysis of ITS1 identified that the most abundant phyla were Ascomycota and Saccharomycetales order (Figure 2). The genus present in fermented beverages were Pichia (the predominant yeast genera with abundance higher than 70%), Saccharomycetales (> 20%) and *Brettanomyces* (5%) in all samples.

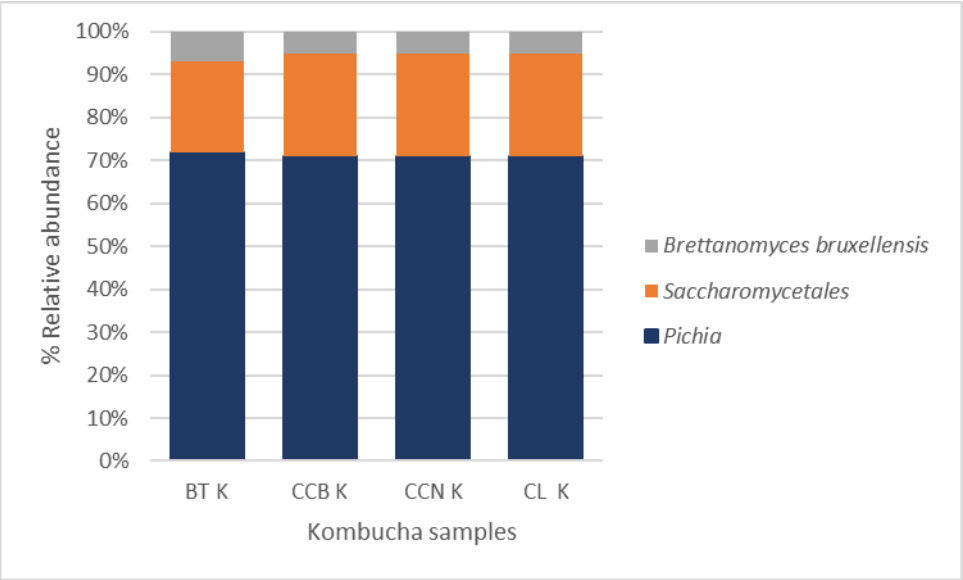


Figure 3. Yeast composition of the solid and liquid phases of the black tea, coffee cascara and coffee leaves kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha; CCB K. Brazil coffee cascara kombucha; CCN K. Nicaragua coffee cascara kombucha; CL K. coffee leaves kombucha

4.2. Physicochemical composition

The pH, total acidity and soluble solids values for all fermented beverages are presented Figures 4, 5, 6 and 7. In black tea kombucha, total acidity increased until day 9 (0.3 mEq/L), when pH was 3.4. Considering the kombuchas elaborated with coffee cascara, while in CCB kombuchas acidity remained stable (0.1 mEq/L) with no statistical differences in pH (3.7 to 3.5), in CCN kombuchas acidity increased during fermentation (from 0.04 mEq/L and pH 3.8 on day 0 to 0.4 mEq/L and pH 3.5 on days 6 and 9). In coffee leaves kombuchas, pH values ranged from 3.9 (day 0) to 3.4 (day 9) for coffee leaves kombucha, while for titrable acidity, the values increased from 0.1 mEq/L to 0.3 mEq/L.

After addition of sucrose and fermentation, a decrease in soluble solids occurred in all beverages from day 0 to day 9. In black tea kombucha, values decreased from 10.6 °Brix to 9.3 °Brix. In coffee cascara kombuchas, the decrease was similar (11.6 °Brix to 9.9 ° Brix). For coffee leaves kombuchas, the values decreased from 12.2 °Brix to 9.2 °Brix.

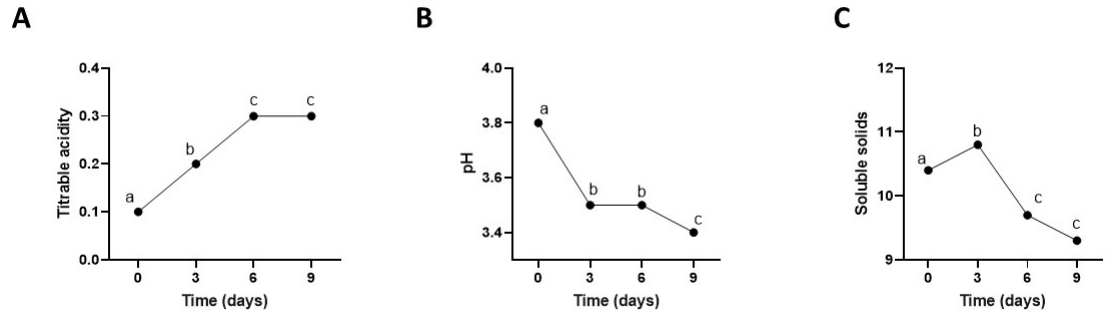


Figure 4. Titrable acidity (A), pH (B) and soluble solids (C) of black tea kombuchas. Data are expressed as mean for triplicate analyses; different letters on the same line for the same graph indicate significant difference ($p<0.05$)

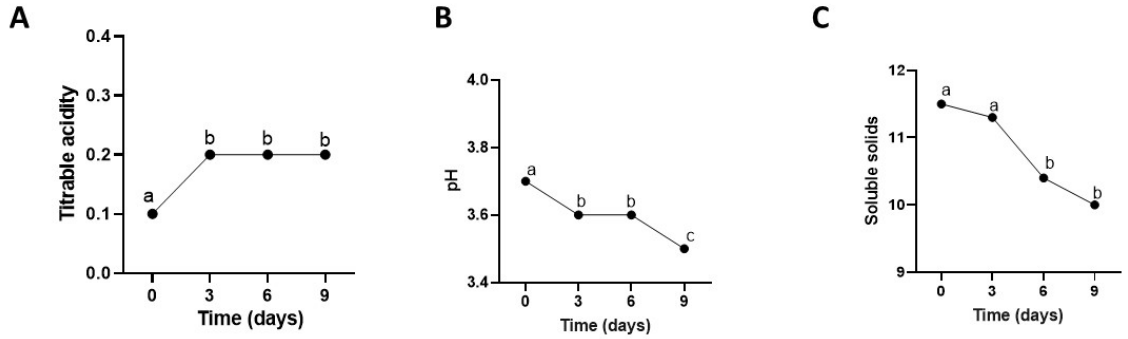


Figure 5. Titrable acidity (A), pH (B) and soluble solids (C) of coffee cascara from Brazil kombuchas. Data are expressed as mean for triplicate analyses; different letters on the same line for the same graph indicate significant difference ($p<0.05$)

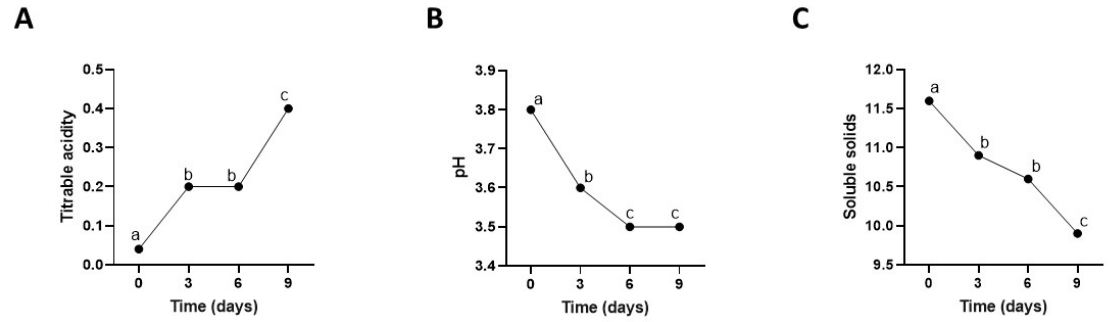


Figure 6. Titrable acidity (A), pH (B) and soluble solids (C) of coffee cascara from Nicaragua kombuchas. Data are expressed as mean for triplicate analyses; different letters on the same line for the same graph indicate significant difference ($p<0.05$)

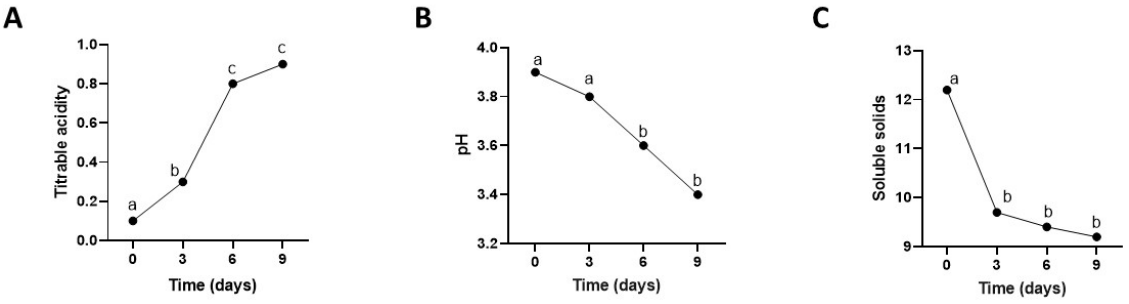


Figure 7. Titrable acidity (A), pH (B) and soluble solids (C) of coffee leaf kombuchas. Data are expressed as mean for triplicate analyses; different letters on the same line for the same graph indicate significant difference ($p<0.05$)

4.3. Chemical composition

4.3.1. Sugars analysis

A few monosaccharides were quantified in the unsweetened infusions. In black tea, we identified glucose (0.4 g/100mL), galactose/xylose (0.11 g/100mL), fructose (0.32 g/100mL) and arabinose (0.38 g/100mL). In CCB, cellobiose (0.23 g/100mL), glucose (1.97 g/100mL), fructose (4.35 g/100mL), arabinose (1.86 g/100mL) and glycerol (< 0.1 g/100mL) were identified, while in CCN, glucose (1.63 g/100mL) and fructose (3.47 g/100mL) were identified. Glucose (0.21 g/100mL), fructose (0.47 g/100mL) and arabinose (1.06 g/mL) were identified in CL infusion. During fermentation, part of the sucrose added to the infusions to make kombucha on day 0 was degraded, with initial concentration of 10g/mL on day 0 and 7.98; 8.16; 8.10 and 7g/100mL (on average) on day 9 in BT, CCB, CCN and CL kombuchas, respectively. In all kombuchas, no significant difference was found in glucose (0.62 – 1.47 g/100mL) and fructose (0.25 – 0.93 g/100mL) concentrations (Figure 8).

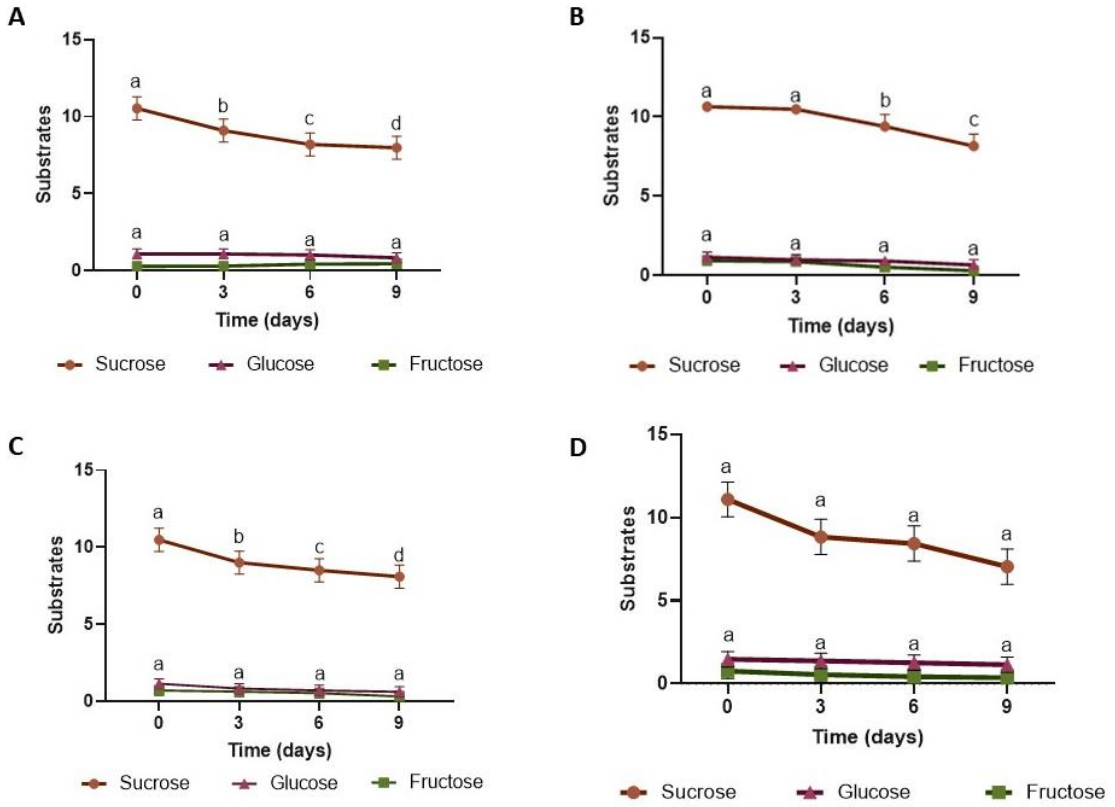


Figure 8. Content of sucrose, glucose, and fructose (g/100mL) in BT (A), CCB (B), CCN (C) and CL (D) kombuchas, from day 0 to day 9 of fermentation. Data were presented as mean of triplicate analysis ± standard deviation. BT: Black tea; CCB: Coffee cascara from Brazil; CCN: Coffee cascara from Nicaragua; CL: Coffee leaves. Different letters on the same line indicate statistical difference by ANOVA followed by Tukey's test ($p < 0.05$).

4.3.2. Catechins in black tea, coffee cascara and coffee leaves kombuchas: The composition of phenolic compounds in black tea, coffee cascara and coffee leaves beverages are presented in table 1. three major catechins were identified: epigallocatechin gallate, epigallocatechin, and epicatechin. Epigallocatechin is the most abundant catechin found in this study. Also, four corresponding stereoisomers, including gallocatechin gallate, gallocatechin, catechin gallate and catechin were identified. In black tea infusion, the major catechins identified were gallocatechin and epigallocatechin followed by epicatechin, catechin and epigallocatechin gallate. The content of gallocatechin and epigallocatechin decreased (on average) 23% and 60%, respectively, from infusion to day 9 of fermentation. We observed an increase of 3% (on average) in epicatechin content from day 0 to day 9. Catechin gallate and gallocatechin gallate were not detected in black tea samples. Along fermentation, the content of catechins decreased 1%, 17%, and 21% after 3, 6, and 9 days, respectively. In coffee cascara and coffee leaves beverages, traces of the seven catechins analyzed were identified (< 0.01 - 0.08 mg/100mL).

4.3.3. Chlorogenic acids in black tea, coffee cascara and coffee leaves kombuchas: The main chlorogenic acid (CGA) found in black tea and its kombuchas is 5-caffeoylquinic acid, which increased 20% from day 0 to day 9. CGA isomers identified in black tea were 3- caffeoylquinic acid, whose concentration increased 41% (on average) from day 0 to day 9, and 4- caffeoylquinic acid, with no changes and its concentration during kombucha fermentation. The content of total CGA increased 30% up to day 9. Minor CGA compounds and related compounds (dicaffeoylquinic acids, diferuloylquinic acids) (Farah and Lima, 2019) were not identified, as well as 3-feruloylquinic acid, 4- feruloylquinic acid and 5- feruloylquinic acid.

All coffee cascara beverages presented higher concentrations of 5-caffeoylquinic acid, followed by 4- caffeoylquinic acid and 3- caffeoylquinic acid than black tea and its

kombuchas, with higher concentration in CCN samples. 5- caffeoylquinic acid concentrations increased (on average) 58% in CCB and 48% in CCN kombuchas up to day 9, as well as 3- caffeoylquinic acid (210% in CCB and 182% in CCN) and 4- caffeoylquinic acid (321% in CCB and 225% in CCN). 3- feruloylquinic acid was not detected in coffee cascara beverages. We also identified 4- feruloylquinic acid, 5- feruloylquinic acid, 3,4-di caffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5- dicaffeoylquinic acid. Compared with coffee cascara infusions, although a 27% (approximately) decreased on day 0 was identified, the content of 4- feruloylquinic acid and 5- feruloylquinic acid increased 318% in CCB and 156% in CCN kombuchas up to day 9. By the way, the content of all dicaffeoylquinic acids decreased from day 0 to day 9: 8% and 17% for 3,4- dicaffeoylquinic acid; 5% and 10% for 3,5- dicaffeoylquinic acid in CCB and CCN kombuchas, respectively., although 4,5- dicaffeoylquinic acid increased 15% in CCB kombuchas and decreased 20% in CCN kombuchas up to day 9.

Table 1. Main bioactive compounds in infusions and black tea, coffee cascara and coffee leaf infusions and kombuchas (mg/100mL).

	BT					CCB					CCN					CL				
	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9
Flavonoids																				
EGC	4.5±0.1[a]	4.5±0.1[a]	3.6±0.1[c]	3.4±0.1[c]	3.6±0.2[c]	<0.3	<0.3	<0.3	<0.3	<0.1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.1	<0.1	<0.1	<0.1	<0.1
GC	11.1±0.00[a]	11.1±0.00[a]	10.5±0.1[a]	8.3±0.47[c]	6.9±0.55[d]	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
EGCG	0.5±0.1[a]	0.5±0.1[a]	0.6±0.0[a]	0.3±0.1[c]	0.35±0.1[c]	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
GCG	n.d.	n.d.	n.d.	n.d.	n.d.	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*	<0.02*
EC	2.7±0.1[a]	2.7±0.1[a]	3.3±0.0[b]	3.1±0.0[c]	3.5±0.0[b]	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
C	0.32±0.0[a]	0.32±0.0[a]	0.4±0.0[c]	0.4±0.0[c]	0.4±0.0[b]	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
CG	n.d.	n.d.	n.d.	n.d.	n.d.	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
Rutin	0.5 ± 0.0[a]	0.4 ± 0.0[a]	0.5±0.0[a]	0.5±0.0[a]	0.6 ± 0.0[b]	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.3 ± 0.0[b]	0.4 ± 0.0[c]	0.4 ± 0.0[c]	0.4 ± 0.0[a]	0.4 ± 0.0[a]	0.6 ± 0.0[b]	0.7 ± 0.0[c]	0.6 ± 0.0[b]	0.2 ± 0.0[a]	0.3 ± ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]
QUER	0.1 ± 0.00[a]	0.1 ± 0.01[a]	0.2 ± 0.02[ab]	0.2 ± 0.01[b]	0.2 ± 0.02[b]	< 0.002*	< 0.001*	< 0.001*	< 0.002*	< 0.001*	< 0.002*	< 0.002*	< 0.002*	< 0.002*	< 0.003*	0.1 ± 0.0[a]	0.1± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]
KAEMP	< 0.002*	< 0.001*	< 0.001*	< 0.002*	< 0.002*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1. continued

	BT					CCB					CCN					CL				
	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9
Phenolic acids																				
GA	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.7 ± 0.0^{b}	0.8 ± 0.0^{c}	0.9 ± 0.0^{d}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.5 ± 0.0^{c}	0.5 ± 0.0^{c}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}	0.5 ± 0.0^{d}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}
CA	n.^{d}.	n.^{d}.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{c}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
3-CQA	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.6 ± 0.0^{a}	0.8± 0.0^{c}	0.9 ± 0.0^{d}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.0^{b}	1.0 ± 0.0^{c}	1.2 ± 0.3^{d}	0.5 ± 0.0^{a}	0.4 ± 0.0^{b}	0.9 ± 0.0^{c}	1.1 ± 0.0^{d}	1.3 ± 0.0^{e}	0.5 ± 0.0^{a}	0.4 ± 0.0^{b}	0.6 ± 0.0^{c}	0.9 ± 0.0^{d}	1.3 ± 0.0^{e}
4-CQA	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.9 ± 0.0^{c}	1.1 ± 0.3^{d}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.7 ± 0.0^{b}	0.9 ± 0.0^{c}	1.2 ± 0.0^{e}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.8 ± 0.0^{c}	1.2 ± 0.0^{d}
5-CQA	1.9 ± 0.0^{a}	1.5 ± 0.0^{b}	1.6 ± 0.0^{c}	1.8 ± 0.0^{d}	2.0 ± 0.0^{e}	3.7 ± 0.0^{a}	3.1 ± 0.0^{b}	4.2 ± 0.0^{c}	4.9 ± 0.0^{d}	5.4 ± 0.6^{d}	5.0 ± 0.0^{a}	4.1 ± 0.0^{b}	5.8 ± 0.0^{c}	6.3 ± 0.0^{d}	6.7 ± 0.0^{e}	7.2 ± 0.0^{a}	6.5 ± 0.0^{b}	7.6 ± 0.0^{c}	8.3 ± 0.0^{d}	8.9 ± 0.0^{e}
3-FQA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-FQA	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.08 ± 0.0^{b}	0.2 ± 0.0^{c}	0.4 ± 0.0^{d}	0.6 ± 0.3^{d}	0.1 ± 0.0^{a}	0.07 ± 0.0^{b}	0.3± 0.0^{c}	0.3 ± 0.0^{c}	0.4 ± 0.0^{d}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.7 ± 0.0^{c}
5- FQA	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.5 ± 0.0^{b}	0.7 ± 0.0^{c}	0.8 ± 0.2^{d}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.6 ± 0.0^{b}	0.8 ± 0.0^{c}	0.9 ± 0.0^{d}	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}	0.6 ± 0.0^{c}	1.0 ± 0.0^{d}
3.4-diCQA	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	1.0 ± 0.0^{a}	0.8 ± 0.0^{b}	0.9 ± 0.0^{c}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}
3.5-diCQA	n.d.	n.d.	n.d.	n.d.	n.d.	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.8 ± 0.0^{a}	0.7 ± 0.0^{b}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	1.9 ± 0.0^{a}	1.1 ± 0.0^{b}	1.0 ± 0.0^{c}	1.0 ± 0.0^{c}	0.9 ± 0.0^{c}

Table 1. continued

	BT					CCB					CCN					CL				
	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9
4.5-diCQA	n.d.	n.d.	n.d.	n.d.	n.d.	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1± 0.0^{b}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.9 ± 0.0^{a}	0.7 ± 0.0^{b}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.5 ± 0.0^{d}
Xanthonoids																				
MG	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}
ISOMG	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TPC	44.9 ± 0.0^{a}	36.3 ± 0.0^{b}	23.6 ± 0.3^{c}	21.3 ± 0.0^{d}	21.4 ± 0.0^{d}	11.9 ± 0.0^{a}	10.1 ± 0.0^{a}	14.9 ± 0.0^{b}	19.1 ± 0.0^{c}	21.9 ± 0.1^{d}	16.3 ± 0.0^{a}	13.9 ± 0.0^{b}	20.4 ± 0.0^{c}	23.3 ± 0.0^{d}	25.9± 0.0e	13.8 ± 0.0^{a}	11.6 ± 0.0^{b}	23.1 ± 0.0^{c}	15.9 ± 0.0^{d}	18.4 ± 0.0e
Alkaloids																				
Caffeine	12.4 ± 0.0^{a}	12.0 ± 0.0^{a}	14.2 ± 0.0^{b}	15.3 ± 0.0^{b}	18.2 ± 0.0^{c}	9.9 ± 0.1^{a}	10.3 ± 0.0^{b}	13.3 ± 0.0^{c}	15.4 ± 0.0^{d}	16.9 ± 0.0e	16.0 0.1^{a}	14.1 ± 0.0^{b}	16.3 ± 0.0^{a}	16.9 ± 0.0^{a}	18.2 ± 0.0^{c}	13.2 0.0^{a}	12.2 ± 0.0^{b}	14.2 ± 0.0^{c}	14.3 ± 0.0^{c}	16.0 0.0^{d}
TG	n.^{d}.	n.^{d}.	n.^{d}.	n.^{d}.	n.^{d}.	2.0 ± 0.0^{a}	1.7 ± 0.0^{b}	1.6 ± 0.0^{b}	1.8 ± 0.0^{c}	1.9 ± 0.0^{c}	2.3 0.0^{a}	1.8 ± 0.0^{b}	1.7 ± 0.0^{b}	1.8 ± 0.0^{b}	1.9 ± 0.0^{b}	1.1 0.0^{a}	1.1 ± 0.0^{a}	1.1 ± 0.0^{a}	1.2 ± 0.0^{b}	1.2 ± 0.0^{b}

Results are mean ± SD; Different letters in the same line for the same sample indicates statiscally differences; BT: black tea; CCB: coffee cascara from Brazil; CCN.: coffee cascara from Nicaragua; CL: coffee leaves INF: infusion; D0. 3. 6 and 9: days 0. 3. 6 and 9 of fermentation. respectively; total phenolic compounds: n.d. values below limit of detection; EGC: epigallocatechin gallate; GC: gallocatechin; EGCG: epigallocatechin gallate; GCG: gallocatechin gallate; EC: epicatechin; CG: catechin gallate. GA: gallic acid; CA: caffeic acid; QUER: quercetin; KAEMP: kaempferol; 3-CQA: 3-caffeoylquinic acid; 4-CQA: 4- caffeoylquinic acid; 5-CQA: 5- caffeoylquinic acid; 3-FQA: 3-feruloylquinic acid; 4-FQA: 4- feruloylquinic acid; 5-FQA: 5- feruloylquinic acid; 3.4-diCQA: 3.4-dicaffeoylquinic acid; 3.5-diCQA: 3.5- dicaffeoylquinic acid; 4.5-diCQA: 4.5- dicaffeoylquinic acid; MG: mangiferin; ISOMG: isomangiferin; TPC: total phenolic compounds- sum of all phenol compounds quantified by chromatography, including chlorogenic acids metabolites; total phenolic compounds; CAF: caffeine; TG: trigonelline

In coffee leaves beverages, the highest chlorogenic acid concentration identified was for 5-caffeoylqunic acid, followed by 3- caffeoylquinic acid and 4- caffeoylquinic acid. In coffee leaves kombuchas, 5- caffeoylquinic acid increased (on average) 25% up to day 9, while 3- caffeoylquinic acid and 4- caffeoylquinic acid increased 138% and 158%, respectively. An increase in feruloylquinic acids was observed: 95% in 5-feruloylquinic acid, 185% in 4-feruloylquinic acid. 3-feruloylquinic acids was not detected in coffee leaves samples. Considering dicaffeoylquinic acids, highest concentrations were found for 3,5-dicaffeoylquinic acid, followed by 3,4-dicaffeoylquinic acids and 4,5- dicaffeoylquinic acids. On average, its contents decreased 9%, 1%; and 16%, respectively, up to day 9.

4.3.4. Chlorogenic acids metabolites in black tea, coffee cascara and coffee leaves kombuchas: Some of the main chlorogenic acids metabolites previously identified in urine (Farah and Lima, 2019) were identified in black tea, coffee cascara and coffee leaves infusions and kombuchas (Table 2). In black tea kombuchas, caffeic acid, hippuric acid, 3,4-dihidroxyphenylacetic acid, 4-hidroxyphenylacetic acid, vanillic acid and dihydrocaffeic acid were identified from 3 days of fermentation, with an increase in its concentrations (25%; 42%; 13%; 36%; 42%; 283%, respectively). Gallic acid was identified in black tea infusion and kombuchas, and its concentration increased 42% up to day 9.

Considering coffee cascara beverages, in CCB beverages caffeic, ferulic and benzoic acids were identified in all samples, and its concentrations increased (on average) up to day 9 (80%; 90%; 33%, respectively). Some compounds were identified from 3 days of fermentation, with an increase (on average) in its concentrations: dihydrocaffeic acid (91%), hippuric acid (79%), p-coumaric acid (50%), 4-hidroxyphenylacetic acid (40%), vanillic acid (34%), 3,4-hidroxyphenylacetic acid (33%) and 3,4-dihidroxybenzoic acid (33%). In CCN beverages, an increase (on average) in caffeic acid (69%), ferulic acid (60%), benzoic acid (46%), 3,4-dihidroxybenzoic acid (75%), hippuric acid (121%), vanillic acid (83%) and dihydrocaffeic acid (118%) was observed from infusions up to day 9. Two phenolic acids were identified from 3 days of fermentation in CCN beverages: p-coumaric acid, with no changes in its concentration up to day 9, and 4-hidroxyphenylacetic acid, which increased (on average) 36%.

Table 2. Chlorogenic acids metabolites in black tea, coffee cascara and coffee leaf infusions and kombuchas (mg/100mL).

	BT					CCB*					CCN*					CL				
Phenolic compounds	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9	INF	D0	D3	D6	D9
CA	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.1± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2± 0.0^{b}
GA	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	0.7 ± 0.0^{c}	0.7 ± 0.0^{c}	0.8 ± 0.0^{d}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}	0.5 ± 0.0^{c}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.5 ± 0.0^{c}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{c}	0.4 ± 0.0^{d}
FA	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
p-CA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	n.d.	n.d.	0.1 ± 0.0^{a}	0.3 ± 0.4^{b}	0.1 ± 0.0^{a}	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
SA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
BA	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.2^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
3.4-diOHbenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.4 ± 0.0^{c}	0.4 ± 0.0^{c}	0.4 ± 0.0^{d}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{c}	n.d.	n.d.	n.d.	n.d.	n.d.
HA	n.d.	n.d.	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	n.d.	n.d.	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	0.3 ± 0.0^{c}	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}
3.4-diOHyphenylacetic acid	n.d.	n.d.	0.2 ± 0.0 ^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	n.d.	n.d.	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}
4-OHphenylacetic acid	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}	n.d.	n.d.	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{b}	n.d.	n.d.	n.d.	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}
VA	n.d.	n.d.	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	n.d.	n.d.	0.3 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.2 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}	0.3 ± 0.0^{c}	0.4 ± 0.0^{d}	n.d.	n.d.	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}
DihydroCA	n.d.	n.d.	0.1 ± 0.0^{a}	0.4 ± 0.0^{b}	0.5 ± 0.0^{c}	n.d.	n.d.	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2± 0.0^{b}	0.3 ± 0.0^{c}	n.d.	n.d.	0.1 ± 0.0^{a}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}

Results are mean ± SD; Different letters in the same line for the same sample indicates statiscally differences; BT: black tea; CCB: coffee cascara from Brazil; CCN.: coffee cascara from Nicaragua; CL: coffee leaves INF: infusion; D0. 3. 6 and 9: days 0. 3. 6 and 9 of fermentation. respectively; total phenolic compounds: sum of all phenol compounds quantified by chromatography, including chlorogenic acids metabolites; total phenolic compounds; n.d. values below limit of detection; CA: caffeic acid; GA: gallic acid; FA: Ferulic acid; pCA: p-coumaric acid; SA: sinapic acid; BA: benzoic acid; 3,4-diOHbenzoic acid: 3,4-dihidroxybenzoic acid; HA: hippuric acid; 3,4-diOHyphenylacetic acid: 3,4-dihidroxyphenylacetic acid; 4-OHphenylacetic acid: 4-hidroxyphenylacetic acid; VA: vanillic acid; DihydroCA: dihydrocaffeic acid

In coffee leaf beverages, caffeic acid and gallic acid were the main metabolites quantified. For caffeic acid, the increase was (on average) 43% in coffee leaves kombuchas. Although a decrease in gallic acid content from coffee leaves infusion to day 0, an increase of 118% (on average) were found in coffee leaves kombuchas. Ferulic acid content decreased in coffee leaves kombuchas (6%, on average).Sinapic acid p-Coumaric acid, hippuric acid, vanillic acid and dihydrocaffeic acid were identified after 3 days of fermentation and its contents increased (on average) 44%; 41%; 14%; and 52%, respectively. The phenolic acids 4-dihidroxyphenylacetic acid and 4-hidroxyphenylacetic acid were identified from 6 days of fermentation, and increased (on average) 71% and 50%, respectively.

4.3.5. Other phenolic compounds identified in black tea, coffee cascara and coffee leaves kombuchas: Rutin and quercetin were also identified in all beverages. Rutin content in black tea kombucha increased 56% up to day 9, while quercetin increased (on average) 61% in black tea kombucha up to day 9. Rutin was also identified in coffee cascara beverages, with 50% increase (on average) from day 0 to 9. Traces of quercetin were identified in coffee cascara beverages (< 0,001 - 0,002 mg/100mL).

In coffee leaf kombuchas rutin content increased 26% up to day 9. Although a slight decrease in quercetin content was found from coffee leaves infusion to its kombucha on day 0, the content increased 77% (on average) up to day 9 in all kombucha beverages. The xanthonoid mangiferin, a C-glycosylated xanthone (Trevisan et al., 2019), was quantified only in coffee leaves infusions and kombuchas. In coffee leaf kombuchas, although a 13% decrease was observed from day 0 to day 6, the content of mangiferin on day 9 was the same to the coffee leaves infusion (0.40 mg/100mL). Isomangiferin, a structural isomer of mangiferin differing only in the position of the C-glucosidic moiety (Trevisan et al., 2019), was not detected in any of coffee leaf beverages. Traces of kaempferol were identified in all beverages studied but the contents were b below the limit of quantification (LOQ).

The total concentration of phenolic compounds in black tea infusion was higher than in kombucha beverages, with a mean decrease of 38% on day 9 . Differently from black tea, the phenolic concentration increased in coffee cascara (116% (on average) in CCB kombuchas and 86% in CCN kombuchas up to day 9) and 38% in coffee leaf kombuchas.

Caffeine was quantified in black tea, coffee cascara and coffee leaf beverages. In black tea, caffeine content increased during kombucha fermentation (51 % from day 0 to day 9). Caffeine was identified in all coffee cascara beverages increasing, on average, 44%. In coffee leaf beverages, a 21% increase (on average) in caffeine content was observed up to day 9, although a slight decreased was observed found in coffee leaf kombuchas on day 0.

Trigonelline was also identified in coffee cascara and coffee leaves beverages, but not in black tea beverages. No differences in trigonelline content were identified in both samples.

4.4. Antibacterial activity

The antibacterial activity of the studied infusions and kombuchas against the pathogenic microorganisms tested and *L. plantarum* is presented in table 3. Acetic acid, used as a control, showed the highest inhibition of pathogenic bacteria for *S. enteritidis* (12.33 ± 0.58 mm), followed by *L. monocytogenes* (11.33 ± 0.58 mm) and *E. coli* (10 ± 0.00 mm), while sterilized water showed no inhibitory activity. Acetic acid and sterilized water did not exhibit antimicrobial activity against *L. plantarum*.

For black tea infusion, the highest inhibition was observed for *L. monocytogenes* (10 mm) and *E. coli* (10 mm) followed by *S. enteriditis* (9 mm). No growth inhibition was observed for *L. plantarum*. When the black tea kombuchas were added to the Mueller-Hinton agar, the highest inhibition was for *L. monocytogenes* on day 0 (10 mm), while a decrease on inhibition was observed up to day 9 (9 mm, on average). The same behavior was observed for *E. coli* and *S. enteritidis*. After kombuchas were adjusted to neutral pH with NaOH 5M (pH = 7.0), an increase, on average, in inhibition zone were found for *E. coli* (10 mm), followed by *L. monocytogenes* (9.5 mm) and *S. enteritidis* (9.0 mm). No antimicrobial activity was found for *L. plantarum* after incubation with black tea kombuchas adjusted to neutral pH.

In coffee cascara infusion, the highest antimicrobial activity was against *L. monocytogenes* after CCB and CCN infusions (10 mm for CCB and 9.0 mm for CCN) incubation, followed by *S. enteritidis* (9 mm for both infusions) and *E. coli* (8.0 mm for CCB and CCN). In relation to coffee cascara infusions, an increase in the antimicrobial activity of cascara kombuchas was observed against *E coli* (9.4 mm, on average), and a slight increase in *S. enteritidis* (9.5 mm) inhibition, while for *L. monocytogenes* the highest inhibition zone was

observed for cascara kombuchas on day 9 (10 mm for both kombuchas). No antimicrobial effect was observed for *L. plantarum*.

Considering coffee leaf teas, the highest inhibition was observed for *L. monocytogenes* (9.0 mm) followed by *S. enteretidis* (8.0 mm) and *E. coli* (8.0 mm). When coffee leaves kombuchas were administered, an increase of antimicrobial activity was observed up to day 9. For coffee leaves kombuchas, the highest antimicrobial activity was for *E. coli* (10 mm) and *L. monocytogenes* (10 mm) and *S. enteritidis* (9.0 mm). No statistical differences in inhibition zone were observed in kombuchas adjusted to neutral pH (pH = 7.0) for *E. coli* and *L. monocytogenes*. No antimicrobial activity was observed for *L. plantarum*.

Discussion

The Kombuchas' final microbiota is the result of the characteristics of the SCOBY used, as well as the microbiological characteristics of the utensils and raw materials. In addition, the results obtained depend directly on the methodology used, since some microorganisms grow easily, others cannot or are difficult to grow. In the present study, the methodology used allowed identify the genera and species present in the studied kombuchas. The results on bacterial composition in kombuchas analyzed agree with previous studies (Chakravorty et al., 2016; Arikan et al., 2020; Marsh et al., 2014; Fabricio et al., 2022). The acetic acid bacteria from the genus *Komagataeibacter* characterizes kombucha cultures (Chakravorty et al., 2016; Ryngajłło et al., 2020; Harrison and Curtis, 2021; Andresen et al., 2022). This genera is the most efficient bacterial cellulose producer (Canazza et al., 2021). *Komagataeibacter rhaeticus* is known to be one of the most abundant bacterial members of the kombucha fermenting agents (Landis et al., 2022). It is able to biosynthesize bacterial cellulose membranes in kombucha (Machado et al., 2016). *K. europaeus* has previously been identified in black tea kombucha (Barbosa et al., 2021; Andresen et al., 2022). This strain is capable of synthesizing bacterial cellulose (Nagmetova et al., 2020). *K. intermedius* (dos Santos et al., 2015, Coton et al., 2017; Gaggia et al., 2019; Villarreal-Soto et al., 2020) and *Gluconoacebacter* genera (Marsh et al., 2014; Chakravorty et al., 2016; Villarreal-Soto et al., 2020) have been previously detected in black tea kombucha and lastly, *G. entanii* has been detected in rooibos tea kombucha (Gaggia et al., 2019).

Table 3. Antibacterial activity of black tea, coffee cascara and coffee leaf infusions and kombuchas

Target bacteria	Controls							Kombuchas																
	AA	Water	Infusion					BT				CCB				NCC				CL				
			BT	CCB	CCN	CL		D0	D3	D6	D9	D0	D3	D6	D9	D0	D3	D6	D9	D0	D3	D6	D9	
***E.coli* ATCC 25922**	++	-	++	+	+	+	Kombucha natural pH	++	++	++	+	+	++	++	++	++	++	++	++	++	++	++	++	
							Kombucha neutralized	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	
***S.enteritidis* ATCC 13076**	+++	-	++	++	++	+	Kombucha natural pH	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
							Kombucha neutralized	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
***L. monocytogenes* ATCC 11229**	+++	-	+++	++	++	++	Kombucha natural pH	++ +	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
							Kombucha neutralized	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
***L. plantarum* ATCC 8414**	-	-	-	-	-	-	Kombucha natural pH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
							Kombucha neutralized	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Results are mean of triplicate analysis of diameter of halo zone. (-): no inhibition; (+): 8 - 9mm of inhibition; (++): 9 - 10mm of inhibition;(+++): > 10mm of inhibition. Acetic acid (AA) was prepared at 2% (v/v); water was sterilized at 121 °C during 15 min. Kombucha natural pH refers to the pH value of the sample without any adjustment.

Lactic acid bacteria was previously identified in kombucha liquid and pellicule (Marsh et al., 2014; Gaggia et al., 2019; Fabricio et al., 2022; Landis et al., 2022). *L. sakei* strains have been identified as the dominant microbial population in fermented products, playing a major role in the preparation of foods such as kimchi and sausages fermented at low temperatures (Kim et al., 2020). Its percentage was higher in the black tea kombucha starter culture than in all other beverages. *P. pentosaceus* has been isolated from fermented foods, aquatic products, raw animal, plant products and faeces. It appears to have probiotic potential (Jiang et al., 2021).

Staphylococcus carnosus and *Staphylococcus xylosus* are the two main staphylococcal species used worldwide as starter cultures in food fermentation, either alone or in combination with defined lactobacilli or other microorganisms. Starter cultures protect the food from undesirable bacteria and make the fermentation process more reliable. They also suppress food spoilage and poisoning by unwanted microorganisms and the whole fermentation process can be better controlled (Löfblom et al., 2017).

The family Enterobacteriaceae comprises a very large group of morphologically and physiologically similar bacteria. They are of great importance, while some of these organisms are involved in food spoilage, others are food-borne pathogens, and some are indicators of fecal contamination of food products (Singh and Anand, 2020). The genus and species of the most common human pathogenic Enterobacteriaceae are *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia* (Rock and Donnenberg, 2014). Some studies have identified this family of bacteria in kombucha liquid or pellicule (Villarreal-Soto et al., 2020; Fabricio et al., 2022). An additional strain from Enterobacteriaceae family identified in starter and CCB kombucha was *Pantoea septica*, which have been isolated mostly from the environment, particularly from plants, seeds and vegetables, fruits, soil, water and human feces (Cooney et al., 2014; Lo et al., 2015).

Regarding yeasts, *Pichia*. is one of the main yeast genera identified in Kombuchas (Marsh et al., 2014; Gaggia et al., 2019; Fabricio et al., 2022; Harrison and Curtis, 2021; Fabricio et al., 2022). *Brettanomyces bruxellensis* is the most common yeast identified in kombucha tea and SCOBY (Teoh et al., 2014; Gaggia et al., 2019; Tran et al., 2020; Villarreal-Soto et al., 2020; Harrison and Curtis, 2021; Torán-Pereg et al., 2021; Fabricio et al., 2022; Landis et al., 2022). It has also been identified in starter and fermented beverages. In kombuchas, *B. bruxellensis* can stimulate acetic acid bacteria biofilm production (Landis et al., 2022).

The percentage of Saccharomycetales sp. was similar to that observed by Landis et al. (2022). Saccharomycetales sp. genera and strains have been previously identified in the liquid and pellicule of kombuchas (Marsh et al., 2014; Chakravorty et al., 2016; Coton et al., 2017). Saccharomyces sp. is the major yeast genus involved in the production of alcoholic beverages (Copetti, 2019).

Concerning their physicochemical composition, pH and titratable acidity values are within the range of values observed in the literature (Cardoso et al., 2020; de Noronha et al., 2022). The increase in acidity is caused by the fermentation process, when bacteria and yeasts metabolize sucrose into a number of organic acids, mainly acetic acid, in addition to glucuronic acid, lactic acid and citric acid (Jayabalan et al., 2007 de Noronha et al., 2022). For coffee leaves kombuchas, the values are similar to those found by Fibrianto et al. (2020), who obtained pH = 3.4 after 3 days of fermentation for robusta coffee leaf kombucha. At this moment, there are no reports on the physicochemical composition of coffee cascara kombuchas for comparison.

Both results are in accordance with previous studies using novel ingredients to prepare kombucha beverages (Ulusoy and Tamer, 2019). The stabilization of pH values during fermentation agrees with the the buffer effect caused by the organic acids and carbon dioxide formed in the fermentation process (Ulusoy and Tamer, 2019; De Noronha et al., 2022). According to Ulusoy and Tamer (2019), the obtained aqueous solution of carbon dioxide dissociates and produces the amphiprotic hydrocarbonate anion (HCO_3^-), which easily reacts with hydrogen ions (H^+) from organic acids, preventing further changes in the (H^+) concentration and contributing to a buffer character of the system. Also, the pH values found in this study are considered safe for human consumption of kombuchas (Nummer, 2013). Values below pH 2.5 indicate hazardous concentrations of acetic acid. Likewise, pH values higher than 4.2 may affect the beverage's microbiological safety (Nummer, 2013). The decrease in soluble solids is probably because of the decrease in sugars concentration in the culture medium overtime (da Silva Junior et al., 2021). At the beginning of the fermentation process, yeast produces invertase which cleaves the disaccharide sucrose to its monosaccharide components, glucose and fructose (May et al., 2019).

The monosaccharide composition of infusions and kombuchas agree with previous studies. Glucose, fructose, galactose and arabinose have been previously identified in black tea leaves and infusion (Wen et al., 2021; Piyasena et al., 2022), while arabinose, galactose, glucose,

xylose have been identified in coffee husk (Cangussu et al., 2021) and in soluble dietary fiber extracted from coffee peel (Dong et al., 2020). Cellobiose were apparently identified for the first time in these infusions. Glucose, galactose, xylose and arabinose were previously identified in coffee leaves (Lima et al., 2013).

As aforementioned, the activity of the enzyme invertase increases along fermentation (Jafari et al. 2020). In all kombuchas, no significant difference was found in glucose and fructose concentrations. In the case of kombucha fermentation, both monosaccharides were consumed without accumulating in the fermented medium because of the multiple microorganisms and biochemical pathways that were occurring simultaneously (Villarreal-Soto et al., 2019).

In relation to catechins, in black tea, gallocatechin is derived from epimerization of epigallocatechin, caused by heat in domestic brewing or production of instant tea (Clifford et al., 2013). Gaggia et al (2019) found epigallocatechin gallate as major catechin in black tea infusion and lower content of epigallocatechin and gallocatechin. Di Lorenzo et al. (2013) and Koch et al. (2017) found epigallocatechin as the main catechin in black tea infusion, but they did not analyze gallocatechin. Also, epicatechin was previously identified in plasma and urine as a result of human microbiota fermentation of black tea (Clifford et al., 2013). The release of catechins from the acid-sensitive microbial cells might be the reason for the increased concentration of epicatechin isomers observed (Jayabalan et al., 2007). Furthermore, it is known that the hydrolysis of epicatechin gallate by microbial metabolism result in gallic acid and epicatechin (Chen and Sang, 2014).

Heeger et al. (2016) could not detect catechin and epicatechin in coffee cascara and pulp. According to them, it is possible that epicatechin was degraded during processing and storage. On the other hand, Ramirez-Martinez et al. (1988) and Mullen et al. (2013) identified epicatechin as one of the abundant phenolic compounds in fresh coffee pulp. Regarding coffee leaves, Trisch et al. (2022) found catechins in fresh coffee leaves, while in dried coffee leaves the content was variable, from not detected to contents varying from 60 to 650 mg/100g of dry weight. According to the authors, this can be related to leaf age and to the oxidation of catechins by polyphenol oxidase to theaflavins or similar oligomeric structures. As our samples were commercial and consequently, the leaves were dried, this could explain why catechins were not identified in our study. Although all initial kombuchas (day 0) had 10% of black tea kombucha, and catechins were identified in all black tea kombucha samples, this small quantity of black tea

kombucha did not interfere in the catechins content of coffee cascara and coffee leaf kombuchas.

Regarding chlorogenic acids, Villarreal-Soto et al. (2019; 2020) quantified chlorogenic acid (5-caffeoylquinic acid) in black tea infusion and kombuchas and found similar results to ours. The higher chlorogenic acids content in CCN samples compared to CCB could be related to many factors such as plant variety, origin, agricultural practices, and, importantly, post harvest processing. (Farah 2019). CCN was wet processed, while CCB was dry-processed. During wet processing, fermentation by yeasts can increase 5- caffeoylquinic acid content. This result is probably related to the production of pectinases, β-glucanases and other hydrolytic enzymes by the yeasts that can facilitate the release of the 5- caffeoylquinic acid out of plant cells (Da Silveira et al., 2019) or release chlorogenic acids bound to other compounds.

Clifford and Ramirez-Martinez (1991) found 5-feruloylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid in wet processed coffee pulp, as well as Mullen et al. (2013) in arabica and robusta coffee fruits. High contents of feruloylquinic acids were found in human urine after coffee consumption (Duarte and Farah, 2011).

Lower content of 3-caffeoylqunic acid in coffee leaves were observed by Chen et al. (2018) (0.007 – 0.039 mg/100mg), but higher content was detected by Monteiro et al. (2020) for 3- caffeoylqunic acid (40 - 300mg/100g) and 4-caffeoylqunic acid (90 – 820 mg/100mg) than in our study. Campa et al. (2012) also did not detect 3-feruloylquinic acid in coffee leaves. 5-caffeoylqunic acid was reported as the most abundant chlorogenic acid present in coffee leaves (Chen et al., 2018). Differences in the contents observed in literature can be due an interaction with the processing method and the age of leaves can influence the content chlorogenic acids in coffee leaves.

Mondolot et al. (2006) also identified 3,5-dicaffeoylquinic acid as the most abundant phenolic acid in coffee leaves. The content of dicaffeoylquinic acids found by De Almeida et al. (2019) were lower than those found in our study. Chen et al. (2018) processed young and mature coffee leaves to mimic white, green, oolong and black tea production. They observed that processed young coffee leaves have more 3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic acids than processed mature coffee leaves. According to the authors, the oxidation process affected the phytochemical content in young and mature leaves differently, but this was dependent on the duration of oxidation.

Gallic acid is one of the main phenolic components of tea and occurs in free and esterified forms in black tea. Most of the gallic acid in black tea is derived originally from epicatechin gallate and epigallocatechin gallate. Also, the content of free gallic acid was higher in black tea and possibly more available for uptake and metabolism (Hodgson et al., 2000). The increase in gallic acid content from black tea infusion to kombuchas was expected. This can explain the reduction of epigallocatechin gallate, probably due the action of the SCOBY microorganisms. In the case of epigallocatechin gallate, the microbial metabolism usually starts with the galloyl ester hydrolysis by microbial esterases, giving rise to gallic acid (Liu et al., 2018). Villarreal-Soto et al. (2019; 2020) also found an increase in gallic acid content, comparing black tea infusions with respective kombuchas.

Considering the phenolic acids identified in black tea kombuchas, hippuric acid had the highest concentration during fermentation. This metabolite was previously identified in urine samples after green or black tea consumption (Clifford et al., 2000; Olthof et al., 2003; Mulder et al., 2005), and is the main product of black tea phenolic compounds. Hippuric acid probably originates in part from the catechins and their condensed polymers such as theaflavins and thearubigins, which are an important group of phenols in tea (Olthof et al., 2003). Clifford et al. (2000) suggested that the origin of the hippuric acid is thought to be gut microbiota metabolism of black tea polyphenols (including the thearubigins) to 3-phenylpropionic acid, followed by endogenous β-oxidation and conjugation to glycine. Microbial metabolites originated by green or black tea consumption may be responsible for at least some of the health effects attributed to tea consumption (Mulder et al., 2005).

Most of the studies that evaluated the excretion of phenolic compounds after green or black tea consumption did not consider the metabolism of chlorogenic acid. A study identified 4-hidroxyphenylacetic acid, another chlorogenic acid metabolite, in urine after black tea consumption (Olthof et al., 2003), also identified in black tea kombuchas after 3 days to 9 days of fermentation. This compound is also a colonic metabolite of quercetin (Crozier et al., 2010). So, we can hypothesize that the other metabolites found in black tea kombucha could be derived from chlorogenic acid metabolism by microorganisms in kombucha consortia. Caffeic, ferulic and p-coumaric acid were previously quantified in black tea kombuchas (Villarreal-Soto et al., 2019; 2020), but we did not identify ferulic acid in our black tea kombuchas.

Dihydrocaffeic acid followed by vanillic acid, 3,4-dihidroxibenzoic acid, hippuric acid, caffeic acid, 4-hidroxiphenylacetic acid, ferulic acid, p-coumaric acid and benzoic acid are known as colonic metabolites of chlorogenic acids (Gonthier et al., 2006) (Figure 9) and were identified in urine and plasma samples after coffee consumption (Farah et al., 2008; Duarte and Farah, 2011). A previous study indicate that human fecal samples contain an esterase capable of hydrolysing chlorogenic acid to caffeic and quinic acids (Plumb et al., 1999). The metabolism of caffeic acid can originate p-coumaric acid and ferulic acid, which originate sinapic acid. Quinic acid can be metabolized to gallic acid, which originate vanillic acid and also 3 and 4-hidroxibenzoic acid, and then originate benzoic and hippuric acid (Farah and Lima, 2019).

Rutin can be metabolized by colonic microbiota to quercetin (Riva et al., 2020) Quercetin is the also the aglycone form of quercetin-4-O-glucoside, that may also be metabolized by colonic bacteria (Crozier et al., 2010), and the two routes may explain its increase during kombucha production, through the action of the microorganisms in the SCOBY. The content of mangiferin found in this study were lower than those found by Campa et al. (2012), Trevisan et al. (2016), Segheto et al. (2018) and De Almeida et al. (2019), although we found higher mangiferin content than Acidiri et al. (2020). Differences in mangiferin content can be explained to differences in coffee species analyzed (Campa et al., 2012) or the region of the coffee tree that leaves were obtained (De Almeida et al., 2019).

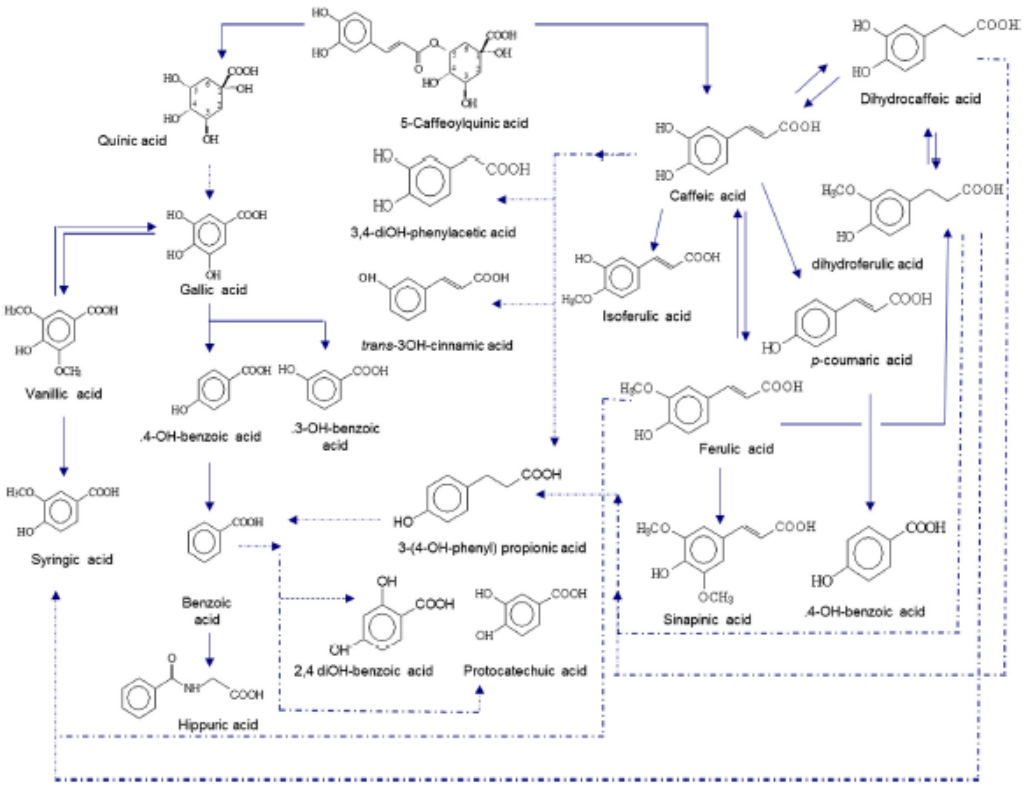


Figure 9. Simplified proposed scheme for the metabolic route of chlorogenic acids, represented by 5-caffeoylquinic acid. Dotted lines represent more than one reaction until the final product. Reproduced from Farah and Lima (2019), with permission from Royal Society of Chemistry.

Regarding caffeine, this compound behaves as a good complexing agent with phenolic compounds and other biomolecules that could affect biological properties such as bioavailability, cellular absorptivity, and biochemical activities (Nemzer et al., 2022). Caffeine-chlorogenic acid complex, mainly with 5- caffeoylquinic acid (Farah and Donangelo, 2006) was probably hydrolyzed by kombucha consortia and can explain the caffeine increase, and consequently, 5-caffeoylquinic acid increase. For trigonelline, at this moment, there is no studies that evaluated its content in coffee husk. For coffee leaves, this compound was previously quantified in coffee leaves (Chen et al., 2018; Monteiro et al., 2020).

Concerning antimicrobial activity of infusions and kombuchas, Battikh et al. (2012) also observed inhibition on pathogenic bacteria growth after administration of unfermented black tea, but a decrease in antimicrobial activity for neutral kombuchas against pathogenic bacteria. According to the authors, such behavior would explain the contribution of the organic acids and

phenolic compounds of the beverages against the tested bacteria. We did not find differences between natural and neutral kombuchas.

Damaging the bacterial cell membrane, inhibiting the fatty acid synthesis and preventing enzyme activity are among the antimicrobial characteristics of phenolic compounds (Demir, 2021), which can explain the highest antimicrobial activity for *L. monocytogenes* for black tea infusion. This Gram-positive bacterium can be more sensitive to tea extracts than Gram-negative bacteria, and the negatively charged epigallocathechin galate may act by binding to the positively charged lipid bilayer in Gram-positive bacteria (Kohda et al., 2008), and then inducing cell wall damage (Yoda et al., 2014). Considering *S. enteritidis* and *E. coli*, two Gram-negative bacteria, the low antimicrobial activity, comparing with *L. monocytogenes* is probably due to cell membrane composition. Gram-negative bacteria have several layers of peptidoglycan. These layers are overlaid by an outer membrane that is mainly composed of lipopolysaccharides (LPS), which is a very important permeability barrier to protect bacteria from various antibacterial materials. Epigallocathechin galate from black tea has low affinity with LPS, limiting the binding of this phenolic compound to peptidoglycan, thereby reducing the susceptibility of Gram-negative rods to catechins (Yoda et al., 2004). Since black tea infusion and its kombucha on day 0 has more total catechins than other kombuchas, this can explain the highest inhibition zone for *L. monocytogenes*.

Furthermore, coffee cascara and coffee leaves have several bioactive compounds that was previously studied for antimicrobial activity of coffee extracts (Almeida et al., 2006). Caffeine, trigonelline, chlorogenic acids, caffeic acid, 3,4-dihydroxybenzoic acid, p-coumaric acid and ferulic acid can exert inhibitory activity mainly for *E. coli*. With exception of ferulic acid, these compounds also inhibited *L. monocytogenes*. Caffeine can pass easily to the cell wall of the bacteria and it can inhibit DNA synthesis. Chlorogenic acid can increase the outer and plasma membrane permeability, causing loss of barrier function and also leakage of nucleotide. It is also possible that they could inhibit enzyme activity, by reacting with sulfhydryl groups or through interaction with proteins (Gloria et al., 2019).

Duangjai et al. (2016) also found antimicrobial activity of coffee pulp against E. coli, and also for *S. aureus, S. epidermidis* and *P. aeruginosa*. Essential oil of coffee husk also exhibited antimicrobial activity against *E. coli* and other pathogenic bacteria (Al-Yousef and Amina,

2018). No statistical differences were found after administration of coffee cascara kombuchas adjusted to neutral pH and no inhibitory activity on *L. plantarum* was observed.

In coffee cascara kombuchas, we observed an increase in antimicrobial activity up to day 9. This could be due to the increased in phenolic compounds in kombuchas from day 0 to day 9 due the action of the microorganisms in SCOBY (see manuscript 4). On the other hand, Khochapong et al. (2021) observed high inhibition on *E. coli* and *S. aureus* strains after administration of coffee pulp extract, while the digested coffee pulp sample showed low inhibitory activity. According to them, the reduction of antimicrobial potency of the digested coffee pulp extract may be due to the reduction of bioactive compounds as well as structural disruption by the digestive enzymes and low pH in the digestive system. In addition, both coffee pulp extract and its digested extract have no inhibitory effect against Lactobacillus strain.

Methanolic extracts of arabica and robusta coffee leaves exhibited activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* (Nayeem et al., 2011). Mesquita Júnior et al. (2022) found a bactericidal effect against the same *E. coli* strain and bacteriostatic effect against *Staphylococcus aureus* after administration of a lyophilized ethanolic extract of arabica coffee leaves, while Kenconojati et al. (2019) found an antibacterial activity in an ethyl acetate extract of *C. canephora* coffee leaves against *Edwardsiella tarda*, which presented the highest inhibition zone, and *Streptococcus agalactiae*. They suggested that coffee leaves extract is more effective against Gram negative than against Gram positive bacteria. In our study, the highest inhibition was found for Gram positive bacteria (*L. monocytogenes*). At this moment, there is no studies evaluating the antimicrobial activity of coffee leaves kombuchas.

Conclusions

The predominance of acetic acid bacteria belonging to the genera *Komagateibacter* and yeasts from *Pichia* genera were observed in the microbial composition of black tea, coffee cascara and coffee leaves kombuchas The raw material did not change the microbial composition between samples. The fermentation process by the consortia increased titrable acidity, lowered the pH and soluble solids. The decrease in sucrose content and consequently increase in glucose and fructose

in kombuchas also show the action of the symbiosis between acetic acid bacteria and yeasts in kombucha consortia due the action of enzymes in the microorganisms.

The main bioactive compounds in black tea kombucha were catechins, whose content decreased during fermentation, followed by chlorogenic acids, with increase in their contents. Although traces of catechins were found in coffee cascara and coffee leaves kombuchas, high concentrations of chlorogenic acids were found during the fermentation process. Furthermore, compounds known as colonic metabolites of chlorogenic acids were identified, reveling that the microorganisms in kombucha consortia can metabolize these phenolic compounds, since their concentrations increased up to day 9 of kombucha fermentation.

For antimicrobial activity, black tea, coffee cascara and coffee leaf infusions showed higher inhibitory activity for *Listeria monocytoges* ATCC 11229. The incubation with its respective kombuchas led to an increase in antimicrobial activity against *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076, with no differences with kombuchas adjusted to neutral pH (pH = 7). No inhibitory activity was found against the lactic acid bacteria *Lactiplantibacillus plantarum* ATCC 8414.

The results obtained show that coffee cascara and coffee leaves are a suitable raw material to produce kombuchas from new substrates, improving the sustainability in coffee production and lowering environmental impacts caused by these byproducts.

The effect of phenolic compounds degradation before kombucha consumption as opposed to in the gut should be evaluated in bioavailability studies.

ESTUDO 2: Caracterização volátil, perfil microbiológico e aceitação sensorial de kombuchas de cascas dos frutos do cafeeiro.

Devido a elevada produção mundial de café, toneladas de cascas de café, fontes de compostos bioativos, são descartadas anualmente. Utilizando este subproduto para produzir alimentos com boa aceitação potencialmente benéficos à saúde é uma prática sustentável que agrega valor a produção de café e pode auxiliar a melhora da vida da população. Este estudo teve como obejtivo a eleboração de kombuchas a partir das infusões de cascas de café, avaliar seu perfil microbiológico, e acompanhar as mudanças no perfil volátil durante a fermentação, associado com atributos sensoriais e aceitação por consumidores no Rio de Janeiro (n=113). Cascas de café arábica do Brasil e Nicarágua foram utilizadas para preparar as infusões, nas quais kombucha de chá preto, um cultura simbiótica de bactérias e leveduras (SCOBY), e sacarose foram adicionados. A fermentação de kombucha de chá preto também foi acompanhada para comparação. O perfil volátil foi analisado após 0, 3, 6, e 9 dias de fermentação através da microextração do headspace em fase sólida CG-EM. Um total de 81 compostos foram identificados considerando todas as bebidas, 59 nos kombuchas de cascas de café e 59 nos kombuchas de chá preto, com 37 compostos em comum. Um aumento principalmente em ácidos e ésteres ocorreu durante a fermentação. Apesar da similaridade com o kombucha de chá preto, alguns aldeídos, ésteres, alcóois e cetonas nos kombuchas de cascas de café não foram identificados no kombucha de chá preto. Potenciais compostos de impacto nas cascas de café foram linalool, decanal, nonanal, octanal, dodecanal, etanol, 2-etilhexanol, acetato de etila, butirato de etila, beta-damascenona, gama-nonalactona, óxido de linalool, alcóol de feniletila, acetona de geranil, fenilacetaldeído, alcóol de isoamila, ácido acético, ácido octanóico, ácido isovalérico, isobutirato de etila, hexanoato de etila e limoneno. As notas médias de aceitação para os kombuchas de casca de café variaram entre 5,7 + 0,53 e 7,4 + 0,53 em uma escala hedônica de 9 pontos, com o kombucha elaborado com a casca da Nicarágua com 3 dias de fermentação com a maior média de aceitação, associado com a doçura e aromas e sabores de frutas fermelhas, mel, amadeirado e de ervas. Os resultados indicam que a casca de café é um subproduto promissor para elaboração de bebidas fermentadas, exibindo características exóticas e singulares que podem ser exploradas para aplicações na indústria de alimentos.

Palavras-chave: subprodutos de café, compostos voláteis, cascas de café, chá preto, fermentação, bebidas fermentadas, novos alimentos

1. Introduction

Coffee is among the most consumed foods globally. In 2021/2022, the coffee world production was, approximately, 10 million tons (ICO, 2022). Several steps are involved in coffee production. After the fruits are harvested, they may undergo different types of processing to release the seeds that are traditionally roasted and ground for the coffee beverage extraction. While in the wet processing the skin and pulp are fermented or enzymatically digested to release the seeds, in the dry and semi-dry processing, they are mechanically separated from the seeds after washing and drying. On a dry weight basis, coffee pulp alone corresponds to approximately 28 % of coffee fruit, the skin approximately 12 %, and the seeds 50 – 55 % (Heuzé et al., 2023). Therefore, a significant amount of biomass, which has been mainly considered as "waste" material by the coffee industry, is currently discarded without further valorization (Esquível et al., 2020).

Coffee cascara is rich in bioactive compounds such as chlorogenic acids, anthocianins and other flavonoids, and soluble fibers (Rios et al., 2020, De Paula et al 2022), In Europe, coffee cascara was considered a novel food from 2005 until recently, when it received authorization by the European Food Safety Authority (EFSA) to be used as a food in the European market (EFSA, 2021). Since then, it has been incorporated in bread production as flour (Rios et al., 2020), added to food matrices like yogurts (Iriondo De-Hond et al., 2020), or used for infusions preparation (Heeger et al., 2016; DePaula et al., 2022). In the USA, it has been market as natural cascara tea.

Fermented foods are defined as foods made through desired microbial growth and enzymatic conversions of food components. Microorganisms determine the course and outcome of fermentation processes and contribute to the development of the characteristic properties of the final fermented food (Marco et al., 2021). Kombucha is a fermented beverage traditionally consumed in Asia for centuries and, as trade routes expanded, it reached Europe (Jayabalan et al., 2014) and other Western countries. It is mainly made of sweetened green and or black *Camellia sinensis* tea and a symbiotic culture, short named as SCOBY and comprised of acetic acid bacteria and yeasts, in addition to minor microorganisms (Leal et al., 2018).

Several potential benefits of traditional kombucha have been reported *in vitro* and in animal studies, such as antibacterial activity (Kaewkod et al., 2019; Cardoso et al., 2020), anticarcinogenic activity for colon breast, lung and prostate cancers (Villareal-Soto et al., 2019;

Kaewkod et al., 2019; Cardoso et al., 2020, Srihari et al., 2013a), hypoglicemiant effect and weight loss in diabetic rats (Srihari et al., 2013b). Most of these effects are related to the relevant antioxidant (Jayabalan et al., 2008; Bhattacharya et al., 2011; Kaewkod et al., 2019; Sales et al., 2023) and anti-inflammatory (Ramani et al., 2019; Villareal-Soto et al., 2019, Sales et al., 2023) activities inherent to *Camelia sinensis*. But kombucha brings about the benefits of being a cold beverage that can be consumed at any time in warm weather, replacing cold drinks and increases bioactive compounds bio-accessibility.

In recent years, kombucha popularity has exploded globally along with the healthier foods trend. The global kombucha market size was valued at USD 2.64 billion in 2021 and is expected to expand at an annual growth rate (CAGR) of 15.6% from 2022 to 2030 (Grand View Research, 2021). Recently, new raw materials have been added or even replacing *C. sinensis* in the production of kombucha and kombucha-like products. This includes food industry by-products, as sustainable and healthy alternatives (Leonarski et al., 2022). So far, there are no reports on coffee cascara kombucha, other than our recent publication on its potential bioactivity (Sales et al., 2023). Considering the potential health promoting qualities of coffee cascara and the prospect of sustainability in coffee production, this study aimed to elaborate kombuchas from coffee fruit cascara infusions with different fermentation intensities and characterize the SCOBY microbial profile and changes in the beverages' volatile profile during fermentation, asso-ciating these changes with their sensory attributes. We also evaluated the acceptance of the beverages by consumers in Rio de Janeiro.

1. Material and Methods

The experimental design of the study is shown in Figure 1.

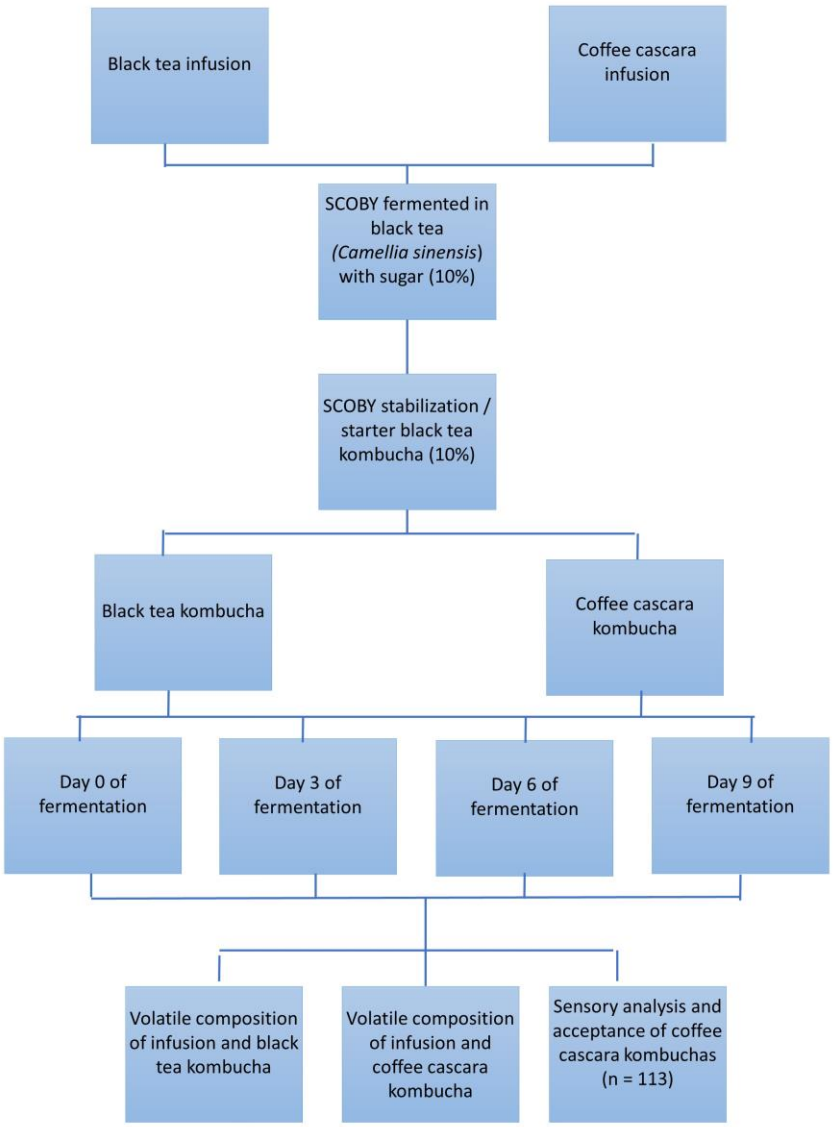


Figure 1. Experimental design of study 2.

2.1 Samples

The lead commercial *C. sinensis* black tea (BT) was purchased in a food market of Rio de Janeiro, Brazil; dry and wet processed *Coffea arabica* cascara (CC) teas were acquired directly

from producers in Domingos Martins, in the Caparaó Mountains region of Espírito Santo, Brazil (CCB), and from Matagalpa, Nicaragua (CCN), respectively.

2.2 Kombucha consortium, black tea and coffee cascara kombucha

Please see study 1.

2.3. pH, total titratable acidity, total soluble solids determination and sugars analysis

Please see study 1.

2.4. Analyses of volatile organic compounds

Extraction of the volatile organic compounds from the infusions and kombuchas was performed by headspace solid-phase microextraction (HS-SPME), using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS, Supelco®), and analyzed by a gas chromatographer (Agilent, 6890 Little Falls, DE, USA) coupled to a mass spectrometer (Agilent 5975) (GC-MS), according to the methodology described by Wang et al. (2019). Before use, the fiber was conditioned according to the manufacturer's recommendations. Two mililiters of each sample were placed in a 20 mL SPME vial, which was immediately sealed with silicone septa and conditioned for 5 min at 50 °C under continuous agitation at 400 rpm. Then, the fiber was exposed to the vial headspace for 30 min, in agitation, and heated at 50 °C. After this period, the fiber was retracted, and inserted into the chromatographic injector, in splitless mode, for 2 min, for desorption of volatile compounds, with the aid of a carrier gas (helium) and transferred directly into the chromatographic column (SPB-5, 60 m × 0.32 mm, film thickness df = 1 μm 5% diphenyl – 95% dimethyl polysiloxane, Supelco, Bellefonte, USA) at 1 mL min/ for 10 min, 250 °C. The chromatographic separation conditions were: 40 °C for 3 min, ramped to 200 °C at 5 °C/min, subsequently ramped to 250 °C at 10 °C/min, and held at final temperature for 3 min. The transfer line, ion source, and MS quadrupole temperature were 250, 230, and 150 °C, respectively. Electron impact mass spectra were measured at the acceleration energy of 70 eV. Data acquisition was performed in full-scan mode from m/z 50 to 550. Analytes were tentatively identified by the linear retention indices (LRI) and confirmed by the National Institute of Standards and Technology (NIST V2.2, USA) library database (NIST Standard Reference Data, 2022). Agilent Chem Station (Agilent Technologies, USA) was used for data collection and processing. In addition, high purity external standards, when available,

were injected. The LRI of each compound was calculated using the respective retention time (RT) compared against the RTs of a series of standard n-alkanes. The compounds were identified based on their LRI, the mass spectra of the NIST library (NIST Database, 2022), or authentic standards measured under the same conditions. To identify the compounds, substances with a probability greater than 50% were selected. To improve the accuracy of compounds' identification, only those substances that provided a match factor higher than 600 and a match factor versus reversed match factor ratio greater than 0.8 were selected for data processing (Galvan-Lima et al., 2021; DePaula et al., 2022). LRI available from previous publications were also used for comparison. Analyses were performed in triplicate samples.

2.5. DNA extraction, amplicon sequencing data analysis and library preparation

Please see study 1.

2.6. Sensory analysis

The study was approved (# 4.513.606) by the Ethical Committee of Clementino Fraga Filho University Hospital at Federal University of Rio de Janeiro and fully explained to the subjects who gave their written informed consent prior to participation. One hundred and fourteen consumer assessors took part in the Acceptance, Purchasing Intention, and Rate All That Apply (RATA) tests. They were students, teachers, visitors, and employees at the Federal University of Rio de Janeiro-UFRJ Health Sciences Center. Eligible criteria were people who consumed kombucha or sparkling beverages, such as sparkling water, soft drinks, cider and sparkling wine. Because part of the test was performed during the COVID-19 pandemic, we asked if the person had a positive diagnosis for the disease and, consequently, the loss of taste and/or smell. These subjects were excluded from the study, as well as any other subjects with a condition that could affect sensory evaluation.

Assessors performed the tests on individual benches in the UFRJ Food and Dietetics Lab under white light. Before receiving the samples, demographic information was collected, including gender, age, educational level, occupation, family monthly income, frequency, and habits of kombucha and sparkling beverages consumption. Approximately 30 mL of kombucha were presented at 4 °C in 40 mL acrylic cup with dimensions 43 x 56 mm, coded with three-digit random numbers, and distributed in a balanced way to avoid the consistent influence of

neighboring samples on the sensory sensation. Crackers and spring water at room temperature were offered between samples to clean the palate.

2.6.1. Consumer acceptance and purchase intention

Assessors evaluated the infusions using the nine-point hedonic scale ranging from 1 (extremely disliked) to 9 (extremely liked), followed by a five-point purchase intention scale ranging from 1 (certainly would not buy) to 5 (certainly would buy) (Meilgaard et al., 2016). The Acceptability Index (AI) was calculated using the following equation: AI = (X * 100)/N, where: X = Average score given by assessors and N = Highest score given by assessors. AI equal to or greater than 70% was considered satisfactory (Meilgaard et al., 2016).

2.6.2 Rate All that Apply (RATA)

After marking the hedonic scales, assessors were given a pre-prepared checklist with 34 sensory attributes related to appearance, aroma, flavor, and mouthfeel, which were identified in a preliminary session performed by a trained panel. Following, they were required to select all terms they considered appropriate to describe the infusions. Given that CCN infusions had a more pronounced aroma, flavor, and taste attributes than CCB, and that aroma intensity increased with fermentation, we investigated whether the assessors perceived such differences by asking them to score the attributes according to their intensity (RATA scores: 1 = low intensity; 2 = medium intensity; and 3 = high intensity). The attributes used in the study were organized by alphabetical order as follows: For aroma, citric, fermented, floral, herbal, honey, raisin, red fruits, rosé wine, syrup, woody, yellow fruits; for taste, acid/sour, bitter, sweet; for flavor, alcoholic, apple vinegar, beer yeast, hibiscus, prune, vinegar; and for mouthfeel, texture and aspect, adstringent, brown color, clear, fizzy, full bodied, opaque/matte, refreshing, sparkling and watery.

2.7. Statistics

Analysis of variance (ANOVA), followed by Tukey's test was used to compare physicochemical analysis and acceptance and purchase intention tests results. Differences were considered significant when $p \leq 0.05$ (Version 8.4.2, Informer Technologies, Los Angeles, CA, USA).

RATA scores were analyzed as continuous data, with Principal Component Analysis (PCA), through the arithmetic mean values of the sensory descriptors for all assessors. Non-applicable attributes were marked as intensity 0 (Ares et al., 2014; Meyners et al., 2016). Cluster analysis based on the hierarchical grouping of acceptance scores was carried out to identify segments of consumers with similar likings (DePaula et al., 2022). Principal Component Analysis (PCA) was performed to understand the evolution of volatile compounds identified as relevant for RATA attributes on CCB and CCN samples. For this purpose the free software R (R version 4.2.2, RStudio team, 2022) was used.

3.Results and Discussion

3.1. Physicochemical parameters

Please see study 1.

3.2. Microbial taxonomy

The microbial community of the starter culture (BT K d14) and the final liquid and biofilm composition of CCKs d9 were characterized (Figure 1). Data analysis of 16S rRNA gene sequence revealed two bacterial phyla in all samples, Proteobacteria and Firmicutes. Proteobacteria was the most abundant phyla, especially in CCKs, with percentage higher than 90%, in agreement with previously black tea kombuchas (Chakravorty et al., 2016; Arikan et al., 2020; Marsh et al., 2014).

In all kombuchas, *Komagataeibacter* - an acetic acid bacteria and the most efficient bacterial cellulose producer (Canazza et al., 2021) - was the most abundant genus observed in the liquid and biofilm, which is also in accordance with previous studies characterizing kombucha cultures (Chakravorty et al., 2016). In the starter culture, only the specie *Komagataeibacter rhaeticus* was identified, with about 40% of the total number of bacteria. This is known to be one of the most abundant bacterial members among the kombucha fermenting agents (Villarreal-Soto et al., 2020; Landis et al., 2022). *Komagateibacter* genus has been positively correlated to the presence of ketones and aldehydes in a type of vinegar (Zhu et al.,

2018). In wine, it was associated to cream, green grass, jams, and tropical fruits' attributes (Zhang et al., 2022).

In CC Ks, *K. rhaeticus* comprised more than 80% and 60%, respectively, of CCB and CCN K microorganisms contained in the liquid and solid cultures. *K. rhaeticus, K. europaeus* (15% - 36%), *K. intermedius* (0.2%), *K. europaeus* and *K. intermedius* have previously been identified in black tea kombucha (Barbosa et al., 2021; Coton et al., 2017; Villarreal-Soto et al., 2020). *Gluconacetobacter entanii* (0.5%) was identified in all CC Ks. *Gluconacetobacter* genus has been previously detected in BT Ks (Marsh et al., 2014; Villarreal-Soto et al., 2020) but it can also be isolated from other fermented food matrixes (Sengun and Karabiyikli, 2011), including *G. entanii* in rooibos tea kombucha (Gaggia et al., 2019). Also, this genus have useful characteristics to be combined with yeast strains for glucuronic acid production (Nguyen et al. 2015).

In the present study, Staphylococcus (24%), Enterobacteriacea (18%), Lactobacillus (15%), and Pediococcus (0.4%) were observed in BT K and not in CC K, showing that these microorganisms were in the BT leaves. The family Enterobacteriaceae comprises a very large group of morphologically and physiologically similar bacteria. They are of great importance, while some of these organisms are involved in food spoilage, others are food-borne pathogens, and some are indicators of fecal contamination of food products (Singh and Anand, 2020). Because near boiling water is used for infusions preparation, we can consider that the beverages were microbiologically safe in relation to Enterobacteria contamination. In the study by DePaula et al. (2022), the infusion of contaminated coffee cascara tea presented zero count of viable thermo-tolerant. These bacteria are heat sensitive and are not viable at temperatures above 45 °C. An additional Enterobacteriaceae strain identified in the starter and in CCB K was *Pantoea septica* (Walterson and Stavrinides, 2015). *Pantoea* spp. genus was previously identified in grape cultivar for wine production and was positively correlated with straight-chain fatty alcohols, aromatic aldehydes, and terpenes in wine (Zhang et al., 2022).

Low percentages of *Lactobacillus* (0.08% - 0.9%), Enterobacteriaceae (0.06% - 0.6%) and *Staphylococcus* (1.3%) were observed in CC Ks. Two lactic acid bacteria were identified in BT K and in CCB K, *Latilactobacillus sakei* and *Pediococcus pentosaceus*. In a model kimchi, *L. sakei* produced volatiles compounds such as hexanal, acetic acid and geranyl acetone (Choi et al., 2019). *Pediococcus pentosaceus* has been used to ferment tilapia surimi and some of the

main volatile compounds were the aldehydes hexanal, nonanal, heptanal, octanal, decanal, undecanal and benzaldehyde (Li et al., 2022). *Staphylococcus carnosus* and *Staphylococcus xylosus* were identified in BT K and CCKs. They are coagulase-negative *Sthaphylococcus* spp. strains commonly found in diversified fermented food products as an integral part of the natural flora, and often recognized as non-infective microbiota. They can also attribute acidic and buttery taste to fermented meats (Khusri and Aarti, 2022).

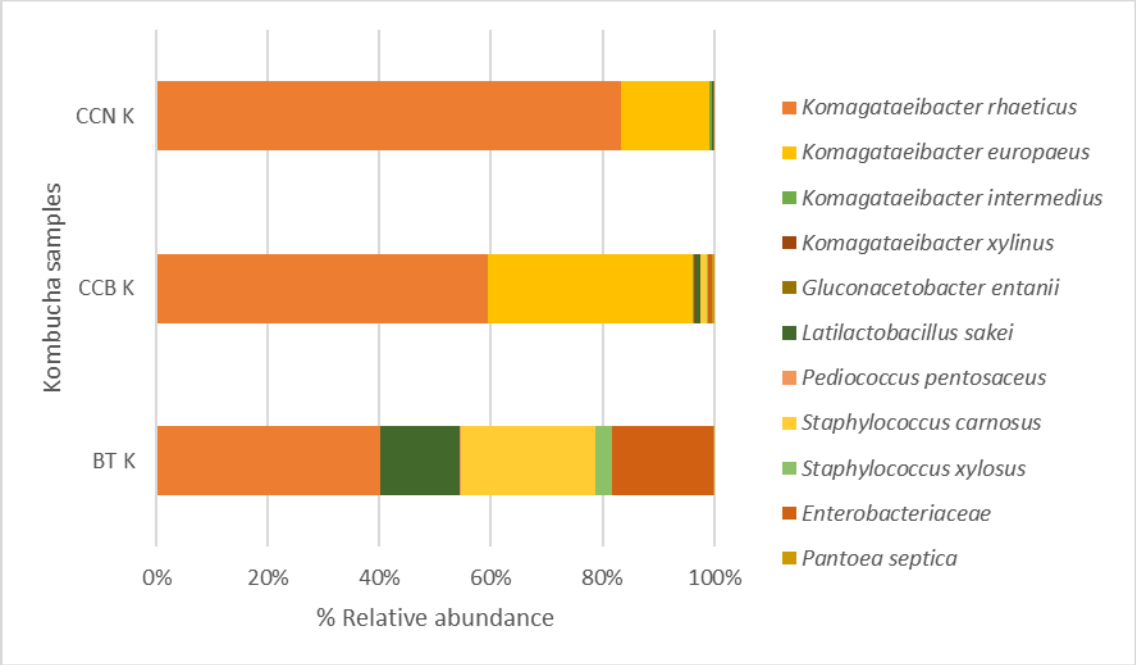


Figure 2. Bacterial composition of the solid and liquid phases of the black tea and coffee cascara tea kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha; CCB K- coffee cascara kombucha from Brazil; CCN K. Nicaragua coffee cascara kombucha

Regarding yeasts, their metabolism is not only responsible for the production of ethanol but also for the formation of several hundreds of flavor-active compounds, imparting their characteristic aroma and flavor to fermented beverages. The production and concentration of metabolites, desirable or not (off-flavors), formed during fermentation depends on the contribution of particular yeast species or strains. Thus, yeast communities have great potential to shape the aroma and flavor of fermented beverages (Varela, 2016).

ITS1 analysis indicated that the most abundant phyla was Ascomycota (Figure 2). Pichia was the predominant yeast genera with abundance higher than 70%, followed by Saccharomyces (>2%). *Brettanomyces bruxellensis* strain (5%) was present in all kombuchas. Other non-

saccharomyces strains comprised 0.4% of total yeasts. They were present in all fermented beverages.

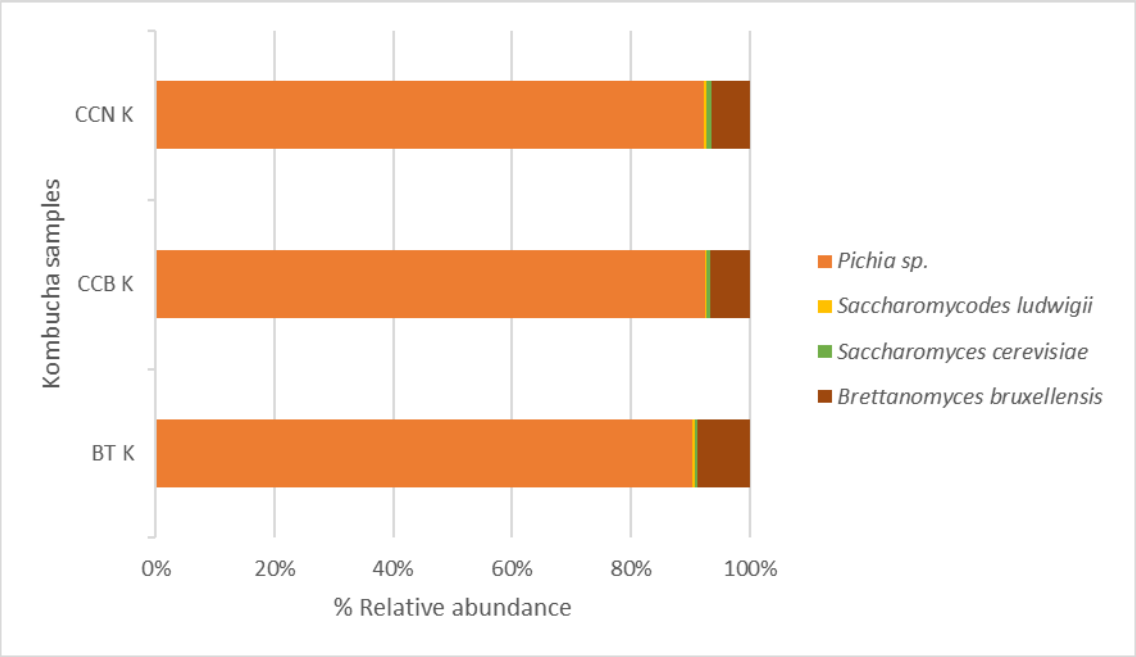


Figure 3. Yeast composition of the solid and liquid phases of the black tea and coffee cascara tea kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha; CCB K- Brazil coffee cascara kombucha; CCN K. Nicaragua coffee cascara kombucha

Pichia sp. is one of the main yeast genera found in Kombuchas (Marsh et al., 2014, Coton et al., 2017; Chakravorty et al., 2016). The main Pichia strains identified in BT and CC kombuchas were *Pichia fermentans* and *Pichia kluyveri*. *Pichia* species are generally applied in winemaking to improve aroma composition (Vicente et al., 2021).

Brettanomyces bruxellensis is the most common yeast identified in kombucha tea and SCOBY (Teoh et al., 2014; Villarreal-Soto et al., 2020; Landis et al., 2022). *Brettanomyces* yeasts can strongly affect the aroma of fermentation products. Many different positive and negative attributes, including apple, floral, tropical fruit, citrus and/or spicy, cracker biscuit, clove, mousy, barnyard, smoky, plastic, phenolic, medical, “band-aid”, metallic, humid leather, sweaty, goat-like are used to describe the (often pungent) aroma profile of these strains (Steensels et al., 2015). In kombucha, *Brettanomyces bruxelensis* can produce high amounts of alcohols and acids, such as isoamyl alcohol, phenylehtyl alcohol, isovaleric acid, hexanoic acid, octanoic acid, lauric acid (Leali et al., 2022).

Saccharomyces sp. is the major yeast genus involved in the production of alcoholic beverages (Copetti, 2019). *Saccharomyces* sp. strains have been previously identified in the liquid and pellicule of kombuchas (Marsh et al., 2014; Chakravorty et al., 2016; Coton et al., 2017). During fermentation, *Saccharomyces cerevisiae* (0.4% - 1% in our kombuchas) produces a broad range of aroma-active substances that are vital for the complex flavor of fermented beverages, with esters being the most important compounds with industrial purposes, since they are responsible for fruity, candy and perfume-like aroma of alcoholic fermented beverages (Saerens et al., 2010), Regarding non-saccharomyces yeasts present in the SCOBY, *Saccharomycodes ludgwigii* is considered detrimental to the winemaking process and contaminate ciders because it can produce in fermented beverages the volatiles ethyl acetate, isoamyl acetate and acetaldehyde that may confer negative undertones to wine when exceeding their respective thresholds of perception (Vejarano, 2018; Ellis et al., 2022).

It is worth noting that some bacteria and genera identified in CC K in this study (*Gluconoacetobacter, Acetobacter, Pediococcus pentosaceus,* Enterobacteria and *Saccharomyces* sp.) are common to coffee fruit and seeds, since CC is a postharvest by-product (Vaughan et al., 2015; De Bruyn et al., 2017; Zhang et al., 2019; Martinez et al., 2022). However, as aforementioned, these microorganisms were probably not viable to take part in the fermentation process given that CC is subjected to near boiling temperature during the infusion preparation.

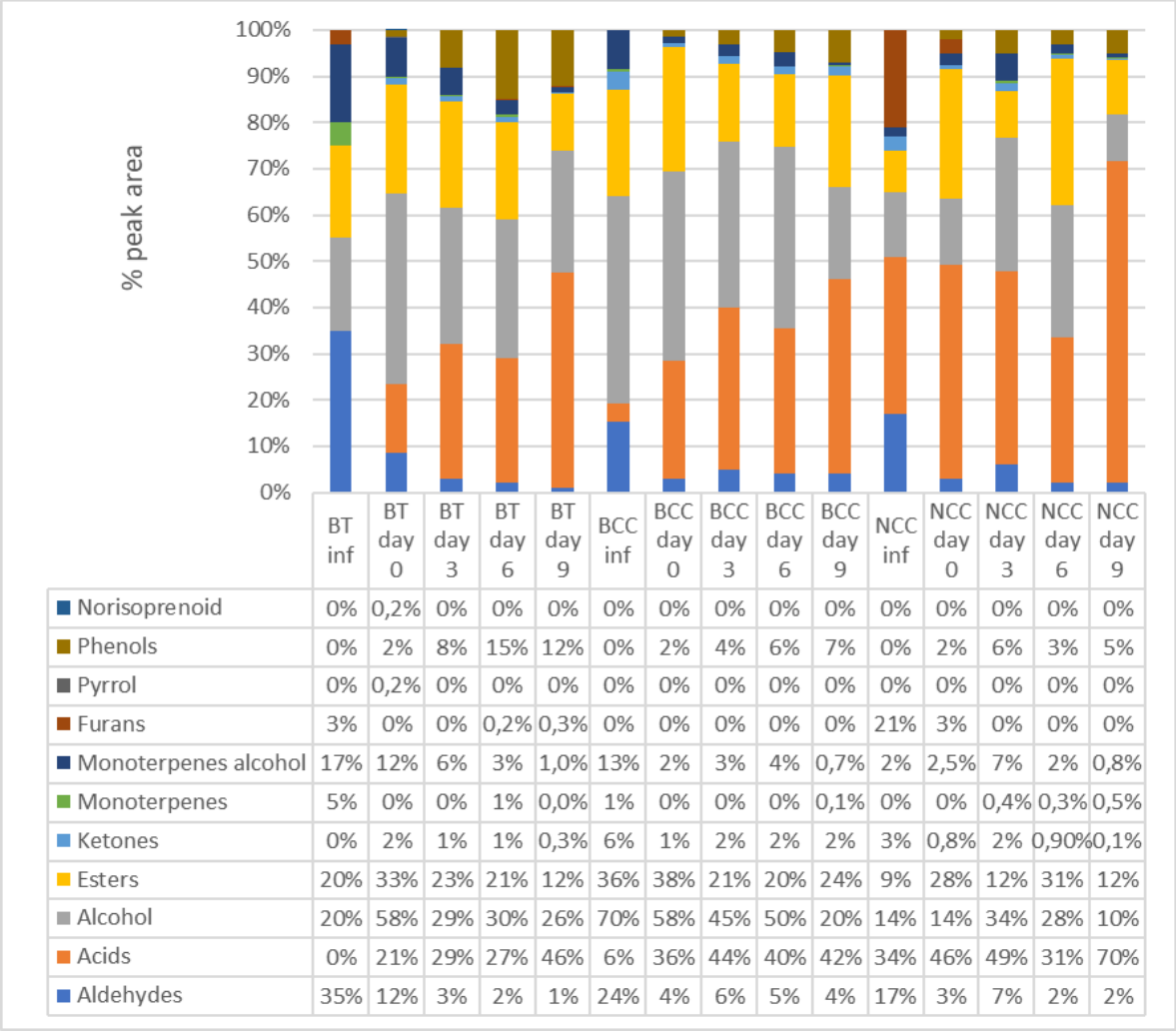
3.3 Volatile organic compounds

Figure 3 compares the relative peak areas of organic volatile compounds (grouped into classes) in infusions and kombuchas made with black tea and coffee cascara.

Aldehydes represented 1 - 12% of total peak areas in BT K and 2- 6% in coffee cascara kombuchas, decreasing as fermentation progressed in all samples, given the transformation into the corresponding derived alcohols (Ubeda et al., 2019). Alcohols represented 26 - 58% of total peak area in BT K and 10 - 58% in CCKs, decreasing up to d9. The largest areas were observed in CCB kombuchas (58%; 45%; 50% and 20% on d0, d3, d6 and d9, respectively). The high content of alcohols at d0 of fermentation in both kombuchas is derived from BT and CC raw material and from the starter (Tran et al., 2020; Bishop et al., 2022). Acids showed the largest peak areas (21- 46% of total peak area in BT K and 31 - 70% in coffee cascara kombuchas), with

higher percentage in CCN K d9 (70%). Volatile acids are produced during alcoholic and acetic fermentation, by the symbiosis between acetic acids bacteria and yeasts in SCOBY (Tran et al., 2020). Esters represented 12- 33% of the total peak areas in BT K and 9-38% in CC Ks. In all CC Ks, an increase in the number of esters was observed during fermentation, although the area of this chemical class decreased from d0 to d9.

Ketones comprised 0.3-2% of total peak areas in BT K and 0.1- 3% in CC Ks, monoterpenes represented 0-1% in BT K and 0-0.5% in CC Ks, and monoterpene alcohols 1-12% in BT K and 0.8-7% CC Ks



	BT inf	BT day 0	BT day 3	BT day 6	BT day 9	BCC inf	BCC day 0	BCC day 3	BCC day 6	BCC day 9	NCC inf	NCC day 0	NCC day 3	NCC day 6	NCC day 9
Norisoprenoid	0%	0,2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Phenols	0%	2%	8%	15%	12%	0%	2%	4%	6%	7%	0%	2%	6%	3%	5%
Pyrrol	0%	0,2%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Furans	3%	0%	0%	0,2%	0,3%	0%	0%	0%	0%	0%	21%	3%	0%	0%	0%
Monoterpenes alcohol	17%	12%	6%	3%	1,0%	13%	2%	3%	4%	0,7%	2%	2,5%	7%	2%	0,8%
Monoterpenes	5%	0%	0%	1%	0,0%	1%	0%	0%	0%	0,1%	0%	0%	0,4%	0,3%	0,5%
Ketones	0%	2%	1%	1%	0,3%	6%	1%	2%	2%	2%	3%	0,8%	2%	0,90%	0,1%
Esters	20%	33%	23%	21%	12%	36%	38%	21%	20%	24%	9%	28%	12%	31%	12%
Alcohol	20%	58%	29%	30%	26%	70%	58%	45%	50%	20%	14%	14%	34%	28%	10%
Acids	0%	21%	29%	27%	46%	6%	36%	44%	40%	42%	34%	46%	49%	31%	70%
Aldehydes	35%	12%	3%	2%	1%	24%	4%	6%	5%	4%	17%	3%	7%	2%	2%

Figure 3. Relative peak area (%) of volatile organic compounds in black tea and coffee cascara infusion and kombucha beverages, grouped into chemical classes. Note. BT: black tea; Inf: infusion; CCB: Coffee Cascara from Brazil; CCN: Coffee Cascara from Nicaragua.

3.3.1. Black tea kombucha

The volatile compounds identified in the infusions and kombuchas are presented in Table 2. In BT infusion, 22 volatile compounds were identified: 6 aldehydes, 3 alcohols, 4 esters, 2 ketones, 2 monoterpenes, 2 monoterpenes alcohols. 1 pyrrol, 1 furan and 1 norisoprenoid. No acids and phenols were observed in black tea infusion. The total chromatogram peak areas can be observed in Figure 3.

The importance of aldehydes, alcohols, and esters for BT aroma has been reported (Kang et al., 2019). In the present study, the following key odorant compounds were identified in BT infusion: (Z)-3-Hexenol, 1-octen-3-ol, benzyl alcohol, trans-linalool oxide, linalool, phenylethyl alcohol, 1-octen-3-one, 2-nonanone, hexanal, nonanal (Magagna et al., 2017; Kang et al., 2019), benzaldehyde and β-ionone (Magagna et al., 2017). Other common compounds in BT identified in the infusions and in BT K d0 were: dihydroactinidiolide, theaspirane and geranyl acetone (Ho et al., 2015). Theaspirane and the furan dihydroactinidiolide are carotenoid-derived aroma compounds in BT (Ho et al., 2015).

In BT K, the number of compounds increased from 22 to 59 from day 0 to 9. They included 13 aldehydes, 8 acids, 11 alcohols, 12 esters, 4 ketones, 3 monoterpenes, 2 monoterpenes alcohols, 2 furans, 1 pyrrol, 2 phenols and 1 norisoprenoid. During fermentation, microorganisms consume carbon sources, mainly sugar and similar molecules, to produce acids, alcohols, and other volatiles (Kliks et al., 2021). In BT K d0, only 4 acids were identified (acetic acid, decanoic acid, nonanoic acid, octanoic acid). As fermentation continued, the number of acids, alcohols, and esters in the chromatograms increased, although the areas of alcohol and esters decreased. Regarding aldehydes, the percentage and number of volatile compounds decreased as fermentation progressed till d9. Furans and pyrrols were identified in the infusions and kombuchas. These volatile compounds can be generated by the Maillard reaction during tea manufacturing process (Ho et al., 2015).

High concentrations of caproic, octanoic and lauric acids and of the terpenoids α-terpineol were previously identified in traditional kombucha fermented by its native microbial consortia (Leali et al., 2022). In the present study, the presence of the phenols 4-ethylguiacol and 4-ethylphenol in black tea kombuchas is possibly derived from the action of *Brettanomyces* yeasts and other microorganisms on non-volatile polyphenols (Farah, 2019). These phenols can impart undesirable odors to wine (Nieto-Rojo et al., 2014). Phenylethyl ethanol and isovaleric

acid were previously identified in black tea kombuchas (Savary et al., 2021; Tran et al., 2022; Zhang et al., 2021).

Table 1. Volatile compounds identified in black tea kombucha

Volatile compound	Odor description	CAS[a]	ELRI[b]	LRI[c]	BT	KBT t0	KBT t3	KBT t6	KBT t9
Aldehydes									
2-Hexenal	Apple, green, sweet, almond, fruity, leafy, plum, vegetable[1,2]	505-57-7	914	939	□	■	□	□	□
2-Methylbutyraldehyde	Musty, cocoa, phenolic, coffee, nutty, malty, fermented, fatty, alcoholic[2]	96-17-3	924	927	■	■	□	□	□
Benzaldehyde*	Almond, burnt sugar, tropical fruit[1,2]	100-52-7	904	914	□	■	□	□	□
Dodecanal	Soapy, waxy, aldehydic, citrus, green, floral[2]	112-54-9	927	958	■	■	■	■	■
Ethanal	Pungent, ether, fresh, fruity, musty[1,2]	75-07-0	956	983	□	■	■	■	□
Heptanal	Fat, citrus, rancid, fresh, aldehydic, green, herbal, wine-lee, ozone[1,2]	111-71-7	784	864	□	□	□	□	■
Hexanal*	Grass, tallow, fat, fresh, green, aldehydic, leafy ,fruity, sweaty[1,2]	66-25-1	920	943	■	■	■	□	□
Isobutyraldehyde	Pungent, malt, green, fresh, aldehydic, floral, green[1,2]	78-84-2	910	916	□	■	□	□	□
Isovaleraldehyde	Ethereal, aldehydic, chocolate, peach, fatty[2]	590-86-3	910	912	□	■	■	□	□
Nonanal*	Fat, citrus, fresh, orange, green[1,2]	124-19-6	875	897	■	■	■	■	■
Octanal	Citrus, soap, lemon, herbal, green, honey[1,2]	124-13-0	856	927	■	■	■	■	■
2-Phenylethanal	Honey, floral, rose, sweet, powdery, fermented, chocolate, earthy, hawthorne, green, hyacinth, clover, cocoa[1,]	122-78-1	925	942	■	■	■	■	□
Tetradecanal	Fatty, waxy, amber, incense, dry, citrus, peel, musk[2]	124-25-4	705	826	□	□	□	■	□
Acids									
2-methylbutanoic acid	Pungent, acid, roquefort cheese[2]	116-53-0	806	832	□	□	□	■	□
Caproic acid	Sweat, sour, fatty, cheese[1,2]	142-62-1	884	906	□	□	■	■	■
Decanoic acid	Rancid, fat, unpleasant, rancid, sour, fatty, citrus[1,2]	334-48-5	907	914	□	■	■	□	■
Isovaleric acid	Sweat, acid, rancid, sour, stinky, feet, cheese, tropical[1,2]	503-74-2	876	887	□	□	■	■	□
Lauric acid	Metal, mild, fatty, coconut, bay, oil[1,2]	143-07-7	755	841	□	□	□	□	■
Nonanoic acid	Green, fat, waxy, dirty, cheese, cultured, dairy[1,2]	112-05-0	892	898	□	■	■	■	■

Octanoic acid	Acid, sweat, cheese, fruit notes[1,2]	124-07-2	917	922	□	■	■	■	■

Table 1. continued

Volatile compound	Odor description	CAS[a]	ELRI[b]	LRI[c]	BT	KBT t0	KBT t3	KBT t6	KBT t9
Alcohol									
1-penten-3-ol	Ethereal, horseradish, green, radish, chrysanthemum, vegetable, tropical, fruity[2]	616-25-1	737	869	□	■	□	□	□
2-Ethylhexanol	Rose, green, citrus, fresh, floral, oily, sweet[1,2]	104-76-7	954	954	■	■	■	□	■
(S)-(-)-2-methyl-1-butanol	Ethereal, fresh[2]	1565-80-6	807	858	□	□	□	□	■
2-methyl-1-butanol	Malt, wine, onion, ethereal, fusel, alcoholic, fatty, greasy, whiskey, leathery, cocoa[1,2]	137-32-6	918	923	□	■	■	■	□
2-Phenylethyl alcohol*	Honey, spice, rose, lilac, floral, fresh[1,2]	60-12-8	917	939	□	■	■	■	■
3-methyl-1-butanol	Whiskey, malt, burnt, fusel, oil, alcoholic, fruity, banana[1,2]	123-51-3	903	909	□	□	□	■	■
(E)-2-Hexenol*	Green, leaf, walnut, fresh, fruity, unripe, banana[1,2]	928-95-0	774	799	□	■	□	□	□
Amyl alcohol	Fusel, oil, sweet, balsam[2]	71-41-0	811	870	□	□	■	□	□
Cedrol	Cedarwood, woody, dry, sweet, soft[2]	77-53-2	611	753	■		■	□	
Ethanol	Sweet[1]	64-17-5	973	973	□	■	■	■	■
Octanol	Moss, nut, mushroom, waxy, green, orange, aldehydic, rose[1,2]	111-87-5	810	870	□	□	□	■	□
Esters									
2-methylbutyl acetate	Fruit, over ripe, sweet, banana, juicy[1,2]	624-41-9	845	899	□	□	□	□	■
Ethyl 2 – methylbutyrate	Sharp, sweet, green, apple, fruity[2]	7452-79-1	772	871	□	□	□	□	■
Ethyl acetate	Pineapple, ethereal, fruity, sweet, weedy, green[1,2]	141-78-6	951	961	□	■	■	■	■
Ethyl decanoate	Grape, sweet, waxy, fruity, apple, oily, brandy[1,2]	110-38-3	947	983	□	□	■	■	■
Ethyl isobutyrate	Sweet, rubber, ethereal, fruity, alcoholic, fusel, rummy[1,2]	97-62-1	754	786	□	□	□	□	■
Ethyl laurate	Leaf, sweet, waxy, floral, soapy, clean[1,2]	106-33-2	876	901	■	□	□	■	■
Ethyl miristate	Sweet, waxy, violet, orris[2]	124-06-1	815	848	■	□	□	■	□
Ethyl octanoate	Fruit, banana, pear[1,]	106-32-1	873	930	□	□	□	■	■
Ethyl phenylacetate	Fruit, sweet, floral, honey, rose, balsam, cocoa[1,2]	101-97-3	840	870	□	□	□	■	■
Isopropyl myristate	Faint, oily, fatty[2]	110-27-0	681	767	■	□	□	□	□
Isopropyl palmitate	Fat, bland, oily[1,2]	142-91-6	780	735	□	□	■	■	□
Methyl salicylate	Peppermint[1]	119-36-8	897	958	■	■	■	■	□

Ketones									
3,5-Octadienone	Fruity, fatty, mushroom[2]	38284-27-4	680	835	□	■	□	□	□

Table 1. continued

Volatile compound	Odor description	CAS[a]	ELRI[b]	LRI[c]	BT	KBT t0	KBT t3	KBT t6	KBT t9
Acetylpropionyl	Pungent, sweet, butter, creamy, caramel, nutty, cheese[2]	600-14-6	834	912	□	□	■	□	□
Geranyl acetone	Magnolia, green, fresh, fruity, waxy, rose, woody, tropical[1,2]	3796-70-1	648	802	□	■	□	□	□
β-damascenone*	Apple, rose, honey, tobacco, sweet[1,2]	23726-93-4	832	927	□	■	■	■	■
Monoterpenes									
(Z)-Sabinene hydrate	Balsam[2]	15537-55-0	818	828	■	□	□	□	□
γ-Terpinene	Terpineol, lilac[2]	586-81-2	871	884	□	■	□	□	□
β-Ionone*	seaweed, violet, flower, raspberry, woody, sweet, fruity, berry, tropical, beeswax[1,2]	14901-07-6	815	825	■	■	■	■	■
Monoterpenes alcohols									
α-Terpineol	Oil, anise, mint, lemon, citrus[1,2]	98-55-5	801	820	□	■	■	■	□
1-Terpinen-4-ol	Turpentine, nutmeg, must, pepper, woody, earth, musty, sweet[1,2]	562-74-3	803	868	□	■	■	□	□
Linalool*	Citrus, flower, lavender, sweet, green[1,2]	78-70-6	921	925	■	■	■	■	■
Linalool oxide*	Flower, wood, musty, camphor, fenchyl, alcohol[1,2]	60047-17-8	730	778	■	■	■	■	■
Furans									
Dihydroactinidiolide	Musk, coumarin[2]	17092-92-1	772	851	■	□	□	■	□
Furfuryl alcohol	Burnt, alcoholic, chemical, musty, sweet, caramel, bread, coffee	98-00-0	806	849	□	□	□	□	■
Pyrrol									
1-Ethyl-1H-pyrrole-2-carboxaldehyde	Burnt, roasted, smoky[2]	2167-14-8	792	832	□	■	□	□	□
Phenols									
4-Ethylguaiacol	Spice, clove, smoky, bacon, phenolic[1,2]	2785-89-9	898	918	□	■	■	■	■
4-Ethylphenol	Phenolic, castoreum, smoke, guaiacol[2]	123-07-9	917	922	□	■	■	■	■
Norisoprenoid									
Theaspirane	Tea, herbal, green, wet, tobacco, leaf, metallic, woody, spicy[2]	36431-72-8	805	913	□	■	□	□	□

Note: BT: Black tea; KBT: Kombucha black tea; [a]CAS# (Chemical Abstracts Service) Registry Number, available in the NIST database; [b]ELRI: Experimental Linear Retention Index; [c]LRI: Linear Retention Index based on literature and NIST database (https://webbook.nist.gov/chemistry/name-ser/); 100% of the compounds in the chromatogram were identified. Alkanes were excluded. * Impact compounds according to Magagna et al., 2017; Kang et al., 2019. [1]http://www.flavornet.org; [2]http://www.thegoodscentscompany.com; ■ compound identified in the sample; □ not identified

3.3.2. Coffee cascara kombuchas

The volatile compounds identified in CC infusions and kombuchas are presented in Table 3. Considering CCB and CCN infusions, 24 and 28 volatile compounds were identified, respectively. The main chemical groups in CC infusions were alcohols, esters, aldehydes, acids, and ketones, in accordance with Pua et al. (2021) and DePaula et al, (2022). Benzaldehyde, decanal, and 2-ethylhexyl salicilate were only identified in CC infusions and not in the kombuchas.

CC infusions contained key odorants such as ethyl octanoate, nonanal, linalool, benzaldehyde, methyl salicylate, β-damascenone and γ-nonalactone, previously identified in dried fruits such as prunes or raisins (Nunes et al., 2008, Javed et al., 2019), and in cascara infusions (DePaula, 2022).

The volatile profiles (Table 2) were similar in both CC infusions and kombuchas. Considering the kombucha beverages elaborated with CCB and CCN, 59 volatile compounds were identified: 14 aldehydes, 8 acids, 15 alcohols, 17 esters, 2 ketones, 2 monoterpenes, 6 monoterpene alcohols, 1 furan and 2 phenols. To date, there are no similar studies using coffee cascara as raw material for kombucha production, and therefore only comparison with similar beverages is possible. As observed in BT K, a progressive increase in the number of acids, alcohols and esters and other minor classes of volatile compounds was observed during fermentation, while the number and area % of aldehydes decreased.

Only 39 volatile compounds were common to both BT K and CC Ks: 10 aldehydes (2-methylbutyraldehyde, isovaleraldehyde, benzaldehyde, dodecanal, acetaldehyde, heptanal, hexanal, nonanal, octanal, tetradecanal), 5 acids (decanoic acid, isovaleric acid, lauric acid, nonanoic acid, octanoic acid), 6 alcohols (2-ethylhexanol, 2-methyl-1-butanol, (S)-(-)-2-methyl-1-butanol, cedrol, ethanol, octanol), 4 monoterpenes alcohols (1-terpin-4-ol, α-terpineol, linalool, linalool oxide), 9 esters (ethyl acetate, ethyl decanoate, ethyl isobutyrate, ethyl laurate, ethyl miristate, ethyl octanoate, ethyl phenylacetate, isopropyl palmitate, methyl salicylate) and 2 ketones (geranyl acetone and β-damascenone). Most of them were probably from the BT K starter.

Terpineol, trans-linalool oxide, isovaleric acid, isoamyl acetate and hexanoic acid have been associated with significant aroma contributions to BT K because of their considerable concentrations (Wang et al., 2022). Trans-linalool oxide, linalool, phenylethyl alcohol, hexanal,

nonanal, benzaldehyde and β-ionone, identified in CC Ks, have been reported as aroma impact compounds in BTs and BT Ks (Magagna et al., 2017; Kang et al., 2019). As aforementioned, the presence of the phenols 4-ethylguiacol and 4-ethylphenol in the cascara kombuchas is probably derived from the fermentative process by yeasts from the genus *Brettanomyces* (Nieto-Rojo et al., 2014).

α-hexylcinnamaldehyde, 1-dodecanol, 1-heptanol, hexadecanol, isopulegol, (E)-linalool oxide, (Z)-linalool oxide, ethyl butyrate, ethyl hexanoate, isoamyl acetate and γ-nonalactone were identified only identified in CC Ks and not in BT K.

Considering the volatile compounds in CC beverages by chemical classes, Nonanal, octanal and dodecanal were aldehydes identified in all CCB and CCN beverages. Acetaldehyde was identified in all cascara kombuchas. This is a key product of fermentation, and an inevitable component in wine (He et al., 2023). Acetaldehyde has also been identified in a distillated fermented coffee pulp beverage (Blumenthal et al., 2022). The number and area of alcohols increased from d3 to d9, especially phenylethyl alcohol and ethanol. Even if not identifiable on the olfactory level, ethanol is an important component of kombucha aromatic profile (Tran et al., 2020). Isoamyl alcohol (3-methyl-1-butanol) and 2-methyl-1-butanol, identified in CCB and CCN kombuchas, have also been identified in distillated fermented coffee pulp beverage (Blumenthal et al., 2022). The contribution of acids to the global aroma depends on their concentration range. At low concentrations, acids with six to ten carbons provide mild and pleasant aroma to wine (Welke et al., 2014). The main acid responsible for kombucha sourness is acetic acid. Its concentration tends to increase with fermentation time (Tran et al., 2020; Bishop et al., 2022). Three volatile fatty acids were identified in CCB K and CCN K, decanoic acid, hexanoic acid (caproic acid) and octanoic acid. They have previously been identified during the production of sparkling wine, but only hexanoic and octanoic acids were mentioned as odor-active compounds (Ubeda et al., 2019).

Table 2. Volatile organic compounds identified in coffee cascara kombuchas

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
Aldehydes														
α-Hexylcinnamaldehyde	Fresh, floral, green, jasmin, herbal, waxy[2]	101-86-0	871	908	□	□	□	□	■	□	□	□	□	□
2-methylbutyraldehyde	Musty, cocoa, phenolic, coffee, nutty, malty, fermented, fatty, alcoholic[2]	96-17-3	644	754	□	■	□	□	□	□	■	□	□	□
Isovaleraldehyde	Ethereal, aldehydic, chocolate, peach, fatty[2]	590-86-3	777	842	■	■	□	□	□	□	■	□	□	□
Benzaldehyde*	Almond, burnt sugar, tropical fruit[1,2]	100-52-7	736	885	■	□	□	□	□	■	□	□	□	□
Decanal	Sweet, citrus, floral, soap, orange peel[1,2]	112-31-2	806	846	■	■	□	□	□	□	□	□	□	□
Dodecanal	Soapy, waxy, aldehydic, citrus, green, floral[2]	112-54-9	929	963	■	■	■	■	■	■	■	■	■	■
Ethanal/Acetaldehyde	Pungent, ether, fresh, fruity, musty[1,2]	75-07-0	980	982	□	■	■	■	■	□	■	■	■	■
Heptanal	Fat, citrus, rancid, fresh, aldehydic, green, herbal, wine-lees, ozone[1,2]	111-71-7	789	863	■	■	■	□	□	□	□	□	□	□
Hexanal	Grass, tallow, fat, fresh, green, aldehydic, leafy ,fruity, sweaty[1,2]	66-25-1	854	892	■	■	□	□	□	■	■	□	□	□
Nonanal*	Fat, citrus, fresh, orange, green[1,2]	124-19-6	911	918	■	■	■	■	■	■	■	■	■	■
Octanal	Citrus, soap, lemon, herbal, green, honey[1,2]	124-13-0	906	937	■	■	■	■	■	■	■	■	□	■
Palmitaldehyde	Cardboard[1,2]	629-80-1	769	843	■	■	□	□	□	□	□	□	□	■

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
Phenylethanal*	Honey, floral, rose, sweet, powdery, fermented, chocolate, earthy, hawthorne, green, hyacinth, clover, cocoa[1,2]	122-78-1	859	930	■	■	□	□	□	□	□	□	□	■
Tetradecanal	Fatty, waxy, amber, incense, dry, citrus, peel, musk[2]	124-25-4	723	852	□	□	■	■	□	□	□	□	□	□
Undecanal	Waxy, soapy, floral, aldehydic, citrus, green, fatty, fresh, laundry[2]	112-44-7	723	865	□	□	□	□	□	■	■	■	■	□
Acids														
Acetic acid	Acidic, sour, ungente, vinegar[1, 2]	64-19-7	943	956	□	■	■	■	■	■	■	■	■	■
Caproic acid	Sweat, sour, fatty, cheese[1,2]	142-62-1	864	898	□	□	□	□	■	□	□	□	■	■
Decanoic acid	Rancid, fat, unpleasant, rancid, sour, fatty, citrus[1,2]	334-48-5	923	948	■	■	■	■	■	■	■	■	■	■
Isobutyric acid	Rancid, butter, cheese, acidic, sour[1,2]	79-31-2	831	861	□	□	□	□	□	■	■	□	□	■
Isovaleric acid	Sweat, acid, rancid, sour, stinky, feet, cheese, tropical[1,2]	503-74-2	851	868	□	□	□	■	■	□	□	□	■	■
Lauric acid	Metal, mild, fatty, coconut, bay, oil[1,2]	143-07-7	689	826	□	□	□	□	■	□	□	□	□	□
Nonanoic acid	Green, fat, waxy, dirty, cheese, cultured, dairy[1,2]	112-05-0	897	904	■	■	■	■	■	■	■	■	■	■
Octanoic acid	Acid, sweat, cheese, fruit notes[1,2]	124-07-2	922	928	□	□	■	■	■	■	■	■	■	■
Alcohol														
1-dodecanol	Earthy, soapy, waxy, fatty, honey, coconut[2]	112-53-8	796	924	■	□	□	□	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
1-Heptanol	Musty, leafy, violet, herbal, green, sweet, woody, peony[2]	111-70-6	796	834	□	□	□	□	□	□	□	■	□	□
2-Ethylhexanol	Rose, green, citrus, fresh, floral, oily, sweet[1,2]	104-76-7	906	953	■	■	■	■	■	■	■	■	■	■
2-Methyl-1-butanol	Malt, wine, onion, ethereal, fusel, alcoholic, fatty, greasy, whiskey, leathery, cocoa[1,2]	137-32-6	858	904	□	□	■	■	□	□	■	■	□	□
(S)-(-)-2- methyl-1-butanol	Ethereal, fresh[2]	1565-80-6	796	847	□	■	□	□	□	□	□	□	□	□
2-Nonen-1-ol	Sweet, fatty, melon, cucumber, vegetable[2]	22104-79-6	636	741	□	■	□	□	□	□	□	□	□	□
(E)-2-Decen-1-ol	Waxy, fresh, air, citrus, rose, rue[2]	18409-18-2	792	796	□	□	□	□	□	□	□	□	□	■
(E)-2-Octen-1-ol	Green, citrus, vegetable, fatty[2]	18409-17-1	602	663	□	□	■	□	□	□	□	□	□	□
2-Phenethyl alcohol	Honey, spice, rose, lilac, floral, fresh[1,2]	60-12-8	825	885	□	■	■	■	■	□	■	■	■	□
3-Methyl-1-butanol*	Whiskey, malt, burnt, fusel, oil, alcoholic, fruity, banana[1,2]	123-51-3	892	899	□	■	□	■	■	□	■	□	□	■
Cedrol	Cedarwood, woody, dry, sweet, soft[2]	77-53-2	653	784	□	■	□	□	□	□	■	■	□	□
Ethanol	Sweet[1]	64-17-5	945	945	□	■	■	■	■	■	■	■	■	■
Hexadecanol	Flower, wax, clean, greasy, floral, oily[1,2]	36653-82-4	932	951	□	□	□	□	■	■	□	□	□	□
Isopulegol	Minty, cooling, medicinal, woody[2]	89-79-2	812	816	□	□	□	□	□	■	■	■	■	□
Octanol	Moss, nut, mushroom, waxy, green, orange, aldehydic, rose[1,2]	111-87-5	712	815	□	□	□	■	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
Esters														
2-Ethylhexyl salicylate	Mild, orchid, sweet, balsam[2]	118-60-5	863	961	■	■	□	□	□	■	■	□	□	□
Ethyl 2-methylbutyrate	Sharp, sweet, green, apple, fruity[2]	7452-79-1	877	895	□	□	□	□	■	□	□	□	□	■
Ethyl Acetate	Pineapple, ethereal, fruity, sweet, weedy, green[1,2]	141-78-6	940	950	□	■	■	■	■	□	■	■	■	■
Ethyl butyrate	Apple, fruity, juicy, fruit, pineapple, cognac[1,2]	105-54-4	765	848	□	□	□	□	□	□	□	□	□	■
Ethyl decanoate	Grape, sweet, waxy, fruity, apple, oily, brandy[1,2]	110-38-3	915	928	□	□	■	■	■	□	□	□	■	■
Ethyl hexanoate*	Apple peel, fruit, sweet, pineapple, waxy, green, banana[1,2]	123-66-0	909	912	□	■	□	□	■	□	■	□	□	■
Ethyl isobutyrate*	Sweet, rubber, ethereal, fruity, alcoholic, fusel, rummy[1,2]	97-62-1	882	895	□	□	□	□	■	□	□	□	□	□
Ethyl laurate	Leaf, sweet, waxy, floral, soapy, clean[1,2]	106-33-2	814	841	□	□	□	■	■	□	□	□	■	■
Ethyl miristate	Sweet, waxy, violet, orris[2]	124-06-1	775	780	□	□	□	□	□	□	□	□	□	■
Ethyl octanoate*	Fruit, banana, pear[1,2]	106-32-1	913	939	□	□	■	■	■	□	□	□	■	■
Ethyl palmitate	Wax, fruity, creamy, milky, balsamic, greasy, oily[1,2]	628-97-7	791	833	■	□	□	□	□	■	□	□	□	□
Ethyl phenylacetate	Fruit, sweet, floral, honey, rose, balsam, cocoa[1,2]	101-97-3	874	894	□	□	□	□	■	□	□	□	□	■
Homomenthyl salicylate	Mild, menthol[2]	118-56-9	792	910	■	■	□	□	□	□	□	□	□	□
Isoamyl acetate*	Banana, sweet, fruity, solvent[1,2]	123-92-2	772	878	■	■	□	□	■	□	□	□	□	□
Isopropyl palmitate	Fat, bland, oily[1,2]	142-91-6	790	882	■	■	□	□	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
Methyl dihydrojasmonate	Floral, oily, jasmin, green, lactonic, tropical, natural[2]	24851-98-7	785	823	□	□	□	□	□	■	■	□	□	□
Methyl palmitate	Oily, waxy, fatty, orris[2]	112-39-0	762	848	□	□	□	■	■	□	□	□	□	□
Methyl salicylate	Peppermint[1]	119-36-8	895	920	■	■	■	■	■	■	■	■	■	□
Ketones														
Geranyl acetone	Magnolia, green, fresh, fruity, waxy, rose, woody, tropical[1,2]	3796-70-1	682	777	□	□	□	□	□	■	■	□	□	■
β-Damascenone*	Apple, rose, honey, tobacco, sweet[1,2]	23726-93-4	878	944	■	■	■	■	■	■	■	□	□	□
Monoterpenes														
Limonene	Lemon, orange, citrus, herbal, terpene, camphor[1,2]	138-86-3	861	893	□	□	■	■	■	□	■	■	□	□
γ-Nonalactone*	Coconut, peach, creamy, waxy, sweet, buttery, oily[1,2]	104-61-0	859	895	■	■	□	□	□	■	■	■	□	□
α-Terpineol	Oil, anise, mint, lemon, citrus[1,2]	98-55-5	871	900	■	■	■	■	■	■	■	■	■	■
1-Terpinen-4-ol	Turpentine, nutmeg, must, pepper, woody, earth, musty, sweet[1,2]	562-74-3	681	804	■	■	■	□	□	□	□	□	■	■
Linalool*	Citrus, flower, lavender, sweet, green[1,2]	78-70-6	919	925	□	□	□	■	■	□	□	□	□	□
Linalool oxide	Flower, wood, musty, camphor, fenchyl, alcohol[1,2]	60047-17-8	891	900	□	□	■	■	■	□	■	■	□	□
cis-Linalol oxide	Flower[1]	5989-33-3	838	852	■	■	□	□	□	■	■	■	□	□
trans-Linalool oxide	Flower[1,2]	34995-77-2	863	878	□	□	□	□	□	■	■	■	□	□
Furans														
Furfural*	Bread, almond, sweet, woody[1,2]	98-01-1	924	924	■	■	□	□	□	■	■	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CCB	CCB t0	CCB t3	CCB t6	CCB t9	CCN	CCN t0	CCN t3	CCN t6	CCN t9
Phenols														
4-Ethylguaiacol	Spice, clove, smoky, bacon, phenolic[1,2]	2785-89-9	862	881	□	■	■	■	■	□	■	■	■	■
4-Ethylphenol	Phenolic, castoreum, smoke, guaiacol[2]	123-07-9	858	889	□	■	■	■	■	□	■	■	■	■

Note: CFC: Coffee fruit cascara; NF: Nonfermented; F: Fermented; KCFC: Kombucha coffee fruit cascara; [a]CAS# (Chemical Abstracts Service) Registry Number, available in the NIST database; [b]ELRI: Experimental Linear Retention Index; [c]LRI: Linear Retention Index based on literature and NIST database (https://webbook.nist.gov/chemistry/name-ser/); 100% of the compounds in the chromatogram were identified. Alkanes were excluded. * Impact compounds according to: Perestrelo et al. (2006), Nunes et al. (2008), Ubeda et al. (2019), Pua et al. (2021) [1]http://www.flavornet.org;[2]http://www.thegoodscentsco.com; ■ compound identified in the sample; □ not identifi

Important esters identified along CC Ks fermentation were ethyl acetate, isoamyl acetate, ethyl octanoate, ethyl hexanoate, ethyl decanoate, and ethyl isobutyrate. Most of them have also been identified in grape musts fermented by different yeasts (Luan et al., 2018). Ethyl acetate was identified in a fermented coffee pulp distillate (Blumenthal et al., 2022). Ethyl decanoate and ethyl octanoate have been reported as abundant in ciders (Kliks et al., 2021). Ethyl isobutyrate, ethyl hexanoate and isoamyl acetate have been reported as impact compounds in sparkling wine (Ubeda et al., 2019). Regarding ketones, β-damascenone has been described as an aroma-active compound in a Robusta coffee pulp puree (Bulk et al., 2021). γ-nonalactone has been reported as the main odorant in red wine (Perestrelo et al., 2006).

Monoterpenes were reported as volatile components of fruits responsible for a wide spectrum of aromas, mostly perceived as very pleasant (Nunes et al., 2008). Limonene was identified in CCB K d9 and in CCN K d0; d3 and d6. Linalool was identified in all CC infusions and kombuchas. It has been previously identified in CC infusions (DePaula et al., 2022).

3.5. Sensory tests

3.5.1. Consumer acceptance and purchase intention test scores

A total of 113 consumers participated in the sensory assessment. The assessors' characteristics are presented in Table 4.

Table 4. Assessor's characteristics

Gender		**Age**			
Male	Female	18 - 24	25 - 34	34 - 44	45 - 59
39%	61%	47%	38%	6%	8%
Level of education					
Basic education	Undergraduated	Incomplete graduation	Complete Graduation	Master's or doctoral degree	
0 %	12 %	40 %	10 %	38 %	
Family income (MW: minimum wages)					
1 MW	2 - 3 MW	4 - 5 MW	> 5 MW		
18 %	39%	9%	7%		
Know kombucha		**Drink Kombucha**			
Yes	No	Yes	No		

68 %	32%	9%		81%	
Sparkling beverages/soft drinks consumption					
Sparkling water	Apple juice	Soda	Tonic water	Sparkling wine	Cider
50%	10%	73%	32%	42%	15%

The mean acceptance was similar for all CCB K, with no statistical difference, with mean score around 6.0. Similar results were obtained for CCN K, except for sample 3d, which scored 7.0, with 89% of the scores between 6 and 9. (Figure 5), therefore all samples were accepted; with purchase intent results followed the same trend (Figure 6). According to Meilgaard et al. (2006), for a sample to be considered "well-accepted", it must obtain Acceptance Index (AI) equal or higher than 70 %. CCN d3 reached 78% AI, while other samples showed AI between 63 and 69%. Therefore CCN K d3 was the only well accepted kombucha. CCN K 3d higher acceptance derives probably from the higher amount of sugar, highlighted by the lower acidity and volatile compounds with sweet, floral and fruity notes in the infusion used to prepare the kombuchas, given that these are suitable attributes for kombucha in general (Andresen et al., 2022). Brazilians in general are used to sweeter beverages, a habit inherited by the Portuguese colonizers who are now educated the European public health agencies to lower sugar consumption, just like other European countries (European Comission, 2021). The United States follow the trend (USDA, 2015). As aforementioned, the amount of sugar contained in the kombucha market in the US and Europe can be considerably low, which makes this beverage an excellent replacement for soft beverages and other nutritionally poor beverages, usually containing more that 10g sugar/100mL. It is worth mentioning that in preliminary tests, the fermentation period to achieve the desired sensory characteristics was related to the proportion of starter and other ingredients, type and variety of raw materials, size of bottle, volume of kombucha, and so forth. Longer fermentation with lower initial amount of sugar and the addition of low glycemic index sweeteners will probably reach the same desirable sensory result to the product developed in this study.

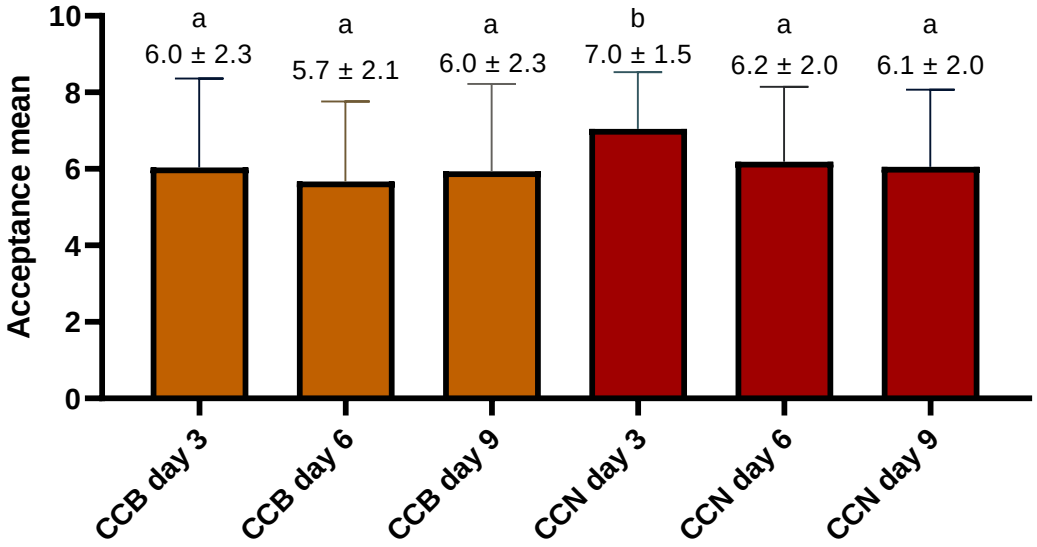


Figure 5. mean acceptance scores of coffee cascara kombuchas by Rio de Janeiro consumers (n=114). Note. CCB: coffee cascara from Brazil; CCN: coffee cascara from Nicaragua.

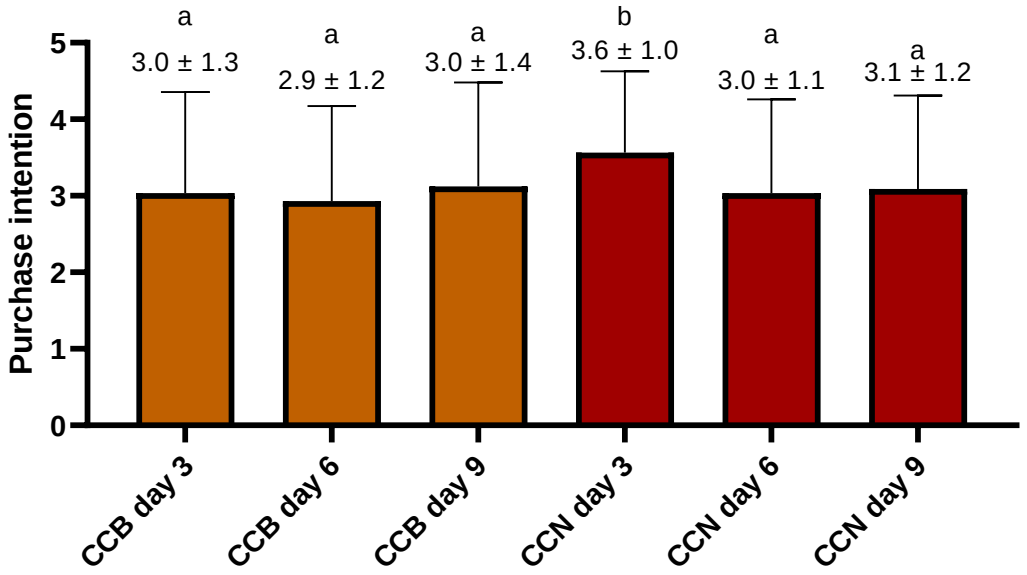


Figure 6. Mean purchase intention scores for coffee cascara kombuchas by Rio de Janeiro consumers n=114). Note. CCB: Brazil coffee cascara; CCN: Nicaragua coffee cascara.

The highest mean scores (7.0 ± 0.19) were given to CCN K d3. Eighty nine percent of the assessors gave scores between 6 and 9 to this sample. The better acceptance of this sample derives probably from the volatile compounds with sweet, floral and fruity notes in the infusion

used to prepare the kombuchas, given that these are suitable attributes for kombucha in general (Andresen et al., 2022).

Because people have different tastes and experiences and cannot be represented by a mean score, we performed cluster analysis to identify different niches of consumers. Two clusters were identified. Cluster 1 (n = 45, mean score = 7.3, and AI = 81%) consistently attributed the highest scores to CCN K d3 (mostly female) and CCB K d9 (mostly male). This cluster was composed of 52% male and 48% female. Forty two percent of them were 18-24 years old. Forty nine percent had higher education, and 38% had family monthly income of 2-3 MW. In this cluster, 8% of the assessors were kombucha consumers. The women's attribution of higher scores to CCN K d3 may be related to the fact they regularly drank sweetened (36%) teas with fruity notes, consumed other sweet beverages such as soft drinks (36 %) and reported low consumption of sparkling water (17 %), sparkling wine/cider (15%) and tonic water (15 %). De Paula et al (2022) also have recently observed that women aged between 18-34 habitually consume fruity teas, confirming data from the Brazilian Institute of Statistics and Geography (IBGE) (IBGE, 2018). Because our assessors were mostly women (61 %), this could be the main reason why CCN K d3 received the highest average score.

The men´s attribution of higher scores to CCB K d9 can possibly be explained by the high consumption of different sparkling and fermented beverages. This sample was not only more acidic. It had high intensity mean for bitter taste and sparkling mouthfeel. Beer, sparkling water and tonic water are bitter and sparkling beverages. Thirty seven percent of men reported consumption of sparkling water, 22% tonic water and 30% sparkling wine and/or cider. According to the IBGE (IBGE, 2018), Young Brazilian men drink more soda, while adult men drink more soda and beer than women.

Cluster 2 (n = 67, mean score = 5.3, and AI = 59%) also attributed the highest scores to CCN K d3. This cluster was primarily composed of females (72%), aged between 25 and 34 (58%), with complete graduate education (54%) and family monthly income between 2 to 3 MW (53%). In this cluster, 9% of assessors were kombucha consumers. Similar characteristics to cluster 1 related to men and women occurred.

Together, these findings suggest that men aged 18–24 and women aged between 25 and 34 years old are potential consumers of cascara kombuchas, although assessors were mostly

young (Table 4). They also indicate that there are potential market niches for kombuchas with different intensities of fermentation.

No similar study was found for comparison but considering the sensory acceptance of kombuchas from other new substrates (black carrot, cherry laurel, blackthorn and red raspberry), in the study by Ulusoy and Tamer (2019) performed in Turkey, the beverages fermented for shorter periods of time (3 and 5 days) obtained scores between 6 and 8, using a 9-point hedonic scale, while beverages fermented for 10 and 12 days received scores below 5. Other studies performed in Brazil and Tunisia reported that assessors liked herbal or and grape kombuchas after 6 days of fermentation, with average acceptance scores between 5 and 7 (Zhang et al, 2021; Ayed et al, 2016)

In the present study, the mean scores for CCB K d6 and CCN K d6 were 6.0 and 6.2 as mean scores, respectively. These are lower than those given for CC K d3, most probably because Brazilians are used to sweeter beverages, a habit inherited by the Portuguese colonizers who are now educated by EFSA and other European public health agencies to lower sugar consumption, just like other European countries (European Comission, 2021). The United States follow the trend (USDA, 2015). As aforementioned, the amount of sugar contained in the kombucha market in the US and Europe is considerably low which makes it an excellent replacement for nutritionally soft beverages and other nutritionally poor beverages, usually containing more that 10g sugar/100mL of sugar. It is worth mentioning that in preliminary tests, the fermentation period to achieve the desired sensory characteristics was related to the proportion of starter and other ingredients, type and variety of raw materials, size of bottle and volume of kombucha, and so forth. Longer fermentation with lower initial amount of sugar and the addition of low glycemic index sweeteners may reach the same desirable sensory result to the product developed in this study.

3.5.2. Rate all that apply (RATA)

Considering that kombucha is a fermented beverage, chemical changes generate different sensory attributes and intensities. Therefore, RATA test was performed to identify these changes during production of coffee cascara kombuchas. The intensity means for aroma, taste, flavor, mouthfeel and appearance checked attributed to CCB Ks and CCN Ks by Rio de Janeiro

consumers are presented in Figures 6 and 7. Significant differences (p = 0.0001) in beverages made with the same raw material were observed mainly between 3d and 9d.

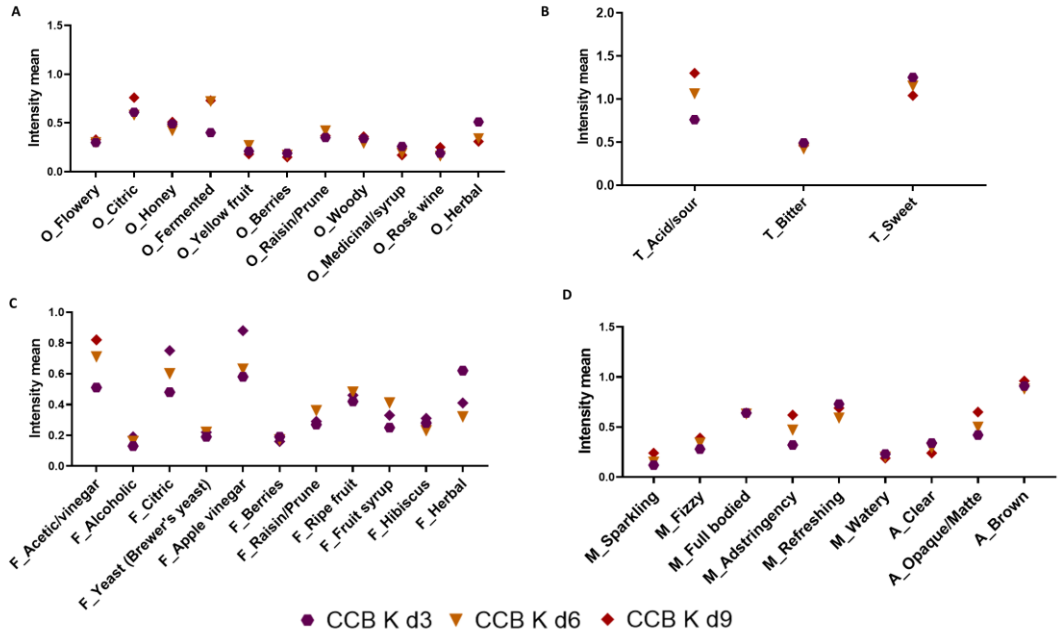


Figure 6. Intensity means for odor (A), taste (B), flavor (C), mouthfeel and appearance (D) for CCB K 3d; 6d and 9d. Note: O: odor; T: taste; F: flavor; M: mouthfeel; A: appearance; CCB: Brazil Coffee Cascara.

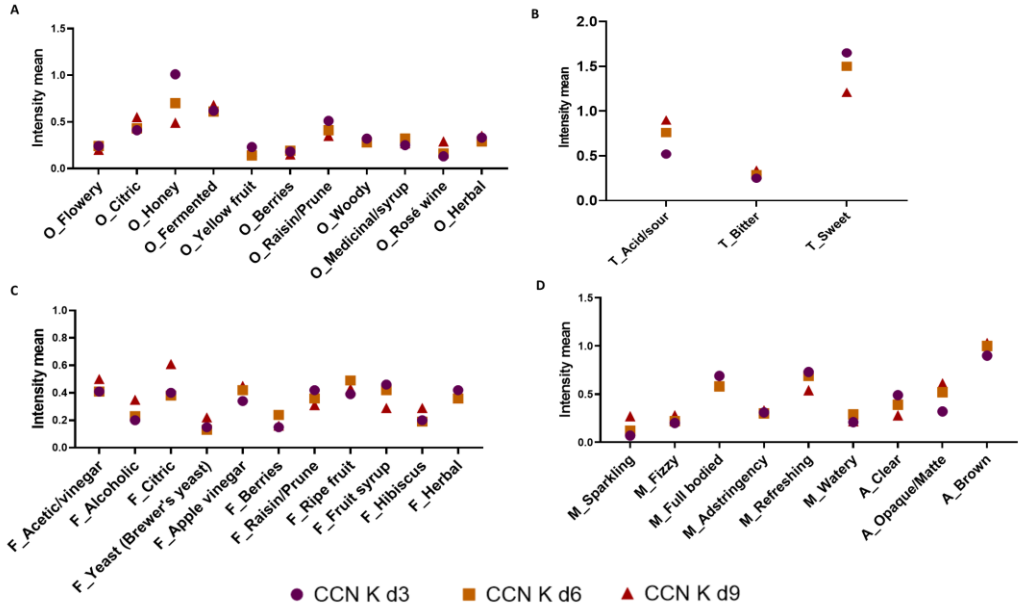


Figure 7. Intensity means for odor (A), taste (B), flavor (C), mouthfeel and appearance (D) for CCN K 3d; 6d and 9d. Note: O: odor; T: taste; F: flavor; M: mouthfeel; A: appearance; CCN: Nicaragua Coffee Cascara

According to Kim and Adhikari (2020) and Tran et al. (2020), the main sensory characteristics of kombucha are developed by acetic acid bacteria activity such as sweet, sour, vinegary odor and flavor), and by yeasts activity, such as cidery odor and flavor and carbonation (from fermentation in general). In addition to the important attributes that characterized CC K on d3, other important attributes with significant intensity were observed in CC K at d9 by the assessors. They were: citric, honey, fermented and herbal for aroma, acid/sour and sweet for taste, alcoholic, apple vinegar, herbal for flavor; slightly astringent and refreshing for mouthfeel; opaque/matte and brown for appearance.

In general, CCB Ks obtained higher intensity means for flowery, citric, fermented for odor; acid/sour for taste, acetic/vinegar, citric, apple vinegar, hibiscus for flavor; fizzy and astringent for mouthfeel. In CCB K d3, the attributes with higher intensities were berries, woody, herbal for odor; sweet for taste; and herbal for flavor. On day 6, CCB K developed more sensory attributes, with high intensities of flowery, yellow fruit, raisin/prune for aroma, acid/sour and bitter for taste, acetic/vinegar, ripe fruit and fruit syrup for flavor; astringency appeared for mouthfeel.

CCN Ks were described as fermented, woody and rosé wine for odor; acid/sour and bitter for taste; alcoholic and citric for flavor. In CCN Ks d3, the attributes showing higher intensities were honey, berries and raisin/prune for odor; sweet for taste; raisin/prune and fruit syrup for flavor; and full bodied for mouthfeel. The sensory complexity attributed to CCN K d3 derives most probably from the fact that CCN was obtained from previous fruit fermentation during post-harvest process (DePaula et al., 2022).

Regarding CCN K d6, assessors marked higher intensity for berries, medicinal/syrup for aroma; sweet for taste; citric, berries and ripe fruit for flavor; and watery for mouthfeel. However, as previously stated, this sample received lower acceptance score than CCB K d3, probably because of the increase in acetic/vinegar flavor together with the positive attributes (Figure 6).

Table 5 contains the RATA attributes that, based on literature data, could correspond to the volatile compounds identified in this study.

Table 5. RATA attributes and the corresponding volatiles compounds identified in the present study for coffee cascara kombuchas (see Table 3)

Aroma and Flavor attributes from RATA test	Respective volatile compounds identified in the literature and in the present study	References
 Alcoholic	Isoamyl alcohol, ethyl acetate, ethyl hexanoate, ethyl decanoate, linalool oxide	(Cardeal et al., 2009)
 Apple	Dodecanal, heptanal benzaldehyde, decanal, decanoic acid, ethyl 2-methylbutyrate, ethyl butyrate, methyl salicylate	(Vrhovsek et al., 2014)
 Berries	Hexanal, decanal, benzaldehyde, octanoic acid ethyl acetate, ethyl hexanoate, ethyl octanoate, linalool	(Vrhovsek et al., 2014; Rey-Serra et al., 2022; Farneti et al., 2017)
 Citric	Nonanal, octanal, dodecanal, limonene α-terpineol, linalool	(Galvan-Lima et al., 2021)
 Fermented	Hexanal, benzaldehyde, nonanal, phenylacetaldehyde, ethanol, octanoic acid	(Pétel et al., 2017)
 Floral/flowery	Phenylethyl alcohol, β-damascenone, linalool, linalool oxide, cis/trans-linalool oxide	(Yue et al., 2022)
 Herbal	Hexanal, heptanal, nonanal, octanal, phenylacetaldehyde, phenylethyl alcohol, linalool	Yin et al., 2022
 Hibiscus	Dodecanal, nonanal, 2-ethylhexanol, acetic acid, linalool	(Zannou et al., 2020)
 Honey	Octanal, benzaldehyde, phenylacetaldehyde, phenylethyl alcohol, isovaleric acid, β-damascenone, cis/trans-linalool oxide, linalool, α-terpineol	(Castro-Vázquez et al, 2007; Moreira et al., 2002)
 Medicinal syrup	4-ethylguiacol, 4-ethylphenol	Milheiro et al., 2017

Table 5. continued

Aroma and Flavor attributes from RATA test	Respective volatile compounds identified in the literature and in the present study	References
 Raisin/prune	Nonanal, benzaldehyde, ethyl octanoate β-damascenone, γ-nonalactone, linalool	(Nunes et al., 2008, Javed et al., 2019
 Ripe fruit	Hexanal, hexanoic acid, octanoic acid, ethanol, ethyl acetate, isoamyl acetate, cis/trans linalool-oxide, linalool	Ménager et al., 2004; Yang et al., 2011
 Rosé wine	Nonanal, 1-dodecanol, 1-heptanol, octanoic acid, decanoic acid, ethyl hexanoate, ethyl octanoate, ethyl decanoate, β-damascenone, linalool	(Ma et al., 2022)
 Sparkling wine	Hexanoic acid, octanoic acid, ethyl isobutyrate, ethyl hexanoate, isoamyl acetate	(Ubeda et al., 2019)
 Vinegar	Acetic acid	(Tran et al 2020)
 Woody	Hexanal, nonanal, cedrol, acetic acid, decanoic acid	(Shen et al., 2022; Ghadiriasli et al., 2021)
 Yeast (Brewer's yeast)	Nonanal, ethanol, octanoic acid, isoamyl alcohol, ethyl decanoate, ethyl hexanoate	(Pinho et al., 2006)
 Yellow fruit	Benzaldehyde, heptanal, nonanal, octanal, ethyl butyrate, ethyl octanoate, ethyl acetate, β-damascenone, limonene, α-terpineol	(Bicas et al 2011; Janzantti and Monteiro, 2014; Zhou et al 2021)

Reports on some spirits prepared with CC were found in the literature. Einfalt et al. (2020) produced fresh coffee cherry spirits, with vegetable, nutty and earthy aroma attributes, while the taste descriptors were vegetable, alcoholic and nutty. Blumenthal et al. (2022) also produced cherry spirits from different arabica coffee varieties and found different sensory results and preferences among the coffee plant varieties. In both studies woody, plum, compote/jam, sweet,

herbs, dried fruits, stone fruit and cherry-like were cited attributes. These descriptors are in accordance with the present study results, partly explaining the differences between CCB and CCN during K production.

Higher intensity means for acid/sour are usually attributed to the organic acids concentration reflected in higher TA and lower pH values (Andresen et al., 2022), which was also observed in the present study. It is known that sourness decreases the ability of humans to detect the initial sweetness of sucrose, given that sucrose threshold stabilize with the increase of acid in the same medium (Mao et al., 2022). This can help explaining the low intensity mean for sweet taste in kombuchas fermented for 9 days. In CCN K some of the attributes with high intensities used to describe d9 samples were brewer's yeast for flavor, sparkling for mouth feel and more opaque/matte for appearance. In CCB K some of these attributes presented similar intensities in all samples. Considering the Brazilian consumer preferences for sweeter beverages, day 9 samples received low acceptance and purchase intention mean scores except for the niche cited in item 3.5.1. In North America and Europe, this type of kombucha would also probably receive higher scores.

Conclusions and final considerations

In the present study, 81 volatile organic compounds were identified, with about 25 (on average) in the CC and BT infusions. The volatile profile changed dramatically during fermentation, 59 compounds were identified simultaneously in all kombuchas. Despite the different origins and post-harvest processing, both groups of CC K presented similar volatile profiles. The content of acids and esters increased progressively due to the symbiosis between acetic acid bacteria and yeasts, represented in the consortia used in this study mostly by the genera *Komagateibacter* sp. and *Pichia* sp., respectively.

CC K were well accepted by the assessors from Rio de Janeiro in general, especially one containing higher amount of sugar and fruity and flowery attributes, resembling the Guaraná soft drink commonly consumed by Rio population. Additionally young adults showed to be potential consumers of CC K, with women preferring early stages of fermentation and men later stages. The sensory characterization was associated with the volatile composition of the beverages. Volatile compounds that seem to have contributed the most to the main characteristics of CC K were: linalool, decanal, nonanal, octanal, dodecanal, ethanol, 2-ethylhexanol, ethyl acetate, ethyl

butyrate, β-damascenone, γ-nonalactone, ethanol, linalool oxide, phenylethyl alcohol, phenylacetaldehyde, isoamyl alcohol, acetic acid, octanoic acid, decanoic acid, ethyl isobutyrate, ethyl hexanoate and limonene. However, this hypothesis needs to be confirmed by studies involving gas chromatography analysis with olfatometric detection.

CC showed to be a suitable raw material to produce aromatic and natural cold beverages that with reduced amount of sugar and considerable amount of bioactive compounds (Sales et al, 2023) can be an excellent replacement for nutritionally poor soft beverages and a way to reduce the environmental pollution caused by the incorrect disposal of coffee cascara after harvest and improve the coffee chain sustainability. Additionally, differently from most kombuchas which are flavored to increase acceptance, CC K does not need flavoring agents like fruits, herbs or species commonly used for the traditional kombucha or kombucha-like beverages. Therefore, the potential of CC K to produce healthy fermented beverages like kombucha is remarkable.

ESTUDO 3: Caracterização volátil, perfil microbiológico e aceitação sensorial de kombuchas de folhas do cafeeiro e folhas do cafeeiro com erva mate

A poda e a colheita dos grãos de café produzem as folhas de café como subprodutos, que são geralmente consideradas sem ou com baixo valor. Preparadas a partir de folhas de café verde secas, o chá de folha de café é consumido por séculos na África e na Ásia, mas não na Europa e na América. Chá mate é comumente cultivado e consumido na América do Sul. Ambas as plantas compartilham diversos compostos bioativos e benefícios à saúde. O objetivo desse trabalho foi elaborar kombuchas com folhas de café,com ou sem erva mate e acompanhar as mudanças na composição volátil do headspace, atributos sensoriais e a aceitação por consumidores do Rio de Janeiro (n=103) após 0, 3, 6 e 9 dias de fermentação. Folha de café comercial da Nicarágua e erva mate tostada do Brasil foram utilizados para preparar as bebidas fermentadas, nas quais foram adicionados kombuchas de chá preto (10%), açúcar (10%) e a cultura simbiótica de bactérias e leveduras (SCOBY) (2,5%). Kombucha de chá preto foi preparados como controles da fermentação. Amostras foram coletadas após 0, 3, 6 e 9 dias de fermentação para caracterização do perfil volátil do headspace por CG-EM. A caracterização sensorial foi realizada pelo teste RATA. Aceitação e intenção de compra também foram avaliados. Um total de 119 compostos voláteis foram identificados considerando todas as amostras de kombuchas, 61 nos kombuchas de folhas de cafée 45 em folhas de café com mate. Um aumento, principalmente em ácidos e ésteres, foi observado durante a fermentação de todas as amostras. Compostos voláteis identificados apenas nos K FC e K FCM, mas não em K CP foram: 2,4-heptadienal, tetradecanal, tridecanal, undecanal, 1-dodecanol, (S)-2-heptanol, , (z)-3-hexanoato de etila, dihidrojasmonato de metila, 3,5-octadien-2-one, (-) carvona, isoforona, mentona, 3-δ-carene, mirceno e canfeno. Esses compostos estão relacionados com notas de verde, herbais, frutadas, oleosas, almíscar, amadeirada, cítrica, doce, floral, jasmin, queijo e refrescante. A maior média de aceitação foi atribuída ao kombucha de folha de café com mate com 3 dias de fermentação (6.6 ± 2.0), provavelmente ao elevado dulçor e presença de aldeídos com aroma doce, associado com a baixa quantidade de ácidos. Elevadas notas foram atribuídas por mulheres com graduação incompleta entre 18-24 anos de idade. As folhas de café mostraram ser um subproduto promissor para a elaboração de bebidas fermentadas, com características

exóticas e específicas, as quais podem ser exploradas para aplicações sustentáveis pela indústria de alimentos.

Palavras-chave: subprodutos do café; colheita de café; planta de café; bebidas fermentadas; chá preto; erva mate; compostos voláteis

1. Introduction

The coffee tree belongs to genus *Coffea*, which is by far the most economically important member of the Rubiaceae family (Farah e Santos, 2014). Coffee is ranked as the second most traded product in the world after petroleum (Maxiselly et al., 2022), with worldwide production, of approximately 10.5 tons (International Coffee Organization, 2021).

Pruning and harvesting during coffee production generates coffee leaves as by-products that are generally considered of no or low value compared with the highly valuable coffee seeds, especially in organic crops. It is estimated that 3.3 tons of leaves per hectare are discarded during coffee harvest in Brazil (Matiello et al., 2010). Prepared from dried green coffee leaves, in a similar manner to *Camellia sinensis* tea, coffee leaf tea has been consumed for centuries, particularly in Africa and Asia, but not as much in Europe and Americas (Novita et al., 2018; Trevisan et al., 2019). Coffee leaves contain various phytochemicals such as chlorogenic acids (Monteiro et al., 2020), mangiferin, isomangiferin , rutin, quercetin, theobromine, caffeine and trigonelline (Campa et al., 2012; Trevisan et al., 2016; Chen et al., 2018; de Almeida et al., 2018) that are known to promote a number of benefits such as antioxidant, anti-inflammatory, antihypertensive, immunomodulatory effects, and relief of gastrointestinal symptoms (Acidiri et al., 2020; Chen et al., 2018; Novita et al., 2018). In Europe, the consumption of coffee leaf tea is increasing, and it is estimated that it will continue to growth to be consumed in addition or to replace coffee or tea beverages in many homes (EFSA, 2020).

Ilex paraguariensis St. Hil., known as yerba mate or maté, is an arboreal species that naturally grows and is cultivated in the temperate and subtropical climatic regions of Argentina, Brazil, and Paraguay (Butiuk et al., 2016). It is found primarily in the southern regions of South America, namely, Brazil (in Mato Grosso do Sul, Minas Gerais, Parana, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, São Paulo), Argentina (in Corrientes, Misiones), Paraguay (Alto Parana, Amambay, Caaguazu, Canendiyu, Central, Guaira, Itapua, Misiones, San Pedro) (Heck and De Meija, 2007). Argentina is the main producing country of yerba mate with 830 million tons in 2019 (Statista, 2022a) and Brazil the second one, with 558 million tons in 2021 (IBGE, 2021). Also, these counties are the main exporters of maté in Latin America (Statista, 2020b).

Due to the presence of methylxanthines caffeine and theobromine, maté is known as a stimulant beverage (Lima and Farah, 2019). Other important bioactive compounds are the

chlorogenic acids (Marques and Farah, 2009) and rutin (Isolabella et al., 2010; da Silveira et al., 2017), in addition to minor compounds such as caffeic acid (Marques and Farah, 2009). Benefits related to maté are antioxidant (Baeza et al., 2016) anti-inflammatory (Arçari et al., 2011) cytotoxic and antiproliferative activities (Boaventura et al., 2015), antimicrobial activity against food pathogens (Martin et al., 2013), adipogenesis inhibition in hepatocarcinoma cell line, (Arçari et al., 2013), improvement on serum glucose, creatinine, urea and total protein levels in diabetic rats (Rocha et al., 2018), and increase in bone mineral density in postmenopausal women (Conforti et al., 2012). Although maté consumption has been linked with esophageal squamous cell carcinoma (Lubin et al., 2013), this is related to people´s habit of drinking it very hot as "chimarrão" (IARC, 2016).

Kombucha is produced by the fermentation of tea and sugar by a symbiotic association of bacteria and yeasts, called SCOBY (Jayabalan et al., 2019). The beverage can be prepared using both black and green tea (*C. sinensis*), with the first being more popular and considered as the preferred ingredient for the beverage preparation (Chakravorty et al., 2019). Several benefits of kombucha have been observed in *in vitro* and animal studies, such as antioxidant (Jayabalan et al., 2008) and anti-inflammatory activities (Villareal-Soto et al., 2019), antiproliferative activity in colon, breast and lung cancer cells (Villareal-Soto et al., 2019; Kaewkod et al., 2019; Cardoso et al., 2020), antibacterial activity (Kaewkod et al., 2019), hypoglicemiant effect and weight loss in diabetic rats (Srihari et al., 2013).

The growing interest in kombucha has attracted the use of new raw materials with black tea for fermentation, such as different types of teas, fruits, herbs, milk, as well as a wide range of agro-industrial materials, mainly by-products of fruit industries (Leonarski et al., 2022), providing that the beverage contains a percentage of *C. sinensis* kombucha. The global kombucha market size was valued at USD 2.64 billion in 2021 and is expected to expand at an annual growth rate (CAGR) of 15.6% from 2022 to 2030 (Grand View Research, 2021). Consumer awareness and popularity are considered the main reason behind the current trend of the flourishing market and active research on kombucha. One of the recognized characteristics of kombucha is its sensory characteristics commonly described as sweet, vinegary sour, and carbonated. (Kim and Adhikari, 2020).

Although the chemical composition of coffee beans has been studied for many years, a limited number of studies have been performed on the coffee leaf properties and their potential

use as a healthy food product (Montis et al., 2021). Furthermore, maté is a rich source of several bioactive chemicals that apparently have their health effects influenced in a synergistic or complementary manner (de Vasconcellos et al., 2022). Considering the taste and composition similarities with *C. sinensis* tea, coffee leaves and mate infusions seem to be promising new raw materials to elaborate kombucha. Furthermore, given the popularity of maté in South America, along with the fact blending with maté has increased acceptance of coffee leaf infusion by Brazilians (Lima, 2020), we hypothesized that maté could be useful in the preparation of fermented beverages to help decrease the waist of coffee leaves. Therefore, the aim of this study was to elaborate kombucha beverages from coffee and coffee-maté leaves and characterize their volatile profiles using a fingerprinting approach. We also investigated their sensory acceptance and profile by Rio de Janeiro consumers.

2. Material and Methods

The experimental design of the study is shown in Figure 1**.**

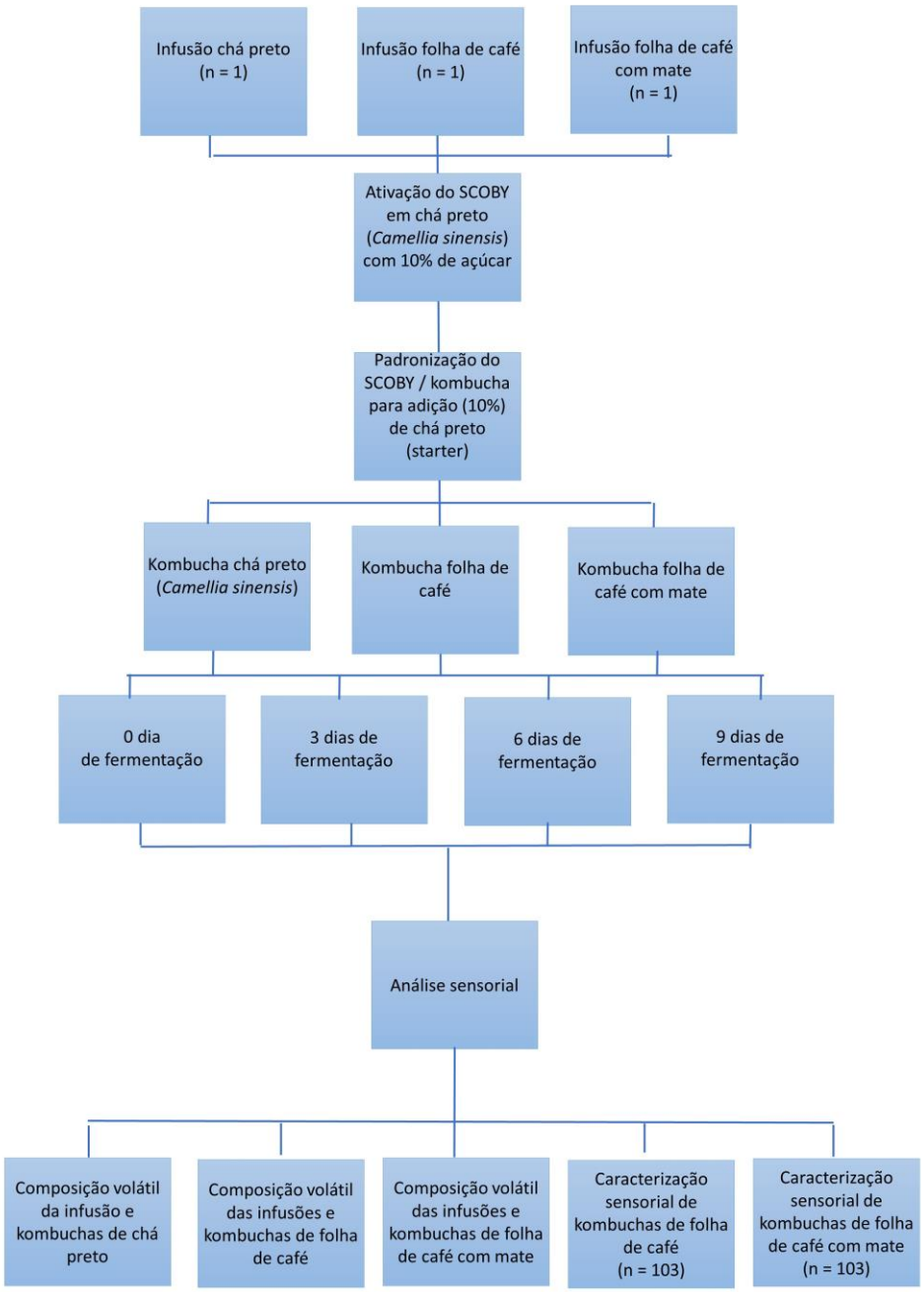


Figure 1. Experimental design of study 3

2.1 Samples

Commercial black tea (*Camellia sinensis*) and toasted yerba mate (*Ilex paraguariensis)* were purchased in bulk in a food store in Rio de Janeiro, Brazil. A leading commercial *C. arabica* leaf tea from Nicaragua was purchased from a reliable trader in Canada. These samples were used to elaborate the infusions for Kombucha preparation.

2.2 Kombucha consortium

The Kombucha consortium was part of the collection of the Microbiology Institute of the Federal University of Rio de Janeiro in Brazil. Previously cultivated in green tea, the consortium was separately fermented three times in black tea, in coffee cascara and coffee leaves teas infusion prior to experimental use in order to stabilize the microbial consortium in these matrixes (Villarreal-Soto et al., 2020). All kombucha beverages were prepared according to the protocol described by Nummer (2013).

Infusions- Infusions of black tea leaves, plain coffee leaves and a mix of coffee leaves (50%) with toasted yerba mate (50%) were prepared at 3%, 2.5% and 2% (weight/volume-w/v), respectively, pouring water at 95°C over the raw material and letting it steep for 10 min.

Black tea kombucha - Black tea kombucha (BT K) was prepared mixing 10% (v/v) of black tea infusion, 10% (w/v) sugar and 2.5% (v/v) of a symbiotic culture of bacteria and yeast (SCOBY) and letting the mixture ferment at 23 °C. Samples were collected before fermentation (day 0) and after 3; 6 and 9 days of fermentation (d0; d3; d6; d9). An extra sample was collected after 14 days of fermentation to be used as a starter (pH = 2.8 ± 0.05) for the coffee leaves with or without yerba mate kombucha production.

Coffee leaves kombuchas - Coffee leaves kombuchas (CL K) were prepared using 2.5% (w/v) of the coffee leaves, 10% (w/v) sugar, 10% (v/v) of the black tea kombucha starter and 2.5% (w/v) of SCOBY. The coffee leaf tea infusion was prepared and after cooling to room temperature, the sugar, the starter and SCOBY were added. The mixture was let ferment at 23 °C. Samples were collected before fermentation (day 0) and after 3; 6 and 9 days of fermentation (d0; d3; d6; d9) for physicochemical and sensory analyses.

Coffee leaves with yerba mate kombucha (CLM K) – The infusion containing 1% (w/v) coffee leaves and 1% yerba mate was prepared and let cool to room temperature, then, 10% (w/v) sugar,10% (v/v) of the black tea kombucha starter and 2.5% (w/v) of SCOBY were added. The

mixture was let ferment in an oven, at 23 °C. Samples were collected before fermentation (day 0) and after 3; 6 and 9 days of fermentation (d0; d3; d6; d9) for physicochemical and sensory analyses.

2.3. pH, total titratable acidity and total soluble solids

Please see study 1.

2.4. Analyses of volatile organic compounds

Please see study 1.

2.5. Amplicon sequencing data analysis and library preparation

Please see study 1.

2.5 Consumer acceptance and sensory characterization

Please see study 2.

2.6.1. Consumer acceptance and purchase intention

Please see study 2.

2.6.2 Rate All that Apply (RATA)

After marking the hedonic scales, assessors were given a pre-prepared checklist with 34 sensory attributes related to appearance, aroma, flavor, and mouthfeel, which were identified in a preliminary session performed by a trained panel. Following, they were required to select all terms they considered appropriate to describe the infusions. considering their intensity (RATA scores: 1 = low intensity; 2 = medium intensity; and 3 = high intensity). The attributes used in the study were organized by alphabetical order as follows: burnt, fermented, fruity, green leaf, herbal, peach, rosé wine, sweet, toasted leaf, white wine(for odor); acid/sour, bitter and sweet (for taste); acetic/vinegar, alcoholic, apple vinegar, fruit syrup, fruity,toasted leaf, green apple,green coffee, herbal, peach, white wine, brewer's yeast (for flavor); adstringency, sparkling, fizzy, full bodied, refreshing, watery (for mouthfeel); and clear, brown and opaque/matte (for appearance).

2.6 Statistics

Physicochemical analyses were performed in triplicate. Results were given as mean ± standard deviation. Analysis of variance (ANOVA), followed by Tukey’s test was performed to compare

the results. ANOVA followed by Tukey's test was used to compare physicochemical analysis and acceptance and purchase intention tests results. Differences were considered significant when $p \leq 0.05$ (Version 8.4.2, Informer Technologies, Los Angeles, CA, USA).

Principal Component Analysis (PCA) of volatile profiles was performed using individual peak areas as variables. Data pretreatment included normalization and scaling, which is a required process for data that present wide scale differences, as is the case of volatiles. Statistical analyses were performed with SPSS for Windows, version 27 (SPSS, Chicago, IL, USA).

Cluster analysis based on the hierarchical grouping of acceptance scores was carried out to identify segments of consumers with similar likings (DePaula et al., 2022).

3. Results and Discussion

3.1. Microbial taxonomy

For discussion see study 2.

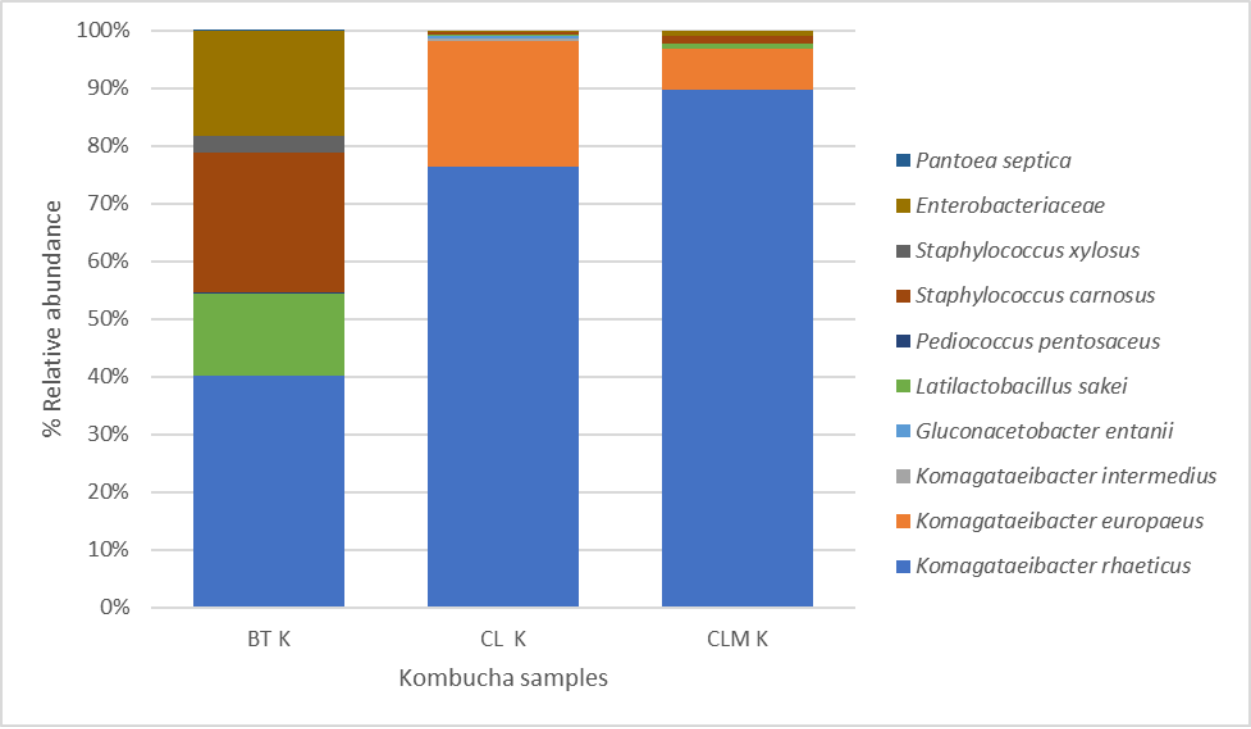


Figure 2. Bacterial composition of the solid and liquid phases of the black tea and coffee leaves kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha; CL K- coffee leaves kombucha; CLM K. coffee-maté leaves kombucha

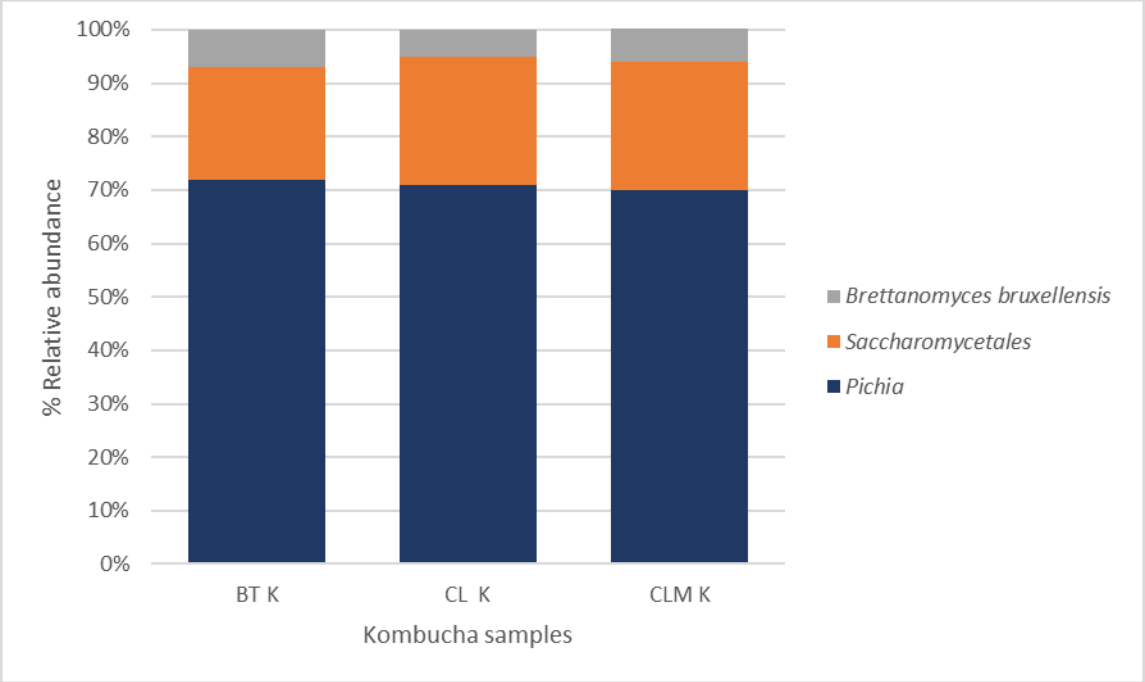


Figure 3. Yeast composition of the solid and liquid phases of the black tea and coffee cascara tea kombuchas consortia after 14 days (starter) and 9 days of fermentation, respectively. Note: BT K. black tea kombucha;CL K-coffee leaves kombucha; CLM K. coffee leaves with maté kombucha

3.2. Physicochemical parameters

The pH, total acidity and soluble solids values for all fermented beverages are presented in Table 1. For discussion, please see study 1.

Table 1. Physicochemical characteristics of black tea kombucha and coffee cascara kombuchas

	Days of fermentation	Titrable acidity (mEq/L)	pH	Soluble solids (° Brix)
Black tea	0	0.1 ± 0.00[a]	3.8 ± 0.07[a]	10.4 ± 0.07[a]
	3	0.2 ± 0.05[a]	3.5 ± 0.00[b]	10.8 ± 0.14[a]
	6	0.3 ± 0.06[b]	3.5 ± 0.00[b]	9.7 ± 0.00[b]
	9	0.3 ± 0.06[b]	3.4 ± 0.00[b]	9.3 ± 0.28[b]
CL	0	0.1 ± 0.04[a]	3.9 ± 0.21[a]	12.2 ± 0.00[a]
	3	0.3 ± 0.06[b]	3.8 ± 0.14[a]	9.7 ± 0.28[b]
	6	0.8 ± 0.00[c]	3.6 ± 0.07[a]	9.4 ± 0.07[b]
	9	0.9 ± 0.10[c]	3.4 ± 0.07[b]	9.2 ± 0.07[b]
CLM	0	0.1 ± 0.04[a]	4.0 ± 0.00[a]	11.8 ± 0.91[a]
	3	0.1 ± 0.04[a]	4.0 ± 0.00[a]	9.9 ± 0.28[a]
	6	0.2 ± 0.05[a]	3.9 ± 0.01[a]	9.6 ± 0.28[a]
	9	0.3 ± 0.06[b]	3.9 ± 0.01[a]	9.1 ± 0.91[a]

Data are expressed as mean ± standard deviation for three replicate analyses; different letters on the same column for the same beverage indicate significant difference ($p<0.05$) by ANOVA followed by LSD test; CL: coffee leaves; CLM: coffee leaf-mate

3.3 Volatile organic compounds

3.3.1. Black tea kombucha

Please see study 2.

3.3.2. Coffee leaf and coffee leaf-maté kombuchas

In CL infusions 32 volatile compounds were identified: 6 aldehydes, 3 acids, 5 alcohols, 7 esters, 4 ketones, 3 monoterpenes,5 monoterpenes alcohol and 1 furan (Table 2). The total chromatogram peak areas included 8% aldehydes, 3% acids, 6% alcohols, 16% esters, 15% ketones, 2% monoterpenes, 46% monoterpene alcohols and 3% furans.

The high peak area for monoterpene alcohols could be explained by the free odor-producing forms of monoterpene alcohols, given the presence of glycosidically bound monoterpene alcohols in tea. Another explanation would be the release of some aroma constituents including monoterpene alcohols when non-volatile materials from tea leaves were fermented (Huafu and Xiaoqing, 1996).

As coffee leaf teas are produced in a similar manner to *C. sinensis* teas (de Almeida et al., 2019), some similarities were found in relation to volatile compounds identified in BT and CL infusions. Key odorant compounds identified in BT and CL were (Z)-3-Hexenol, 1-octen-3-ol, linalool oxide, linalool, phenylethyl alcohol, hexanal, nonanal, β-ionone and dihydroactinidiolide (Ho et al., 2015; Magagna et al., 2017; Kang et al., 2019).

A similar volatile profile was found when beverages were prepared with maté. It is noteworthy mentioning that maté was added to coffee leaf tea given that Lima (2020) observed that adding maté to coffee leaf infusion increased its acceptability. Fermentation of yerba mate alone was tried and the SCOBY consortium did not developed for an unknowing reason. In coffee-maté leaf infusion 29 volatile compounds were identified: 6 aldehydes, 10 alcohols, 1 ester, 4 ketones, 1 monoterpene, 5 monoterpenes alcohols and 1 furan. The total chromatogram peak areas included 12% for aldehydes, 21% for alcohol, 7% for esters, 13% for ketones, 1% for monoterpenes, 44% for monoterpenes alcohol and 2% for furans. It is important to emphasize that we used in this study toasted maté. The heating process (drying and/or roasting), to which

the mate leaves are subjected before consumption, leads to a large amount of degradation products and enhances flavor (Marquez et al., 2013).

Considering toasted yerba mate, Kawakami and Kobayashi, (1991) identified 196 volatile compounds, including β-ionone, methyl palmitate, linalool oxide and dihydroactinidiolide that were also identified in this study. According to them, the composition of the aroma concentrates of mate tea was slightly different from that of *C. sinensis* tea, but both teas contained various similar components. Additional volatile compounds identified in our study and previously identified in maté are α-terpineol and β-damascenone (Machado et al., 2007).

3.3.3. Coffee leaves and coffee leaf-maté kombuchas

The volatile compounds identified in CL K and CLM K are presented in Table 2. In CL K, we identified 61 volatile compounds, while in CLM K 45 volatile compounds were identified. Esters, acids, monoterpenes alcohols, aldehydes and ketones had the highest peak areas. Some compounds were only identified in CL K: pentadecanal, tridecanal, undecanal, methyldihydrojasmonate, menthone, myrcene and camphene.

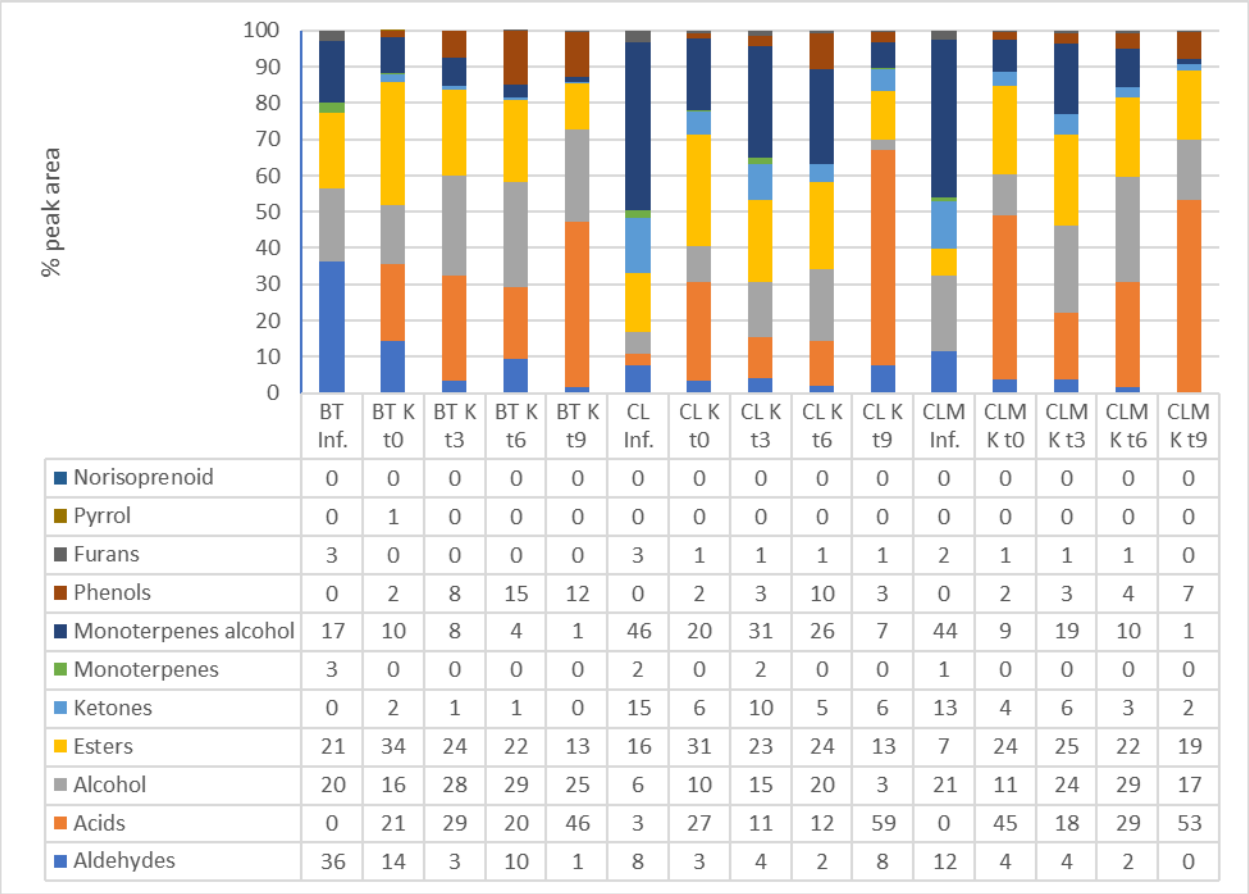
In addition to the similarities between BT and CL infusions, a similar profiles were observed in the volatile profiles of kombuchas, for example, the presence of phenylethyl ethanol and isovaleric acid, previously identified in BT K (Savary et al., 2021; Tran et al., 2022; Zhang et al., 2021).Similarities were also found in CL K and CLM K (Figure 3). When these matrixes were fermented to make kombuchas, only two new volatile compounds were identified: the aldehyde 2,4-heptadienal, a marker volatile compound in green (Araújo et al., 2017) andtoasted maté (Machado et al., 2007). The formation of this volatile compound is favored by some steps in the maté processing such as the *sapecado* and drying, where the material is submitted to heat treatment (Marquez et al., 2013); and the alcohol 2-methyl-1-butanol, probably originated from its aldehyde 2-methylbutyraldehyde, identified in CL K d0. It is important to note that plain maté did not grow as well as with CL, taking a long time to form biofilm.

Identified in CL K and CLM K, terpineol, trans-linalool, isovaleric acid, isoamyl acetate and hexanoic acid were reported to have significant contributions to the aroma of BT K. (Wang et al., 2022) . Also, trans-linalool oxide, linalool, phenylethyl alcohol, hexanal, nonanal, benzaldehyde and β-ionone, identified in CL K and CLM K, , have been reported as aroma impact compounds in BT and BT K (Magagna et al., 2017; Kang et al., 2019).The presence of

the phenols 4-ethylguiacol and 4-ethylphenol in the coffee leaves kombuchas is probably due to the fermentative process by yeasts from the genus *Brettanomyces* (Nieto-Rojo et al., 2014).

Figure 4 presents the relative peak area (%) of volatile organic compounds in black tea, coffee leaf and coffee-maté leaves infusion and kombucha beverages, grouped into chemical classes. Following, the main classes of compounds identified in the coffee leaf kombuchas will be commented.

Aldehydes comprised 2% - 8% of peak areas for CL K and 0% - 4% for CLM K. For CL K, the lowest area occurred on d6 (2 %) and the highest on d9 (8 %), while in CLM K the highest occurred on day 0 (4 %) and the lowest on day 9 (0 %). Benzaldehyde and hexanal are suggested as key odorants in black tea due to their high odor activity value, contributing to a sweet and floral aroma (Yang et al., 2022). These aldehydes were identified in CL K d3 and in CLM K d0 and d3. Safranal, identified in CL K d3 and d6, is suggested to be a key odorant in Pu-erh tea (Wang et al., 2022). Octanal and nonanal were previously identified in maté (Polidoro et al., 2016).



	BT Inf.	BT K t0	BT K t3	BT K t6	BT K t9	CL Inf.	CL K t0	CL K t3	CL K t6	CL K t9	CLM Inf.	CLM K t0	CLM K t3	CLM K t6	CLM K t9
Norisoprenoid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyrrol	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Furans	3	0	0	0	0	3	1	1	1	1	2	1	1	1	0
Phenols	0	2	8	15	12	0	2	3	10	3	0	2	3	4	7
Monoterpenes alcohol	17	10	8	4	1	46	20	31	26	7	44	9	19	10	1
Monoterpenes	3	0	0	0	0	2	0	2	0	0	1	0	0	0	0
Ketones	0	2	1	1	0	15	6	10	5	6	13	4	6	3	2
Esters	21	34	24	22	13	16	31	23	24	13	7	24	25	22	19
Alcohol	20	16	28	29	25	6	10	15	20	3	21	11	24	29	17
Acids	0	21	29	20	46	3	27	11	12	59	0	45	18	29	53
Aldehydes	36	14	3	10	1	8	3	4	2	8	12	4	4	2	0

Figure 4. Relative peak area (%) of volatile organic compounds in black tea and coffee cascara infusion and kombucha beverages, grouped into chemical classes. Note. BT: black tea; Inf: infusion; K: Kombucha; CL: coffee leaf; CLM: coffee leaf-maté

Alcohols represented 3% - 20% of total peak area for CL K and 11 % - 29% CLM K. The highest area was on d6 for both kombuchas. New alcohols were identified in the fermentation process, mainly on d6: cedrol in CL K and 2-methyl-1-butanol in CLM K. Cedrol is an important contributor to Pu-erh tea aroma (Wang et al., 2022). Ethanol was identified in all CL K and CLM K samples. Even if not identifiable on the olfactory level, this compound can impact the aromatic profile of kombucha (Tran et al., 2020). Although ethanol contributes to a sweet aroma, at threshold, ethanol is significantly bitterer than any other attribute (Mattes and DiMeglio, 2001).

Acids represented 27% - 59% of total peak area for CL K and 18% - 53% for CLM K, being on d9 the largest area (59% and 53%, for CL K and CLM K, respectively). The main acid responsible for kombucha sourness is acetic acid (Tran et al., 2020; Bishop et al., 2022), identified only on d9 in both kombuchas, possibly for co-elution with other compounds. Decanoic and octanoic acid were identified in all CL K and CLM K beverages. They are

reported as odor-active compounds in sparkling wine (Ubeda et al., 2019).Decanoic, octanoic and caproic acids have been identified in beers (Pinho et al., 2006).

Esters represented 31% - 13% of total peak area for CL K and 19% - 24% for CLM K. Ethyl acetate and methyl salicylate were identified in all CL K and CLM K samples. Ethyl acetate imparted apple and banana traits to the wine (Kong et al., 2022) while methyl salicylate is an odor-active compound in Pu-erh tea (Wang et al., 2022) and oolong tea (Zhang et al., 2022). Also, methyl salycilate was previously identified in yerba mate tea (Machado et al., 2007). Some esters were identified on d6 and d9 in all samples, such as ethyl decanoate, ethyl hexanoate, ethyl isobutyrate, ethyl laurate and ethyl phenylacetate. Ethyl decanoate and ethyl octanoate have been reported as abundant in ciders, (Kliks et al., 2021). Ethyl isobutyrate and ethyl hexanoate have been reported as impact compounds in sparkling wine (Ubeda et al., 2019). Ethyl phenylacetate is one of the important esters in wine aroma compounds formed during alcoholic fermentation (Kong et al., 2022) and ethyl laurate was positive correlated with aroma in beer (Benucci et al., 2021).

Ketones comprised 5% - 10% of total peak area for CL K and 2% - 6% for CLM K. According to Steger et al. (2022), 3,5-octadien-2-one, α-ionone and β-ionone are key odorants in coffee leaf tea fermented by yeasts and were identified in this study. Also, β-damascenone, α-ionone and β-ionone are odor active compounds in yerba mate (Marquez et al., 2013). Geranyl acetone was previously identified in yerba mate (Machado et al., 2007; Marquez et al., 2013) and is a marker compound in green yerba mate (Araújo et al., 2007). Additional compounds identified in CL K were menthone and carvone, important contributors to Pu-erh tea aroma (Wang et al., 2022).

Monoterpenes and monoterpenes alcohols corresponded 2% and 7% - 31% of total peak area, respectively, for CL K and 0% and 1%-9%, respectively, for CLM K. Linalool and linalool oxide are odor active compound in yerba mate (Marquez et al., 2013). α-terpineol, which was identified in all CL K and CLM K, was previously identified in yerba mate (Kaltbach et al., 2021) and coffee leaf tea (Steger et al., 2022). Furthermore, we identified some monoterpenic compounds that have significant contributors for green, black and Pu-erh tea, such as myrcene, 3-carene and camphene (Su et al., 2022; Yin et al., 2022, Wang et al., 2022; Wang et al., 2022).

Furans represented 1% of total peak area in all coffee leaf and coffee leaf with mate kombuchas samples. The only furan identified in all samples was dihydroactinidiolide. This

volatile compound are viewed as critical aroma in determining the characteristics of black tea (Ho et al., 2015). Also, this compound was previously identified in Pu-erh tea (Wang et al., 2022) and green maté (Araújo et al., 2007).

Table 2. Volatile compounds in coffee leaves and coffee- maté leaves infusions and kombuchas

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLMt 6	CLM t9
Aldehydes															
2-methylbutyraldehyde	Musty, cocoa, phenolic, coffee, nutty, malty, fermented, fatty, alcoholic[2]	96-17-3	644	754	□	□	■	□	□	□	□	□	□	□	□
2,4-Heptadienal	Fried, nut, fat, green, pungent, fruity, spicy[1,2]	5910-85-0	852	865	□	□	□	□	□	■	□	■	□	□	□
Isovaleraldehyde	Ethereal, aldehydic, chocolate, peach, fatty[2]	590-86-3	777	842	□	□	■	□	□	■	□	□	□	□	□
Benzaldehyde	Almond, burnt sugar, tropical fruit[1,2]	100-52-7	736	885	■	■	■	□	□	■	□	■	□	□	□
Dodecanal	Soapy, waxy, aldehydic, citrus, green, floral[2]	112-54-9	917	963	■	■	■	■	■	□	■	■	■	■	■
Ethanal	Pungent, ether, fresh, fruity, musty[1,2]	75-07-0	976	976	□	□	■	■	■	■	□	■	■	□	■
Heptanal	Fat, citrus, rancid, fresh, aldehydic, green, herbal, wine-lee, ozone[1,2]	111-71-7	909	917	□	□	□	□	□	■	■	■	□	□	□
Hexanal	Grass, tallow, fat, fresh, green, aldehydic, leafy ,fruity, sweaty[1,2]	66-25-1	800	855	■	■	■	□	□	□	□	■	■	□	□
Nonanal	Fat, citrus, fresh, orange, green[1,2]	124-19-6	929	931	□	■	□	■	□	□	■	■	■	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLMt 6	CLM t9
Octanal	Citrus, soap, lemon, herbal, green, honey[1,2]	124-13-0	877	907	■	■	□	■	□	□	■	■	■	□	□
Pentadecanal	Fresh, waxy[1,2]	2765-11-9	823	956	□	□	■	□	□	□	□	□	□	□	□
Phenylethanal	Honey, floral, rose, sweet, powdery, fermented, chocolate, earthy, hawthorne, green, hyacinth, clover, cocoa[1,2]	122-78-1	932	954	■	■	■	□	□	■	■	■	■	□	□
Safranal	Herb, sweet, fresh, phenolic, metallic, rosemary, tobacco, spicy[1,2]	116-26-7	889	899	□	□	■	■	□	■	□	□	□	□	□
Tetradecanal	Fatty, waxy, amber, incense, dry, citrus, peel, musk[2]	124-25-4	763	896	■	□	□	□	■	□	■	□	□	□	□
Tridecanal	Flower, sweet, must, fresh, clean, aldehydic, soapy, citrus, petal, waxy, grapefruit peel[1,2]	10486-19-8	781	885	□	□	□	□	■	□	□	□	□	□	□
Undecanal	Waxy, soapy, floral, aldehydic, citrus, green, fatty, fresh, laundry[2]	112-44-7	723	865	□	□	□	□	■	□	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLMt 6	CLM t9
Acids															
Acetic acid	Acidic, sour, pungent, vinegar[1, 2]	64-19-7	943	956	■	□	□	□	■	□	□	□	□	□	■
Caproic acid	Sweat, sour, fatty, cheese[1,2]	142-62-1	897	920	□	□	□	■	■	□	□	■	■	■	■
Decanoic acid	Rancid, fat, unpleasant, rancid, sour, fatty, citrus[1,2]	334-48-5	924	933	□	■	■	■	■	□	□	■	■	■	■
Isovaleric acid	Sweat, acid, rancid, sour, stinky, feet, cheese, tropical[1,2]	503-74-2	818	852	□	□	□	■	■	□	□	□	□	■	■
Lauric acid	Metal, mild, fatty, coconut, bay, oil[1,2]	143-07-7	689	826	□	□	□	□	■	□	□	□	□	□	□
Nonanoic acid	green, fat, waxy, dirty, cheese, cultured, dairy[1,2]	112-05-0	887	900	■	■	■	■	■	□	□	■	■	■	■
Octanoic acid	Acid, sweat, cheese, fruit notes[1,2]	124-07-2	921	934	□	■	■	■	■	□	□	■	■	■	■
Alcohols															
1-dodecanol	Earthy, soapy, waxy, fatty, honey, coconut[2]	112-53-8	799	896	□	□	□	□	□	□	■	□	□	□	□
1-penten-3-ol	Ethereal, horseradish, green radish, chrysanthemum, vegetable, tropical fruity, green, radish, vegetable rummy, truffle, oily, resinous[2]	616-25-1	846	846	□	□	□	□	□	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLMt 6	CLM t9
2-Ethylhexanol	Rose, green, citrus, fresh, floral, oily, sweet[1,2]	104-76-7	930	943	■	■	■	■	■	■	■	■	■	■	■
2-Methyl-1-butanol	Malt, wine, onion, ethereal, fusel, alcoholic, fatty, greasy, whiskey, leathery, cocoa[1,2]	137-32-6	855	876	□	□	□	□	□	□	□	■	■	■	□
(S)-2-Heptanol	Mushroom, oily, fatty, blue cheese, moldy[2]	6033-23-4	800	859	□	□	□	■	□	□	□	□	□	□	□
(S)-(-)-2-methyl-1-butanol	Ethereal, fresh[2]	1565-80-6	796	847	□	■	□	■	□	□	□	□	□	□	□
3-Octenol	Mushroom, earthy, green, oily, fungal, raw chicken, vegetative[2]	20125-85-3	852	878	□	□	□	□	□	□	■	□	□	□	□
Z-3-hexenol	Grass, fresh, green, cut, foliage, vegetable, herbal, oily[1,2]	928-96-1	881	916	■	□	□	□	□	□	■	□	□	□	□
Phenethyl alcohol	Honey, spice, rose, lilac, floral, fresh[1,2]	60-12-8	888	932	■	■	■	■	■	□	□	■	■	■	■
3-Methyl-1-butanol	whiskey, malt, burnt, fusel, oil, alcoholic, fruity, banana[1,2]	123-51-3	877	894	□	■	□	■	■	□	□	■	■	■	■
Cedrol	Cedarwood, woody, dry, sweet, soft[2]	77-53-2	661	825	■	□	□	■	□	□	■	□	□	□	□
Ethanol	Sweet[1]	64-17-5	976	976	□	■	■	■	■	■	■	■	■	■	■
Eugenol	Clove, honey, sweet, spicy, clove, woody[1,2]	97-53-0	864	868	□	□	■	□	□	■	■	□	□	■	■

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
Esters															
2-Methylbutyl acetate	Fruit, over ripe fruit, sweet, banana, juicy[1,2]	624-41-9	677	848	□	□	□	□	□	□	□	□	□	□	■
2-Methylallyl butyrate	Powerful, fruity, ether, sweet, pineapple, apple, plum[2]	7149-29-3	696	774	□	□	□	□	□	■	□	□	□	□	□
Ethyl 2-methylbutyrate	Sharp, sweet, green, apple, fruity[2]	7452-79-1	899	947	□	□	□	■	■	□	□	□	□	□	■
Ethyl 3-hexenoate	Fruity, pineapple, green, tart, candy, metallic, tropical, rhubarb, weedy, cheesy[2]	2396-83-0	909	920	□	□	□	■	□	□	□	□	□	□	□
Ethyl (Z)-3-hexenoate	Green, pear, apple, tropical[2]	64187-83-3	914	933	□	□	□	□	□	□	□	□	□	□	■
Ethyl Acetate	Pineapple, ethereal, fruity, sweet, weedy, green[1,2]	141-78-6	971	977	□	■	■	■	■	□	□	■	■	■	■
Ethyl butyrate	Apple, fruity, juicy, fruit, pineapple, cognac[1,2]	105-54-4	765	848	□	□	□	□	□	□	□	□	□	□	■
Ethyl decanoate	Grape, sweet, waxy, fruity, apple, oily, brandy[1,2]	110-38-3	913	928	□	□	□	■	■	□	□	□	□	■	■
Ethyl hexanoate	Apple peel, fruit, sweet, pineapple, waxy, green, banana[1,2]	123-66-0	921	924	□	□	□	■	■	□	□	□	□	□	■
Ethyl isobutyrate	Sweet, rubber, ethereal, fruity, alcoholic, fusel, rummy[1,2]	97-62-1	882	895	□	□	□	■	■	□	□	□	□	□	■

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
Ethyl laurate	Leaf, sweet, waxy, floral, soapy, clean[1,2]	106-33-2	843	880	□	□	□	■	■	□	□	□	□	□	□
Ethyl nonanoate	Fruity, rose, waxy, rum, wine, natural, tropical[2]	123-29-5	876	899	□	□	□	□	□	□	□	□	□	■	□
Ethyl octanoate	Fruit, banana, pear[1,2]	106-32-1	876	899	□	□	□	□	■	□	□	□	□	■	■
Ethyl palmitate	Wax, fruity, creamy, milky, balsamic, greasy, oily[1,2]	628-97-7	739	820	■	□	□	□	□	□	■	□	□	□	□
Ethyl phenylacetate	Fruit, sweet, floral, honey, rose, balsam, cocoa[1,2]	101-97-3	877	904	□	□	□	■	■	□	□	□	□	□	■
Ethyl valerate	Yeast, fruit, sweet, apple, pineapple, green, tropical[1,2]	539-82-2	798	876	□	□	□	□	□	□	□	□	□	□	■
Isoamyl acetate	Banana, sweet, fruity, solvent[1,2]	123-92-2	759	859	□	□	□	□	□	□	□	□	□	□	■
Isopropyl myristate	Faint, oily, fatty[2]	110-27-0	856	867	■	□	□	□	□	■	■	□	□	□	□
Isopropyl palmitate	Fat, bland, oily[1,2]	142-91-6	826	894	■	■	□	■	□	□	■	□	□	□	□
Linalyl acetate	Sweet, fruit, green, citrus, bergamot, lavender, woody[1,2]	115-95-7	772	809	■	□	□	□	□	□	□	□	□	□	□
Methyl dihydrojasmonate	Floral, oily, jasmin, green, lactonic, tropical, natural[2]	24851-98-7	785	823	□	□	■	■	□	□	□	□	□	□	□
Methyl palmitate	Oily, waxy, fatty, orris[2]	112-39-0	747	798	■	□	□	□	□	□	■	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
Methyl salicylate	Peppermint[1]	119-36-8	889	928	■	■	■	■	■	■	■	■	■	■	■
Ketones															
3,5-Octadien-2-one	Fruity, fatty, mushroom[2]	38284-27-4	879	895	■	■	■	■	□	■	■	■	■	■	□
2-octanone	Earthy, weedy, natural, woody, herbal, dairy, waxy, cheese, woody, mushroom and yeast[2]	111-13-7	842	842	□	□	□	□	□	■	□	□	□	□	□
2,3-butanedione	Strong, butter, sweet, creamy, pungent, caramel, milky[2]	431-03-8	894	894	□	□	□	□	□	■	□	□	□	□	□
(-)-Carvone	Mint, sweet, spearmint, herbal[1,2]	6485-40-1	918	921	■	■	■	■	■	□	■	■	■	■	■
Geranyl acetone	Magnolia, green, fresh, fruity, waxy, rose, woody, tropical[1,2]	3796-70-1	682	777	□	□	□	■	■	■	■	■	■	□	■
Isophorone	Cooling, woody, sweet, green, camphor, fruity, musty, cedarwood, tobacco, leather[2]	78-59-1	699	773	□	■	□	□	□	■	□	□	□	□	□
Ketoisophorone	Musty, woody, sweet, tea, tobacco, leaf, citrus, floral, musty, tea like with green, sweet, fruity nuances[2]	1125-21-9	808	808	□	□	□	□	□	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
β-Damascenone	Apple, rose, honey, tobacco, sweet[1,2]	23726-93-4	898	955	□	■	■	■	■	■	□	■	■	■	■
Monoterpenes alcohol															
1-Terpinen-4-ol	Turpentine, nutmeg, must, pepper, woody, earth, musty, sweet[1,2]	562-74-3	860	898	■	■	□	□	□	□	■	■	□	□	□
Linalool	Citrus, flower, lavender, sweet, green[1,2]	78-70-6	942	945	■	■	■	■	■	■	■	■	■	■	■
Linalool oxide	Flower, wood, musty, camphor, fenchyl, alcohol[1,2]	60047-17-8	900	928	■	■	■	■	■	■	□	■	■	■	■
Trans-Linalool oxide	Flower[1,2]	34995-77-2	880	892	■	■	□	□	□	■	□	□	■	□	□
Cis-Linalool oxide	Earthy, floral, sweet, woody [1,2]	5989-33-3	838	852	□	□	□	■	□	□	■	□	■	□	□
Monoterpenes															
3-δ-Carene	Citrus, terpenic, herbal, pine, solvent, resinous, phenolic, cypress, medicinal, woody[2]	13466-78-9	741	825	□	■	□	□	□	□	■	□	□	□	□
Myrcene	Balsamic, must, spice, peppery, terpene, plastic[1,2]	123-35-3	834	892	■	□	■	□	□	□	□	□	□	□	□
Menthone	Fresh, green, minty[1,2]	89-80-5	844	859	□	□	■	□	□	□	□	□	□	□	□
Camphene	Camphor, woody, herbal, fir, needle, camphor, terpenic[1,2]	79-92-5	815	860	■	□	□	□	■	□	□	□	□	□	■

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
α-Ionone	Wood, violet, sweet, floral, orris, tropical, fruity[1,2]	127-41-3	892	897	■	■	■	■	■	■	■	■	■	■	■
β-ionone	seaweed, violet, flower, raspberry, woody, sweet, fruity, berry, tropical, beeswax[1,2]	14901-07-6	847	849	■	■	■	■	■	■	■	■	■	■	■
α-Terpineol	Oil, anise, mint, lemon, citrus[1,2]	98-55-5	901	921	■	■	■	■	■	□	■	■	■	■	■
γ-Terpineol	Terpineol, lilac[2]	586-81-2	951	956	□	□	□	■	□	□	□	□	□	□	□
1,5,8-p-Mentatriene	Roasted[2]	21195-59-5	735	780	□	□	□	□	□	■	□	□	□	□	□
Furans															
(S)-dihydroactinidiolide	Musk, coumarin[2]	17092-92-1	888	905	■	■	■	■	■	■	■	■	■	■	■
Furfural	Bread, almond, sweet, woody[1,2]	98-01-1	916	945	□	□	□	□	□	■	□	□	□	□	□
5-methylfurfural	Almond, caramel, burnt sugar, spice, maple, sweet, brown, caramellic, grain, maple-like[1,2]	620-02-0	884	911	□	□	□	□	□	■	□	□	□	□	□
2-Ethylfuran	Chemical, beany, ethereal, cocoa, bready, malty, coffee, nutty, ethereal, brown, cocoa, rooty, earthy, musty[2]	3208-16-0	626	766	□	□	□	□	□	■	□	□	□	□	□
Pyrrol															
1-Furfurylpyrrole	Plastic, green, waxy, fruity, coffee, vegetable, vegetative, onion, sharp and metallic[2]	1438-94-4	859	859	□	□	□	□	□	■	□	□	□	□	□

Table 2. continued

Volatile compound	Odor description	CAS#[a]	ELRI[b]	LRI[c]	CL	CL t0	CL t3	CL t6	CL t9	YM	CLM	CLM t0	CLM t3	CLM t6	CLM t9
Phenols															
4-Ethylguaiacol	Spice, clove, smoky, bacon, phenolic[1,2]	2785-89-9	908	939	□	■	■	■	■	□	□	■	■	■	■
4-Ethylphenol	Phenolic, castoreum, smoke, guaiacol[2]	123-07-9	817	856	□	■	■	■	■	□	□	■	■	■	■

Note: CL: Coffee leaves; CLM: Coffee leaf-mate YM: yerba maté; K: Kombucha [a]CAS# (Chemical Abstracts Service) Registry Number, available in the NIST database; [b]ELRI: Experimental Linear Retention Index; [c]LRI: Linear Retention Index based on literature and NIST database (https://webbook.nist.gov/chemistry/name-ser/);[1]http://www.flavornet.org; [2]http://www.thegoodscentscompany.com; ■ compound identified in the sample. □ not identified;

Principal component analysis (PCA) was performed to evaluate the effect of time on the changes of volatile compounds profile throughout fermentation (Figure 5). The data from volatile profile of BT, CL and CLM infusions and kombucha at d0, d3, d6 and d9 were used as variables. Figure 4 presents the first two principal components (Components 1 and 2), which together explained 42.1% of the total variance (22.3% for C1 and 19.8% for C2). PCA allowed a grouping of the observations separating the initial time, intermediate times, and final time of the three fermented matrices (CL, CLM, and BT). On the edge, the volatiles that increase on each component are described (only those with component matrix higher than ± 0.7 were considered). Component 1 is linked with fermentation time, whereas Component 2 is associated with sample origin.

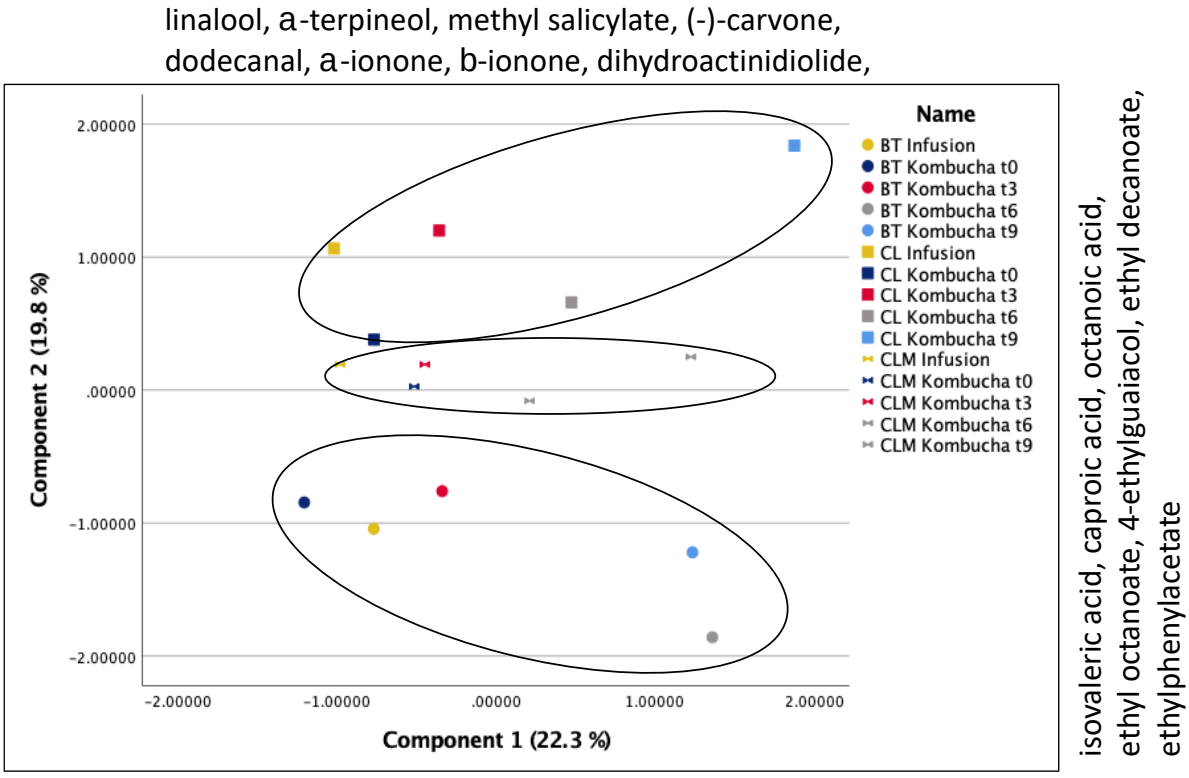


Figure 5. Principal component analysis (PCA) showing the clustering of infusions and kombuchas from black tea, coffee leaves and coffee leaves with mate raw materials and changes in volatile compounds profile. Note: BT: black tea; CL: coffee leaves; CLM: coffee leaves with mate; t0, t3, t6 and t9: days 0, 3, 6 and 9 of fermentation.

According to PCA analysis, CL infusion and all its kombuchas and CLM infusion and its kombuchas on d3 and d6 were positively correlated with linalool, α-terpineol, methyl salicylate, (-)-carvone, dodecanal, α-ionone, β-ionone and dihydroactinidiolide. BT, CL and CLM

kombuchas on d6 and d9 were positively correlated with isovaleric acid, caproic acid, octanoic acid, ethyl octanoate, 4-ethylguiacol, ethyl decanoate and ethyl phenylacetate.

Figure 6 shows the factor map grouping the samples by clusters according to the similarities with volatile compounds. BT infusion and its kombuchas on d0 and d3 (green cluster), CL and CLM infusion and its kombuchas on d0 and d3 (blue cluster) and CL K d6 and CLM K on d6 and d9 (light brown cluster) showed very similar volatile profiles. Therefore, they were clustered together, with great distance from other samples, as BT K on d6 and d9, which were grouped together (red cluster) and CL K on d9 (pink cluster).

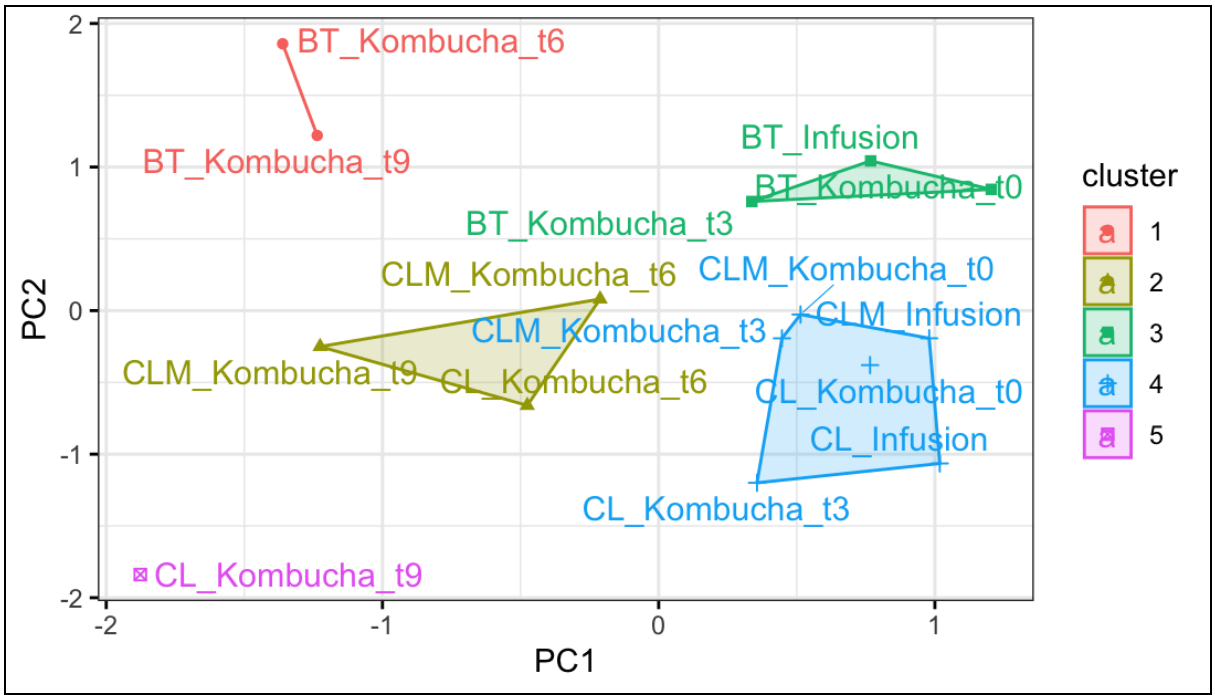


Figure 6. Factor map grouping by cluster the samples with volatile similarity. Note: BT: black tea; CL: coffee leaves; CLM: coffee leaves with mate; t0, t3, t6 and t9: days 0, 3, 6 and 9 of fermentation.

Results indicate that despite the similarity of CL K and CLM K aromas, they shared many impact or odor active volatile compounds identified in coffee leaves tea (Steger et al., 2022), in *C. sinensis* tea (Ho et al., 2015; Wang et al., 2022; Zhang et al., 2022) and maté (Araújo et al., 2007; Marquez et al., 2013). It was possible to distinguish kombuchas according to these matrixes and days of fermentation.

3.4. Sensory analysis

A total of 103 consumers participated in the sensory assessment. The assessor's characteristics are presented in table 3. The mean acceptance scores for CL K at d3, d6 and d9 were 6.4; 5.9 (slightly liked) and 5.4 (neither like nor dislike), respectively. For CLM K were 6.6 (moderate liked) 6.2 and 5.9 (slightly like), respectively (Figure 7).

Table 3. Assessor's characteristics

Gender		**Age**				
Male	Female	18 - 24	25 - 34	34 – 44	45 - 59	≥ 56
32 %	68 %	53 %	32 %	6 %	4 %	5%
Level of education						
Basic education	Undergraduated	Incomplete graduation		Complete Graduation		Master's or doctoral degree
2 %	48 %	15 %		12 %		24%
Family income (MW: minimum wages)						
1 MW		2 - 3 MW		4 - 5 MW		> 5 MW
22 %		37 %		19 %		22 %
Know kombucha				Drink kombucha		
Yes		No		Yes		No
64 %		36 %		16 %		84 %
Sparkling beverages/soft drinks consumption						
Sparkling water	Apple juice	Soda	Tonic water	Sparkling wine		Cider
44%	13%	62%	24%	34%		17%

For a sample to be considered "well-accepted", it must obtain 70 % AI or higher (Meilgaard et al., 1991). Only the samples with 3 days of fermentation had higher AI (above 70%), while the AI for samples on 6 days of fermentation was 67%. Additionally, the low score given to day 9 can be attributed to the higher number and area percent (abundance) of acids. As usual, the purchase intention results were associated with those from the acceptance test (Figure 8).

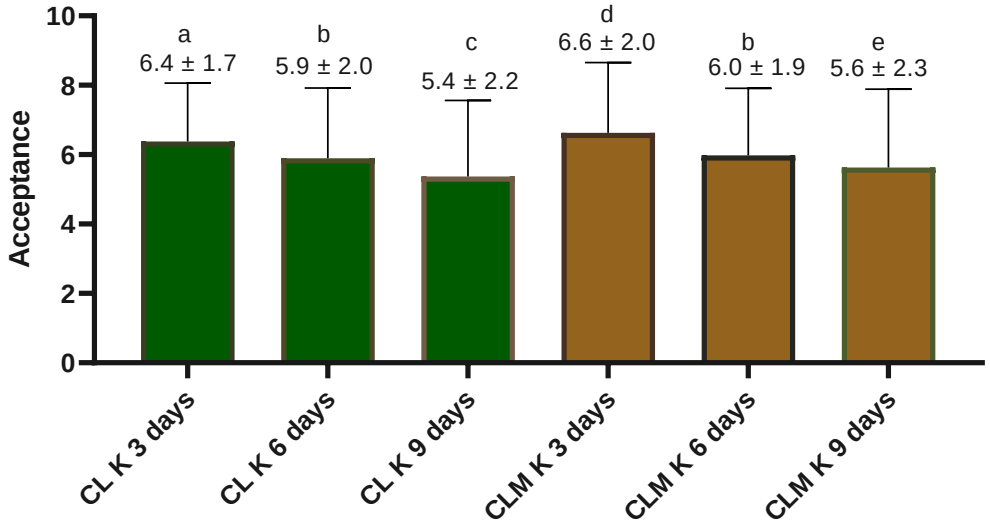


Figure 7. mean acceptance scores of coffee leaves and coffee-mate kombuchas by Rio de Janeiro consumers n=103). Note: CL: coffee leaves; CLM; coffee leaves-mate; K: kombucha

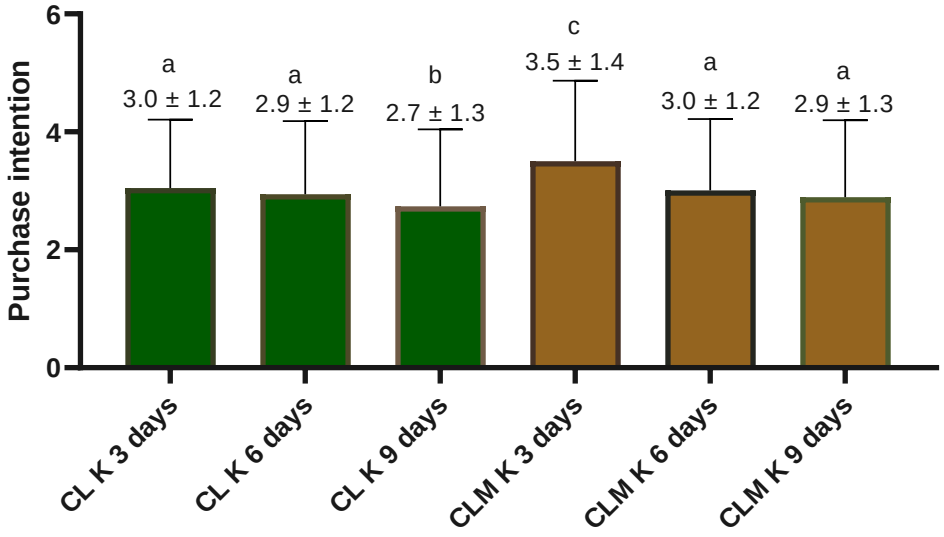


Figure 8. Mean purchase intention scores for coffee leaves and coffee-mate kombuchas by Rio de Janeiro consumers n=103). Note: CL: coffee leaves; CLM; coffee leaves-mate; K: kombucha

The highest mean scores (6.4 ± 1.7 and 6.6 ± 2.0) were given to CL K and CLM K on d3, respectively. Seventy-seven and seventy-five percent of the assessors gave scores between 6 and 9, respectively. This result is probably due to the low acid content and high soluble solids value. When scoring samples, the assessors had space to make comments if they wished. The most

cited attributes by the assessors were sweet for both samples on d3 and d6 and also yerba mate for CLM K. Additional attributes cited by assessors were: soft drink, peach, peach syrup and bitter. The high acceptance mean for CLM K may be explained by food pairing. The "food pairing hypothesis" states that two ingredients that share chemical compounds are more likely to taste (and smell) good together (Arellano-Covarrubias et al., 2022).

No similar study was found for comparison but considering the sensory acceptance of kombuchas from other new substrates (black carrot, cherry laurel, blackthorn and red raspberry) but considering the sensory acceptance of kombuchas from new substrates (black carrot, cherry laurel, blackthorn and red raspberry), in the study of Ulusoy and Tamer (2019) performed in Turkey, the beverages fermented for shorter periods of time (3 and 5 days) obtained scores between 6 and 8, using a 9-point hedonic scale, while beverages fermented for 10 and 12 days received scores below 5. According to the authors, ratings below the 5-point limit value indicate the product will not be consumed. Studies performed in Brazil and Tunisia reported that assessors liked herbal or and grape kombuchas after 6 days of fermentation, with average acceptance scores between 5 and 7 (Zhang et al, 2021; Ayed et al, 2016).

Because people have different tastes and experiences and cannot be represented by a mean score, we performed cluster analysis to identify different niches of consumers. Cluster 1 (n = 41, mean score = 6.9, and AI = 77%) consistently attributed the highest scores to CLM K 3d (acceptance mean = 7.7). This cluster was composed of 54% female. Forty three percent of them were 18-24 years old. Forty one percent had incomplete graduation, and 36% had family monthly income of 2-3 MW. In this cluster, 12% of the assessors were kombucha consumers. This cluster had the highest consumption of sparkling water (46%), apple juice (15%), soda (71%), sparkling wine (34%), cider (20%) and tonic water (24%)

Cluster 2 (n = 30, mean score = 5.1, and AI = 57%) also consistently attributed the highest scores to CLM K 3d (acceptance mean = 7.6). This cluster was composed of 77% female. Sixty three percent of them were 18-24 years old and incomplete graduation, and 33% had family monthly income of 2-3 MW. In this cluster, 16% of the assessors were kombucha consumers. This cluster had the high consumption of soda (50%), and low consuption of sparkling water (30%), apple juice (7%), tonic water (13%), sparkling wine (37%) and cider (17%). According to Lima (2020), the high acceptance of coffee leaves with maté are due to its usual consumption in the Southwest of Brazil.

Cluster 3 (n = 32, mean score = 5.5, and AI = 61%) also consistently attributed the highest scores to CL K 3d (acceptance mean = 6.5). This cluster was composed of 78% female. Fifty six percent of them were 18-24 years old, forty percent with incomplete graduation, and 38% had family monthly income of 2-3 MW. In this cluster, 16% of the assessors were kombucha consumers. This cluster had high consumption of soda (63%) and sparkling water (50%), followed by tonic water (34%), sparkling wine (31%), apple juice (19%) and cider (13%).

3.4.1. Rate all that apply (RATA)

Considering that as kombucha is fermented, chemical changes generate different sensory attributes and intensities, RATA test was performed to identify these changes during CL K and CLM K production.

On d3 of fermentation, CL K presented higher intensity for herbal and sweet odor, sweet taste, fruity, herbal and peach flavors and clear in appearance. On d6 , the intensity of fermented odor increased and decreased herbal and sweet odor, decreased sweet taste and increase acid/sour taste, increase acetic/vinegar, apple vinegar flavor and decreased fruity, herbal and peach flavor, and increased fizzy and adstringency mouthfeel. CL K d9 presented the low intensity mean for sweet and green leaf odor, the highest intensity for acid/sour taste and acetic/vinegar, apple vinegar flavors and fizzy mouthfeel.

For CLM K, herbal and toasted leaf odor had the highest intensity mean, as well as sweet taste, toasted leaf and herbal flavor and was more refreshing. On days 6 and 9 of fermentation, CLM K presented higher intensity mean for fermented odor, while herbal and toasted leaf odor intensities decreased, acid/sour taste increased, and sweet taste decreased. Acetic/vinegar flavor increased while herbal and toasted leaf flavor decreased. These sample were more adstringent than CLM K d3.

These results show that the fermentation progress results in changes in sensory profiles of kombuchas, due the action of microorganisms in SCOBY. Higher intensity for sweet odor and taste on d3 and d6 can be attributed to high soluble solids content, while higher intensity means for acid/sour on d9 are attributed to organic acids concentration reflected in higher TA and lower pH values (Andresen et al., 2022). Figures 9 and 10 show the intensity means for each attribute for odor, taste, flavor, mouthfeel and appearance for all kombuchas.

Considering the Brazilian consumer preferences for sweeter beverage taste, d9 samples had low acceptance and purchase intention means. Considering the existing products in the markets of many European countries and in North America, this type of kombucha would probably receive higher scores in these countries.

It is important to note that our kombuchas were naturally flavored, that is, with no addition of fruits, herbs or species to flavor them. In general, commercial kombuchas, receive additives to increase flavor intensity and variety (Andresen et al., 2022).

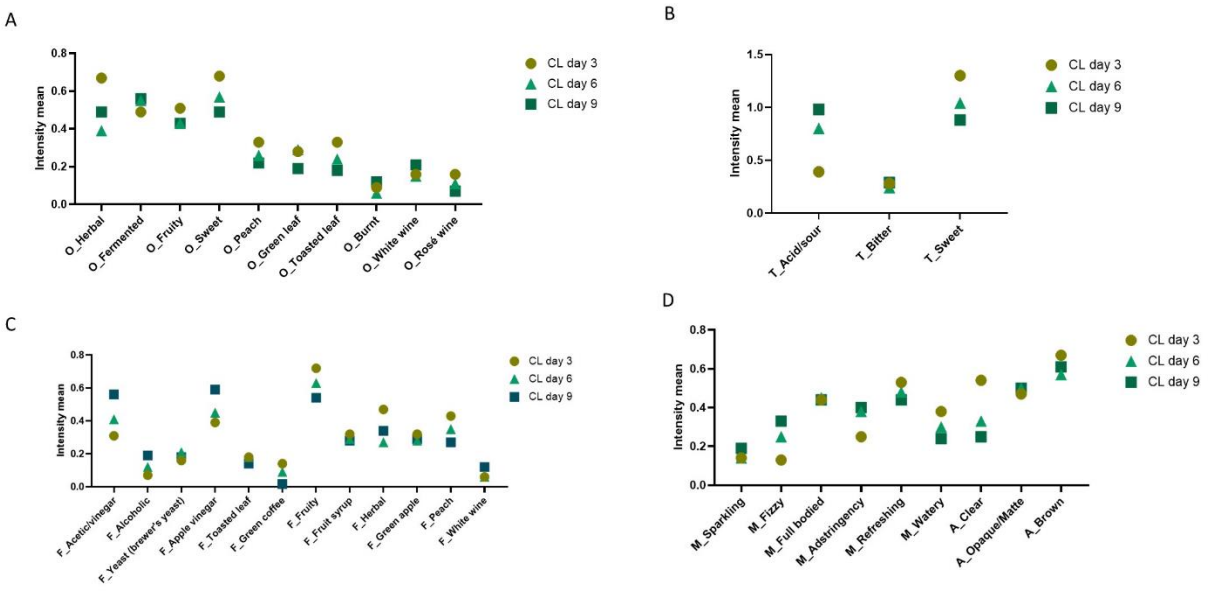


Figure 9. Intensity means for odor (A), taste (B), flavor (C), mouthfeel and appearance (D) for CL kombuchas with 3; 6 and 9 days of fermentation. Note: O: odor; T: taste; F: flavor; M: mouthfeel; A: appearance; CL: Coffee Leaves

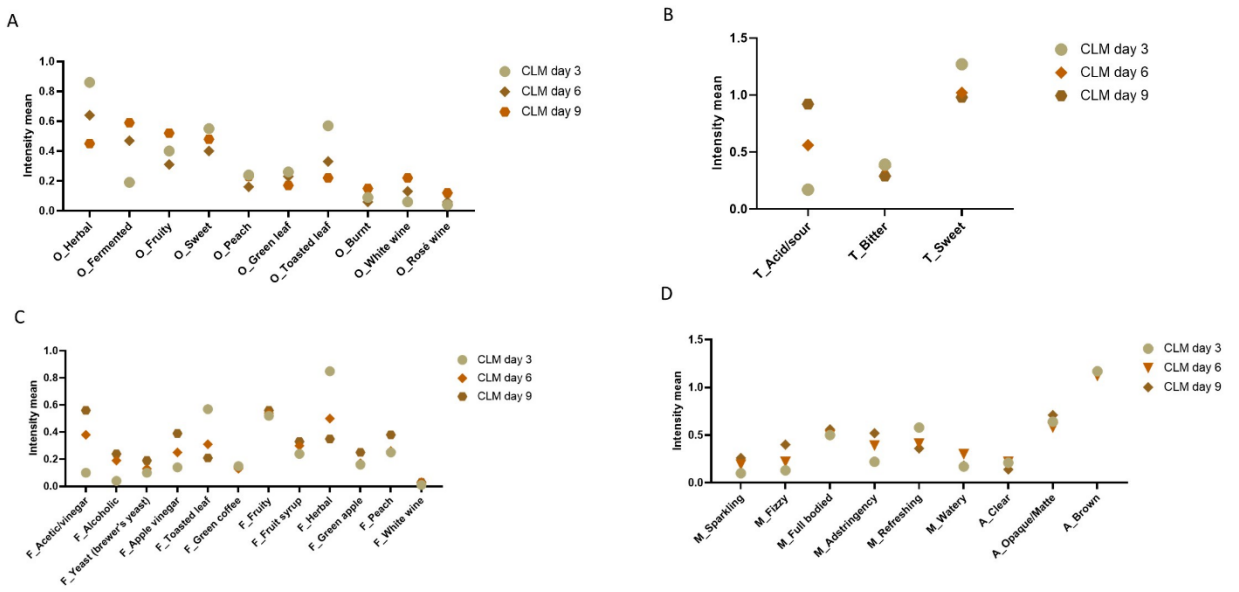


Figure 10. Intensity means for odor (A), taste (B), flavor (C), mouthfeel and appearance (D) for CL kombuchas with 3; 6 and 9 days of fermentation. Note: O: odor; T: taste; F: flavor; M: mouthfeel; A: appearance; CLM: Coffee leaves with yerba mate

Conclusions

In the present study, a total of 119 compounds were identified considering all kombucha samples, 61 in coffee leaves kombucha, 45 in coffee leaf-maté kombucha and 59 in black tea kombucha. Despite the different raw materials, both coffee leaves and coffee-mate leaf kombuchas presented similar volatile profiles. Some volatile compounds were identified only in coffee leaves and/or coffee-mate kombuchas, mainly 2,4-heptadienal, tetradecanal, tridecanal, undecanal, 1-dodecanol, (S)-2-heptanol, 3-octenol, ethyl (Z)-3-hexenoate, linalyl acetate, methyl dihydrojasmonate, 3,2-octadien-2-one, (-) carvone, isophorone, menthone, 3-δ-carene, myrcene e camphene. The production of these compounds seemed to be related to microbial composition and days of fermentation and raw material. Fermentation increased progressively the content of acids and esters, due to the symbiosis between acetic acid bacteria and yeasts, represented in the consortia used in this study by the genera *Komagateibacter* and *Pichia*, respectively.

Coffee-maté leaf kombuchas were more accepted than coffee leaf kombuchas by Rio de Janeiro assessors, especially the ones containing higher amount of sugar and herbal, toasted leaves and sweet attributes. The sensory characterization was supported by the volatile composition of the beverages. Coffee leaves showed to be a suitable raw material to produce

aromatic and natural cold beverages and a way to reduce the environmental pollution caused by the incorrect disposal of coffee leaves after the harvest of coffee tree, improving the coffee chain sustainability.

ESTUDO 4 : Atividade antioxidante intracelular contra espécies reativas de oxigênio e atividade antiinflamatória de infusões e kombuchas elaborados com cascas de frutos de café e chá preto em células induzidas HK-2 e RAW 264.7

Kombucha é uma bebida obtida através da fermentação da infusão açucarada de *Camellia sinensis* pela ação de uma Cultura Simbiótica de Bactérias e Leveduras que exercem ações biológicas benéficas. Matérias-primas alternativas têm sido usadas para criar novos produtos semelhantes ao kombucha. O café é a mais importante commodity alimentar mundial e gera grandes quantidades de subprodutos durante a colheita e pós-colheita. O principal subproduto do café são as cascas de café. Neste estudo, comparamos a captura de espécies reativas de oxigênio e efeitos anti-inflamatórios das infusões e kombuchas elaborados com cascas de café arábica (n=2) e chá preto (n=1) fermentados por 0, 3, 6 e 9 dias. Para tal, utilizamos modelos in vitro de indução de células HK-2 por indoxil sulfato (IS) e alta concentração de glicose (G), e macrófagos RAW 264.7 tratados com lipopolissacarídeos (LPS). Os teores de compostos fenólicos, cafeína e outros parâmetros físicoquímicos também foram avaliados. A fermentação causou a liberação de formas conjugadas de compostos fenólicos, seguida pela degradação. Apesar da concentração de 10-8% de sacarose nos kombuchas, todos os kombuchas de chá preto e cascas de café capturaram as espécies reativas de oxigênio de forma similar (41% de redução, em média) e reduziu (10%-55%) as concentrações intracelulares de ácido úrico nas células HK-2 tratadas com IS+G, revertendo o estresse oxidativo induzido. Todas as bebidas também reduziram (81%-90%) a formação de óxido nítrico em células RAW 264.7 tratadas com LPS, exibindo atividade antiinflamatória. Esses efeitos podem ser atribuídos aos polifenóis e à cafeína, cujas concentrações foram comparáveis em todas as bebidas. As cascas de café mostraram potencial atividade similar a C. sinensis para produzir bebidas saudáveis a apoiar a sustentabilidade na produção de café.

Palavras-chave: chá de cascas de café; subprodutos de café; fermentação; capacidade antioxidante; atividade antiinflamatória, sustentabilidade

1. Introduction

Kombucha tea is defined as a beverage obtained through the fermentation of sweetened black or green (*Camellia sinensis*) infusion by the action of a Symbiotic Culture of Bacteria and Yeast (SCOBY) (Martinez-Leal et al., 2018; Coelho et al., 2020; Diez-Ozaeta e Astiazaram, 2022). The beverage has gained substantial popularity, especially in the West, because of many claims regarding its therapeutic potential against maladies (Chakravorty et al., 2016).

It is well known that *C. sinensis* tea exerts many beneficial biological actions such as antioxidant, anti-inflammatory, and antimicrobial (Aboulwafa et al., 2019). *In vitro* and animal studies showed that such biological activities remain in kombucha (Greenwalt et al., 1998; Jayabalan et al., 2008; Bhattacharya et al., 2011; Srihari et al., 2013b; Ramani et al., 2019; Villareal-Soto et al., 2019; Kaewkod et al., 2019; Cardoso et al., 2020). Additional activities such as antidiabetic and antiproliferative effect in colon, breast, and lung cancer cell lines, were also reported (Villareal-Soto et al., 2019; Kaewkod et al., 2019; Cardoso et al., 2020) have also been reported, mostly as a consequence of the antioxidant and anti-inflammatory activities of the plant.

The consumer demand for healthy foods is continuously increasing. Food processors are reorganizing food product development to this dynamic demand to enhance the biological activities of the incorporated nutrients with proven health benefits and enhanced food properties for better acceptance (Gemechu et al., 2020). Recently, raw materials different from *C. sinensis* tea, for example, fruit or vegetable juices and cocktails, herbal or plant infusions, milk, and food industry byproducts, have been used to create new kombucha-like functional products (Emiljanowicz and Malinowska-Pánczyk, 2019). Coffee is the most important food commodity worldwide and ranks second, after petroleum, among all commodities (Esquível and Jímenez, 2012). In the last 20 years, world coffee production has consistently increased (Statista, 2022). According to the International Coffee Organization (ICO), approximately 10.5 million tons were produced worldwide in 2020 (ICO, 2021), generating large amounts of byproducts during harvest and post-harvest processing (Gemechu et al., 2020; ICO, 2021). With the prospect that coffee can reach a totally sustainable environment, it should be noted that that by-products still have not gained enough popularity to be reused.

The main coffee byproduct is the cascara or husk (Figure 2) generated during separation of coffee beans from the cherry pulp (IriondoDeHond et al., 2019). The coffee pulp alone

corresponds approximately to 28% of the coffee fruit on a dry weight basis, and the skin, approximately 12% (DePaula et al., 2022). These wastes are contamination sources and may lead to water and soil pollution and negative environmental impacts, given the high concentrations of caffeine, tannins, and lower molecular weight polyphenols (Murthy and Naidu, 2012; Janissen and Huynh et al., 2018). On the other hand, cascara has potential as a natural sustainable source of bioactive compounds with antioxidant and anti-inflammatory effects, such as polyphenols, caffeine, and melanoidins, in addition to micronutrients and soluble fibers (Prata et al., 2007; Rios et al., 2019; Iriondo-DeHond et al., 2019; Bondam et al., 2022, Bobkova et al., 2022). Indeed, several potentially beneficial effects of coffee cascara as a whole product, such as antibacterial, anti-inflammatory, adipogenic and lipolytic effects have been observed *in vitro* (Duangjai et al., 2016; Heeger et al., 2016; Magoni et al., 2018; IriondoDeHond et al., 2019; Khochapong et al., 2021).

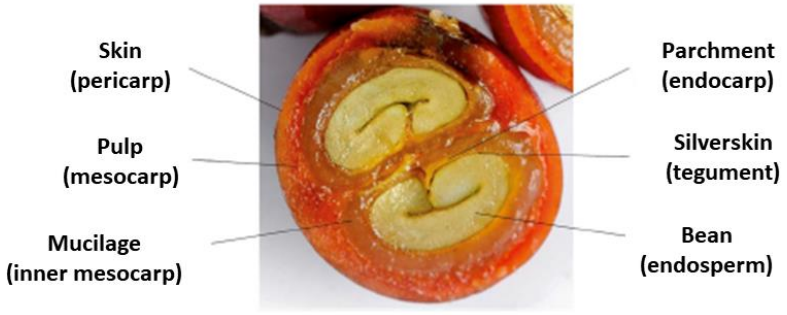


Figure 1. Transversal section of a ripe coffee cherry. Reference: Del Castillo et al., 2019a, with permission from Royal Society of Chemistry.

In Europe, coffee cascara was considered to be a novel food until recently when it received authorization from the European Food Safety Authority (EFSA) to be used in the European market (EFSA, 2021) as a new safe food ingredient with multifunctional properties, being used in bread production as flour (Rios et al., 2020), added to food matrices like yogurts (Iriondo De-Hond et al., 2020), or for infusion preparation (Heeger et al., 2016; Iriondo-DeHond et al., 2019; DePaula et al., 2022). Nevertheless, studies evaluating the bioactivity of the cascara and derivatives still need to be available.

This study aimed to evaluate the ROS scavenging and anti-inflammatory effects of infusions and kombuchas prepared with coffee cascara and black tea. We used human induced proximal tubular (HK-2) and murine macrophage (RAW 264.7) cell models for this.

2. Materials and methods

The experimental design of the study is shown in Figure 1.

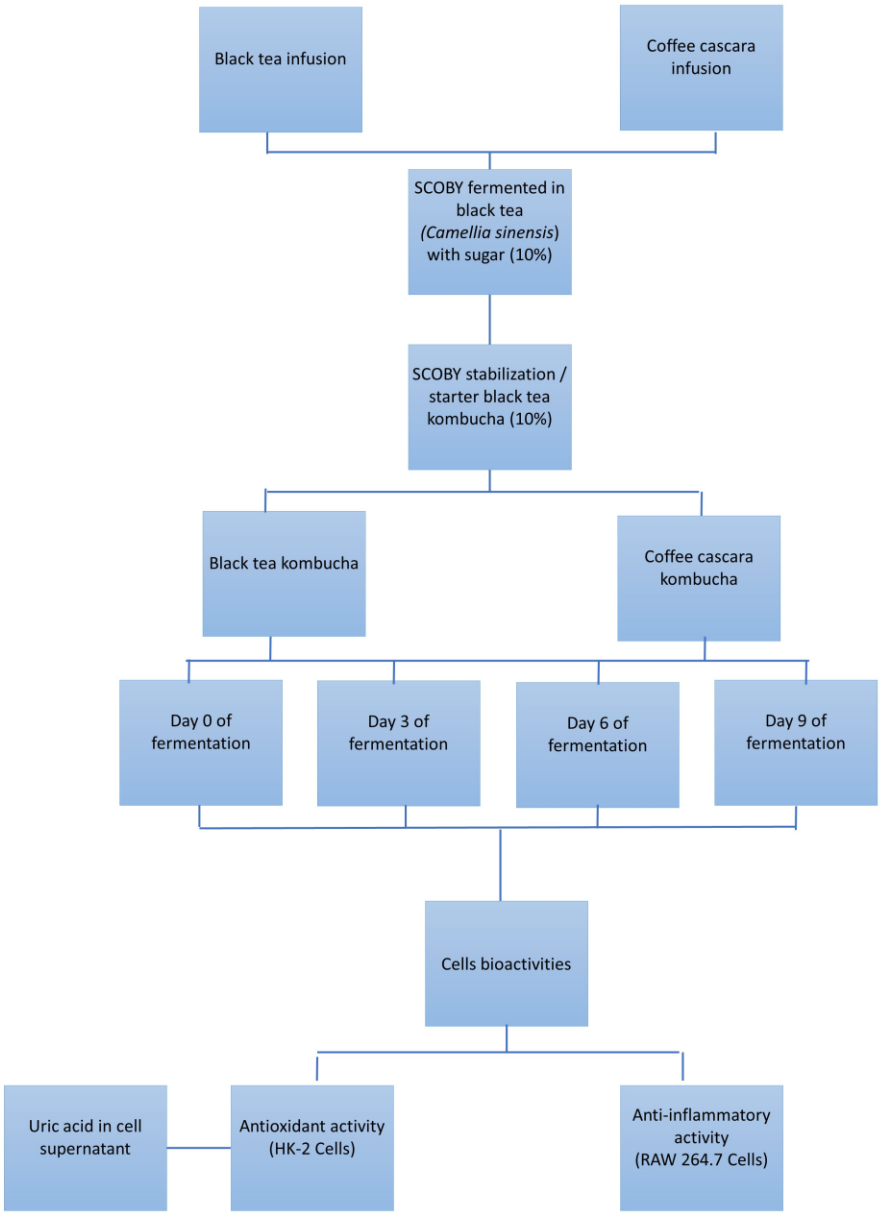


Figure 2. Experimental design of study 4

2.1. Reagents

Indoxyl sulfate (IS), uric acid (UA), glucose, tert-butyl hydroperoxide (tBOOH), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazole-y)-2,5-diphenyltetrazolium bromide

(MTT) and 21,71-dichlorodihydro-fluorescein diacetate (DCFH-DA), sodium nitrite, and lipopolysaccharide from *E. coli* O55:B5 (LPS) were purchased from Sigma Chemical (Sigma-Aldrich, St Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Lonza (Basel, Switzerland). L-glutamine, antibiotics (penicillin and streptomycin), and trypsin were obtained from Gibco (Invitrogen Co., Grand Island, NY, USA) and fetal bovine serum (FBS) was obtained from Hyclone (GE Healthcare, Chicago, IL, USA). Catechins, including (-)-catechin (C, >98%), (-)-epicatechin (EC, >98%), (-)-gallocatechin (GC, >98%), (-)-epigallocatechin (EGC), (-)-catechin gallate (CG, >98%), (-)-gallocatechin gallate (GCG, >98%), (-)-epicatechin gallate (ECG, >98%) and (-)-epigallocatechin gallate (EGCG, >98%), gallic acid (GA, ≥ 99%), 5-caffeoylquinic acid (5-CQA, ≥ 95%) rutin (hydrate, ≥ 94%), quercetin (hydrate, ≥ 95%), kaempferol (≥ 97%), caffeic acid (≥ 98%), ferulic acid (≥ 99%), p-coumaric acid (≥ 98%), sinapic acid (≥ 98%), benzoic acid (≥ 99.5%), 3,4-dihydroxybenzoic acid (≥ 97%), hippuric acid (≥ 98%), 3,4-dihydroxyphenylacetic acid (≥ 98%), 4-hydroxyphenylacetic acid (≥ 98%), vanillic acid (≥ 97%), dihydrocaffeic acid (≥ 98%) and caffeine (≥ 99%) for HPLC references were provided by Sigma Chemical Co. For dicaffeoylquinic acids (diCQA), a mixture of 3,4- diCQA; 3,5- diCQA; and 4,5-diCQA from Carl Roth (Karlsruhe, Germany) was used. Feruloylquinic acids (FQA), were synthesized from 3-feruloylquinide and 4- feruloylquinide (FQL) by hydrolysis in 50% aqueous tetrahydrofuran (Huynh-Ba, 1995). Sucrose was provided by PROQUIMIOS Produtos Científicos, Rio de Janeiro, Brazil; glucose, galactose and fructose were provided by VETEC Química Fina, Rio de Janeiro, Brazil.

2.2. Raw materials

A leading commercial (Chinese) black tea brand was purchased in a Brazilian food market; organic arabica coffee (*Coffea arabica*) cascara samples were acquired directly from producers (in Espírito Santo, Brazil - dry processed, and in Nicaragua - wet processed).

2.3. Infusions preparation, kombucha consortium, and fermentation

Infusions- Black tea and coffee cascara infusions were prepared at 3% (weight/volume-w/v), pouring water at 95°C over the raw material, letting it steep for 10 min, and filtering the mixture using a regular paper filter (Mervilab S.A., Madrid, Spain) for bulk tea.

- *Kombucha consortium and fermentation* - The Kombucha consortium was part of the collection of the Microbiology Institute of the Federal University of Rio de Janeiro in Brazil. Previously cultivated in green tea, the consortium was separately fermented three times in black tea and in coffee cascara tea infusion prior to experimental use in order to stabilize the microbial consortium in these matrixes (Villarreal-Soto et al., 2020). All kombucha beverages were prepared according to the protocol described by Nummer (2013).
- *Black tea kombucha* - Black tea kombucha was prepared by mixing 10% (volume/volume-v/v) of black tea starter, 80% black tea infusion (weight/volume-w/v), 10% (w/v) sugar, and 2.5% (v/v) of a symbiotic culture of bacteria and yeast (SCOBY) and letting the mixture ferment for 14 days at 23 °C (Sanyo™ MIR-154PE, Sanyo Electric Co. Ltd, Japan). Samples were collected before fermentation (day 0) and after 3, 6 and 9 days of fermentation.
- *Coffee cascara kombucha*– Coffee cascara kombuchas were prepared using 80% (v/v) of the coffee cascara infusion, 10% (v/v) of the black tea kombucha, 10% (w/v) sugar, and 2.5% (w/v) of SCOBY. The mixture was let ferment at 23 °C (Sanyo™ MIR-154PE, Sanyo Electric Co. Ltd, Japan). Samples were collected before fermentation (day 0) and after 3, 6 and 9 days of fermentation.

2.4. DNA extraction, amplicon sequencing data analysis and library preparation

Please see study 1.

2.5. Analysis of titratable acidity, pH, soluble solids, and sugars

Please see study 1.

2.6 Analysis of bioactive compounds

Please see study 1.

2.7. Cell culture and treatments

HK-2 human kidney proximal cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 1.0g of glucose and supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin/streptomycin. RAW 264.7 macrophage cells from murine were cultured in DMEM with 4.5g of glucose and supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. The cells were incubated in standard conditions (37 °C, 5% CO_2, in a humidified incubator (BINDER CB series 2010, Tuttlingen, Germany).

All beverages were sterilized with a 0.22 µm pore membrane before the experiments. Then, 3mg/mL of each beverage were added to DMEM culture media without FBS. IS at 2.5mM and glucose at 25mM were diluted in phosphate buffer saline (PBS) and sterilized with a membrane with 0.22µm pore before the experiments.

2.8. Cell viability assays

The effect of the test beverages on cell viability was measured using the MTT assay (Bakondi et al., 2003). HK-2 and Raw 264.4 cells were cultured at a density of 1.0×10^4 and 8.0 x 10^4 cells per well of a 96-well plate, respectively. After 24 h culture, cells were treated with the beverages diluted in DMEM culture medium without FBS (3 mg/ml IS at 2.5mM and glucose at 25mM were diluted in PBS for 3 h. DMSO (50%) was used as death control. Subsequently, cells were incubated in MTT solution (0.5 mg/ml) for 1 h at 37 °C. The supernatant was removed and 100 µL of DMSO was added, and the optical density at 570 nm was measured using a microplate reader (BioTek Synergy HT Multi-Mode Microplate Reader, Winooski, VT, USA). Experiments were carried out three times in triplicate.

2.9. Intracellular ROS scavenging assay in HK-2 cells diabetic nephropathy cell model.

HK-2 cells were cultured at a density of 1.0 x 10^4 cells per well of a 96-well plate. After 24 h, cells were treated with non-fermented and fermented black tea and coffee fruit cascara beverages (3 mg/ml), IS at 2.5mM and glucose at 25mM diluted in DMEM culture medium (3 mg/mL) for 3 h. The analysis was performed by measuring the fluorescence intensity of the DCFH-DA probe, which was proportional to the amount of ROS formed (Gomes, Fernandes, & Lima, 2005) after induction of diabetic nephropathy by IS and high glucose solution. A 10mM solution of DCFH-DA was prepared (5 mg in 1 mL DMSO), and a 50 µL aliquot was separated. Then, 800 µL of DMSO was added to the 50 µL solution. After 24 h of extract incubation, cells

were pre-loaded with 2.5 µL/well of this last solution for 30 min at 37 °C. After incubation, DCFH becomes dichlorofluoresce in (DCF) due to intracellular oxidants and will emit fluorescence. Next, the culture medium was removed; cells were washed with PBS; and the fermented beverages extracts (1 mg/mL) were added for 1 h. tBOOH 1mM was used as a positive control. Then, fluorescence was measured at 485 nm/528 nm (BioTek Synergy HT Multi-Mode Microplate Reader). Analyses were performed in triplicate and for three different cell passages.

2.10. Quantification of uric acid in cells supernatant

To determine cell functionality, the content of uric acid was estimated in HK-2 cells supernatant after treatments with IS and beverages using a commercial kit (Spinreact, Girona, Spain). In a microplate, 5 µL of cell supernatant was mixed with 200uL of reagent. The mixture was incubated at 15-25 °C for 10 min. Finally, absorbance was measured at 520 nm using an Epoch 2 Microplate spectrophotometer (BioTek Winooski, VT, USA). A uric acid standard was used for quantification and reaction control (357 µmol/L). Analyses were performed in triplicate, and the results were expressed in µmol/L.

2.11. Anti-inflammatory activity assay through nitric oxide production determination in RAW 264.7 cell line

The anti-inflammatory properties of black tea and coffee cascara infusions and kombuchas were determined by quantifying the nitrogen oxide (NO) production in macrophages (RAW264.7) as described by Gutierrez-Barrutia et al. (2022). Briefly, RAW264.7 cells were seeded on a 96-well plate (8 x 10^4 cell/well) and cultured in complete medium (DMEM with 4.5 g/L of glucose, 10% v/v of FBS, 1% v/v of L-glutamine and 1% v/v of antibiotics) for 24 h (37 °C, 5% CO^2). Following, cells were treated with 150 µL of medium without FBS containing 1µg/mL lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 and non-fermented and fermented black tea and coffee fruit cascara beverages (3 mg/ml). Then, cells were incubated for 24 h (37 °C, 5% CO2). Negative and positive controls consisted of medium without FBS and 1µg/mL of LPS in medium without FBS, respectively. After the incubation period, 100 µL of supernatants from the wells were removed and combined with 100 µL of Griess reagent (1% (w/v) sulfanilamide and 0.1% w/v N-1-(naphthyl)-ethylenediamine dihydrochloride in 2.5% v/v

H_3PO_4). The mixtures were incubated at room temperature in the dark for 15 min, and absorbance was measured at 550 nm in a BioTek Epoch 2 Microplate spectrophotometer (Winooski, VT, USA). A NO-in-DMEM-without FBS calibration curve was used for quantification (0–10 µg/mL). Analyses were performed in triplicate and for three different cell passages.

2.12. Statistics

All data are reported as mean ± standard deviation. Analysis of variance (ANOVA), followed by Tukey's test, were performed using GraphPad Prism (Version 8.4.2, Informer Technologies, Los Angeles, CA, USA) to determine significant differences between samples at $p \leq 0.05$.

3. Results and discussion

3.1 Beverages characterization

3.1.1 pH, total acidity, soluble solids, and sugars

The results were presented and discussed in study 1.

3.1.2. Microbial taxonomy

The results were presented and discussed in study 1.

3.1.3. Bioactive compounds

3.1.3.1 Black tea

The results were presented and discussed in study 1.

3.1.3.2 Coffee cascara

The results were presented and discussed in study 1.

3.2. Effect of Black tea and coffee cascara beverages on ROS scavenging activity.

HK-2 cells were treated with indoxyl sulfate (IS) and high glucose (G) to increase oxidative stress (ROS production) and simulate the condition of diabetic nephropathy. IS is known as a gut-derived uremic toxin, which induces free radical production in renal tubular and glomerular mesangial cells (Niwa, 2010, Chen et al, 2020). In normal kidney proximal tubular epithelial cells, IS has been shown to reduce proliferation leading to cellular senescence. It

adversely affects redox control and mitochondrial metabolism and promotes pro-fibrotic/inflammatory gene expression (Ellis et al., 2016), leading the cells to apoptosis, hypertrophy, mitochondrial dysfunction, and pro-fibrotic and inflammatory molecules (Ellis et al., 2018). tBOOH 1mM was used as an oxidant control, while ascorbic acid 3µg/mL was used as an antioxidant control.

The induced (nephropatic) cells were submitted to the test infusions and kombuchas. The results are presented in Figure 6. As expected, IS + G treatment induced ROS production compared to DMEM (the negative control). tBOOH 1mM also produced ROS, similar to the treatment IS + G.

Treating HK-2 cells with plain G caused no significant difference in ROS production. This was not expected because chronic hyperglycemia associated with Diabetes *mellitus* should increase oxidative stress related to excess of ROS generation and impaired antioxidant response (Singh et al., 2011). Considering that Wang et al. (2019) were able to increase the level of intracellular ROS when using higher glucose concentrations (30mM), it is possible that higher concentrations than the one used in the present study (25mM) could result in the expected intracellular ROS production.

Treating cells with IS+ G + black tea infusions or kombuchas reversed ROS production (32-42% reduction), producing a lower response than DMEM control but similar to ascorbic acid. Coffee cascara tea infusions and kombuchas were also able to reverse the effect of IS+G, (43% reduction in ROS production, on average) with no difference compared to DMEM. However, in this treatment ROS production was still statistically higher than with ascorbic acid, although very similar to black tea (Figure 3).

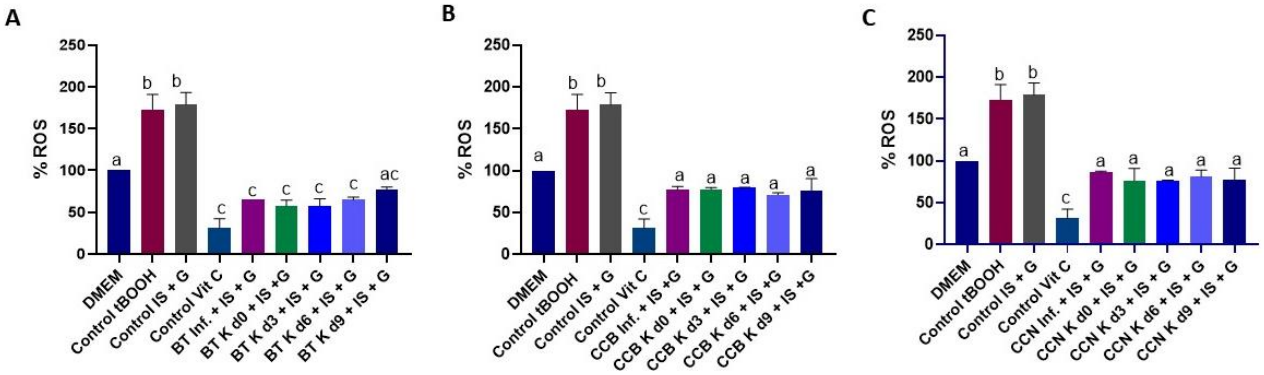


Figure 3. Effect of HK-2 cells treatment with BT (A), CCB (B) or CCN (C), associated with IS and G, on ROS formation, determined by fluorescence. tBOOH: tert-Butyl hydroperoxide (oxidant control); Vit C: ascorbic acid (antioxidant control); IS + G: indoxyl sulfate with high glucose (25mM) solution; d0, d3, d6 and d9: days 0, 3, 6 and 9 of fermentation, respectively. BT: black tea; Inf: plain infusion; K: kombucha; CCB: Coffee cascara from Brazil; CCN: Coffee cascara from Nicaragua. Different letters over the bars indicate statistical difference among treatments by ANOVA followed by Tukey's test (p<0.05).

Bhattacharya et al. (2013) have previously demonstrated the protective effect of black tea kombucha against oxidative stress-mediated damage in different tissues of diabetic rats. To date, this is the first study evaluating the effect of black tea and cascara tea infusions and kombuchas in HK-2 cells model of oxidative stress.

The suppression of ROS production in HK-2 cells by green *C. sinensis* tea has been previously reported by Sun et al. (2018). Catechins are constituents of green and black *C. sinensis* tea. These compounds can inhibit prooxidant enzymes, e.g., NADPH (nicotinamide adenine dinucleotide phosphate)-oxidase, or modulate interactions of ligands with receptors, e.g., tumor necrosis factor alpha (TNF-α). They can also suppress many oxidative stress-related pathways responsible for the inflammation processes. Catechins modulate the activities of redox-sensitive transcription factors such as nuclear factor kappa beta (NF-κB) and activator protein-1 (AP-1), which are very important in the response to pathogenesis-related oxidative stress (Bernatoiene and Kopustinskiene, 2018). In the study by Sun et al. (2018), green tea polysaccharides reduced the production of intracellular ROS and alleviated oxidative damage in cells, compared to oxalate as damage control.

Several cell culture experiments have shown the antioxidative properties of chlorogenic acids at both cellular and molecular levels (Farah and Lima, 2019). As a source of chlorogenic acid, coffee cascara prevented intracellular ROS formation in HepG2 cells (Iriondo-DeHond et al., 2019), in RAW 264.7 cells (Rebollo-Hernanz et al., 2019a; 2019b), and in 3T3-L1 adipocytes (Rebollo-Hernanz et al., 2019b). We can attribute the reduction in intracellular ROS formation mainly to the high content of chlorogenic acids in coffee cascara beverages in addition to caffeine and other minor antioxidant compounds. In black tea this reduction can be attributed to the high content of catechins, associated with other polyphenols, including chlorogenic acids. It is well known that due to their structures, catechins, chlorogenic acids and their primary metabolites caffeic, ferulic and *p*-coumaric acids exert antioxidative activity (Rice-Evans et al,

1996), and have the ability to scavenge ROS *in vitro* (Bernatoiene and Kopustinskiene, 2018; Farah and Lima, 2019).

A high-fat-high-sugar diet composed of sucrose or fructose increased ROS levels in murine kidneys (Rosas-Villegas et al., 2017). In another study, after increasing ROS levels with a high-fat-high-sucrose diet in mice, combining the same diet with raspberry flour extract supplementation reduced hepatic ROS production. This effect was attributed to ellagic acid (a phenolic compound) in raspberry flour extract (Kang et al., 2016). Despite the remaining amount of sucrose and monosaccharides in the kombucha beverages, when HK-2 cells were treated with infusions (with did not contain added sucrose) and kombucha beverages, ROS production was similar, probably because of the high concentration of phenolic compounds and other substances that stimulate the antioxidative responses in cells, such as caffeine (Figures 4 and 5) (Bernatoniene and Kopustinskiene, 2018; Del Castillo et al., 2019). Also, Jafari et al. (2020) observed that in the presence of enzymes secreted by the kombucha microbial consortium, the antioxidant activity was enhanced by the decomposition of polyphenolic compounds, possibly due to the increase in phenolic acids along fermentation.

Microorganisms in the symbiotic culture of bacteria and yeasts for kombucha production are more active because they act in synergism. These microorganisms are more resistant to different intrinsic (e.g., nutrient content, moisture content, pH), extrinsic (e.g., temperature, relative humidity, gases), and biological conditions (e.g., presence, dynamics, and type of interaction of microorganisms) of the ecosystems. They can metabolize nutrients from different unconventional substrates, allowing them to be used in efficient biotechnological processes to obtain bioactive compounds with functional properties (Pihurov et al., 2021).

The effect of all treatments on cell viability is shown in Figure 4. DMSO (50%) (death positive control) caused the cells to die, while DMEM (negative death control) did not affect the cells viability, as expected. The amount of G used in this experiment did not affect the cell viability, while the administration of IS + G decreased cell viability by 30 %. The viability of cells treated with black tea beverages was 27% higher, on average, than DMEM, and 80% higher than IS+G, reversing IS effect. In cascara beverages, viability was 21% lower than DMEM, on average. The viability when cells were treated with cascara + IS + G differed for both samples. While cells treated with CCB had viability similar to DMEM and IS+G, CCN cells were not different from IS+G, which was lower than DMEM. The present results showed that after

administration of black tea and coffee cascara kombuchas, the cell viability mostly neither decreased nor increased.

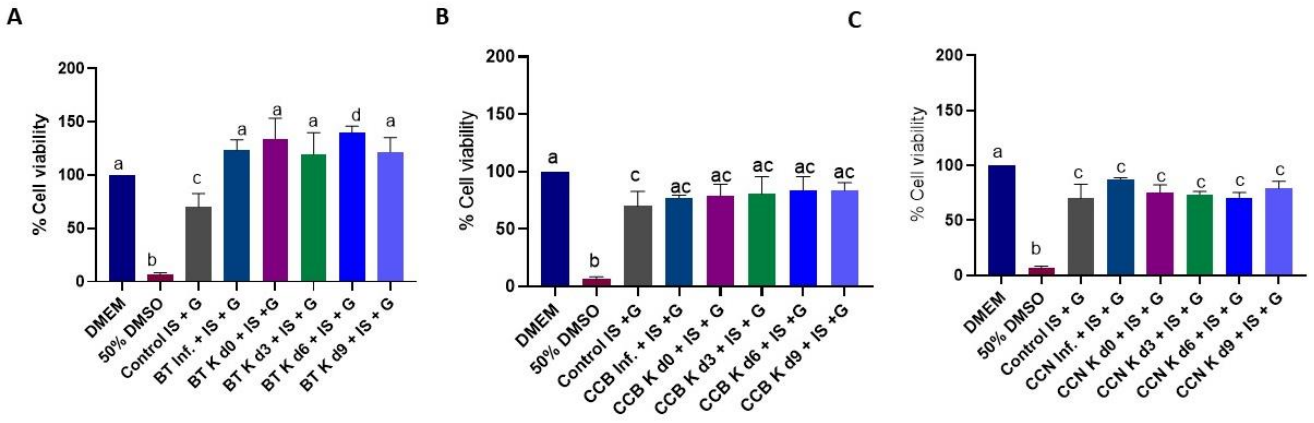


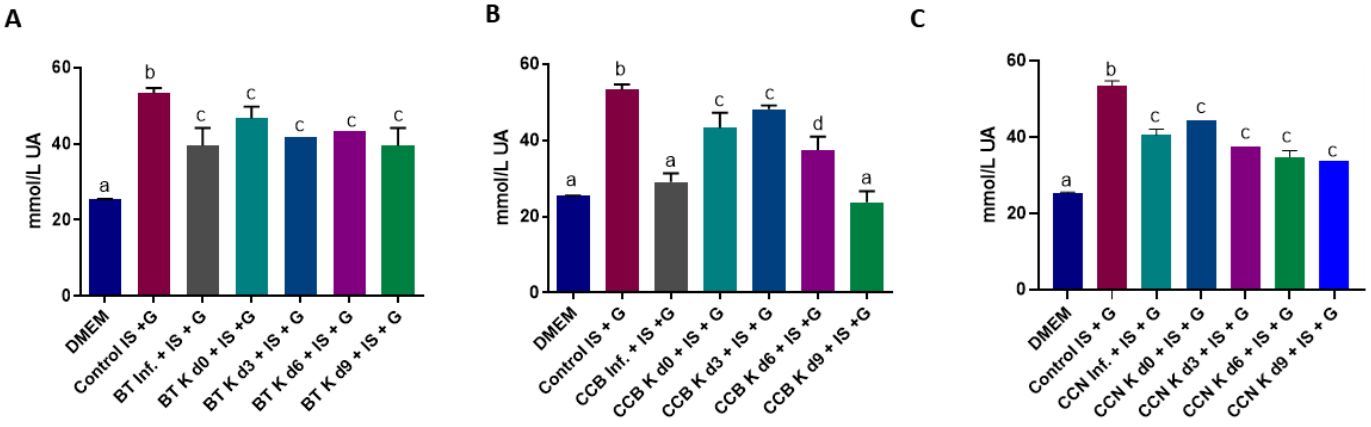
Figure 4. Effect of treatment with BT (A), CCB (B) and CCN (C) beverages, associated with IS and high G, on HK-2 cells viability, determined by MTT assay. Results are average of triplicate of experiments performed three times. Different letters over the bars indicate statistical difference among treatments by ANOVA followed by Tukey's test ($p<0.05$). DMEM: Dulbecco's Modified Eagle Media (life control); DMSO: Dimethyl Sulphoxide (death control). IS + G: indoxyl sulfate with high glucose solution; d0, d3, d6 and d9: days 0, 3, 6 and 9 of fermentation, respectively; BT: black tea; Inf: plain infusion (no starter and sugar); K: kombucha; CCB: coffee cascara from Brazil; CCN: coffee cascara from Nicaragua.

3.3. Effect of black tea and coffee cascara beverages on uric acid concentrations.

Uric acid (UA) production and metabolism are complex processes involving various factors that regulate hepatic production, and renal and gut excretion of this compound. UA is the end product of an exogenous pool of purines and endogenous purine metabolism. The exogenous pool varies significantly with diet; animal proteins contribute significantly to this purine pool. The endogenous production of uric acid is mainly from the liver, intestines, and other tissues like muscles, kidneys, and the vascular endothelium (Chaudhary et al., 2013). In the present experiment, we used UA as a marker for cell damage. UA is also a urine biomarker for oxidative stress and plays an important role in diabetic nephropathy development. In patients diagnosed with type 2 diabetes, increased UA levels may be an important predictor of nephropathy in

diabetic patients (Kocak et al., 2019, Khan et al., 2020). In the same way, reducing UA levels in diabetic patients can also reduce the progression of chronic kidney disease (Khan et al., 2020).

When cells were treated with IS, an increase in the concentration of supernatant UA was observed, as expected (Figure 5), given that IS and other uremic toxins concentrations correlate



inversely with renal function (Mahmoodpoor et al., 2017).

Figure 5. Effect of HK-2 cells treatment with BT (A), CCB (B) or CCN (C) beverages, associated with IS and G, on uric acid concentration. Results are average of triplicate of experiments performed three times Different letters over the bars indicate significant difference among treatments by ANOVA followed by Tukey's test ($p < 0.05$).DMEM: Dulbecco's Modified Eagle Media; IS: indoxyl sulfate; G: culture media with high glucose solution; d0, d3, d6 and d9: days 0, 3, 6 and 9 of fermentation, respectively; BT: black tea; Inf: plain infusion; K: kombucha; CCB: coffee cascara from Brazil; CCN: coffee cascara from Nicaragua.

DMEM and G treatments did not affect UA concentrations in the supernatant of the cells. Black tea kombuchas reduced 13-26% the concentration of UA in the supernatants from day 0 to 9, compared to the cells treated with IS, while cascara beverages reduced 10-55%. UA concentrations in the supernatant are higher than those found by Hou et al. (2019), who used cultured HK-2 cells to establish a stable model of hyperuricemia for long-term studies.

In an *in vitro* test using xanthine oxidase to increase UA and ROS production, Jayabalan et al. (2008) observed that green and black tea infusions and kombuchas were able to scavenge superoxide radicals, showing significant antioxidant activity. Zhu et al. (2021) demonstrated that epicatechin gallate can inhibit xanthine oxidase (and therefore UA and ROS production) in a

dose-dependent manner. Epigallocatechin gallate exerted antihyperuricemic activity and attenuated kidney damage in hyperuricemic rats (Zhu et al., 2018). The combined effect of catechins in black tea infusion and kombuchas can explain the reduction in UA levels compared with cells treated with IS +G.

Polyphenols such as epigallocatechin gallate, quercetin, gallic acid, caffeic acid, chlorogenic acid, sinapic acid, among others, could exert benefits in hyperuricemia, inhibiting the UA enzymes producers (xanthine oxidase and adenosine deaminase) and increasing the excretion of UA. Consequently, they prevented UA reabsorption in the kidneys, and increased the excretion of UA in the intestines (Zhu et al., 2021). Using a murine hyperuricemic model, Zhou et al. (2021) observed a decrease in serum UA concentration after administration of 30 or 60mg/kg of 5-caffeoylquinic acid, the main chlorogenic acid compound in coffee, suggesting that supplementation with this compound could effectively prevent hyperuricemia and mitigate kidney impairment. The high content of chlorogenic acids in coffee cascara kombuchas can explain the decrease in uric acid concentration in cell supernatant. Additionally, caffeine intake through food has been negatively associated with uric acid concentration (Liu et al., 2022).

3.4. Effect of black tea and coffee cascara beverages on anti-inflammatory activity in RAW 264.7 cell line

The effect of the test beverages on NO production in Raw 264.7 cells induced by LPS is shown in Figure 6. Inflammation is an overactive immune response to harmful cell stimuli. In the inflammatory response, activated macrophages produce large amounts of inflammatory factors such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and nitric oxide (NO) to induce tissue injury at the inflammatory site (Zhu et al., 2021). Although macrophages are essential for the effective control and clearance of infections, removal of derbies and dead cells, promotions of tissue repair, and wound healing, they also contribute to tissue damage and pathology during infections and inflammatory diseases (Moghaddam et al., 2018), resulting in a causal association of macrophages with disease states, such as fibrosis, obesity, and cancer (Wynn et al., 2013). Also, macrophages are key inflammatory cells mediating kidney inflammation in experimental and human diabetes (Lim and Tesch, 2012).

Nitric oxide (NO), the smallest cell signaling molecule, participates in diverse physiological functions, such as vasodilation, neural transmission, and immune responses (Wu et

al., 2022). LPS is a component of the cell wall of Gram-negative bacteria. It is a potent activator of the inflammatory response and is a crucial pathogen-associated molecular patterns (PAMPs) in Gram-negative bacteria. Consequently, small amounts of LPS in the blood due to a bacterial infection are sufficient to elicit an inflammatory response through the interaction with toll-like receptors (Mohammad and Thiermermann, 2021).

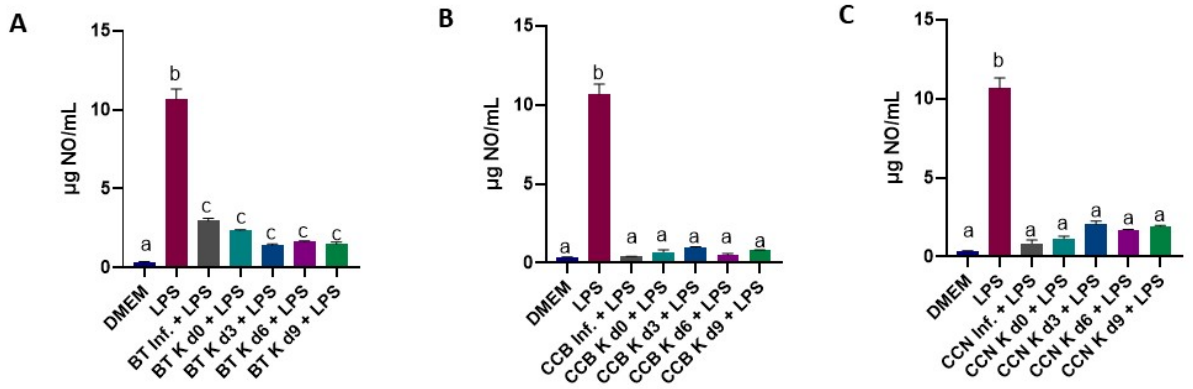


Figure 6. Effect of treatment with BT (A), CCB (B) or CCN (C) beverages, associated with LPS, on NO production in Raw 264.7 cells, determined by Griess Reagent. Results are mean of triplicate of experiments performed three times. DMEM: Dubecco's Modified Eagle Media; LPS: lipopolysaccharide (nitric oxide production control); d0, d3, d6 and d9: days 0, 3, 6 and 9 of fermentation, respectively; BT: black tea; Inf: plain infusion; K: kombucha CCB: coffee cascara from Brazil; CCN: coffee cascara from Nicaragua. Different letters over the bars indicate statistical difference among treatments by ANOVA followed by Tukey's test ($p \leq 0.05$).

DMEM (negative control) did not induce NO production in macrophages, while LPS (positive control) showed the highest NO production. All infusions and kombucha beverages similarly reduced the NO formation in cells treated with LPS (81% lower NO production than with LPS in black tea beverages and 90% lower in cascara beverages, on average), suggesting a potential anti-inflammatory effect. While NO production in cells treated with coffee cascara infusions and kombuchas were similar to DMEM control, NO production in cells treated with black tea infusion and kombuchas was higher than in DMEM cells ($p < 0.0001$). Comparing all treatments, no difference was found between infusions and kombuchas made with the same food material.

All cells were viable after treatment using LPS and LPS + beverages for stimulation, while the cells treated with DMSO (50%) (death control) were not, as expected (Hwang et al., 2016 (Figure 6).

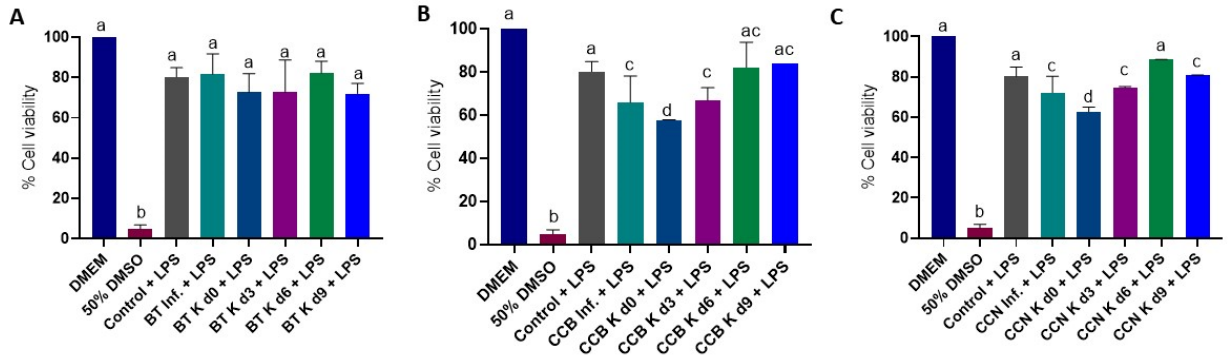


Figure 7. Effect of treatment with BT (A), CCB (B) or CCN (C) beverages, associated with LPS, on Raw 264.7 cells viability, determined by MTT assay. Results are mean of triplicate of experiments performed three times. DMEM: Dulbecco's Modified Eagle Media (life control); DMSO: Dimethyl Sulphoxide (death control). d0, d3, d6 and d9: days 0, 3, 6 and 9 of fermentation, respectively. BT: black tea; Inf: infusion; K: kombucha CCB: Coffee cascara from Brazil; CCN: Coffee cascara from Nicaragua. Different letters over the bars indicate statistical difference among treatments by ANOVA followed by Tukey's test ($p < 0.05$).

Villarreal-Soto et al. (2019) evaluated the anti-inflammatory activity of nonfermented tea and black tea kombucha against the enzyme 5-LOX and suggested an improvement of kombucha in anti-inflammatory activity. Studies with unfermented coffee cascara and inflammation in macrophage cells have previously been performed. Coffee cascara extracts could significantly reduce NO production and PGE2 in RAW 264.7 cells (Rebollo-Hernanz et al., 2019b). Also, coffee cascara extract significantly inhibited LPS-induced macrophage activation in RAW 264.7 cells (Rebollo-Hernanz et al., 2019a). The effect was attributed to the reduced expression of the inflammatory enzymes iNOS and COX-2 induced by LPS. Traditional black tea kombucha exhibited a potential effect against development of systemic inflammatory responses associated with sepsis in mice (Wang et al., 2021). Vázques-Cabral et al. (2017) attributed the anti-inflammatory activity of black tea kombucha to its polyphenols content. In fact, several studies related polyphenols from different sources with anti-inflammatory effect. Quercetin and quercetrin were also able to inhibit LPS-induced inflammation in RAW 264.7 cells (Tang et al., 2019). 3-OH flavone, kaempferol, and quercetin were also effective in the prevention of NO

production induced by LPS in RAW 264.7 cells, even when combined with a heme oxygenase inducer (Lin et al. 2003). The bioactive compounds contained in cascara infusion and green and black tea kombuchas showed anti-inflammatory effects in different studies. Most evidence to date shows that 5-caffeoylquinic acid, the main chlorogenic acid in the coffee cascara, and possibly other chlorogenic acid compounds, exert anti-inflammatory activity by down-regulating pro-inflammatory cytokines, through modulation of key transcription factors, such as tumor necrosis factor-alpha (TNF-α), and interleukins such as IL-8. In a study using RAW 264.7 macrophages, 5-caffeoylquinic acid decreased lipopolysaccharide (LPS)-induced upregulation of cyclooxygenase (COX-2) at protein and mRNA levels, suggesting that 5-caffeoylquinic acid could exert anti-inflammatory effects through inhibiting prostaglandin E2 (PGE2) production (Farah and Lima, 2019). Jang et al. (2021) found that pretreatment with 4,5-dicaffeoylquinic acid, effectively inhibited LPS-induced expression of NO and other inflammatory pathways with no cytotoxic activity. Caffeine also showed anti-inflammatory activity, decreasing the level of NO production in LPS induced RAW264.7 cells in a dose-dependent manner (Hwang et al., 2016). These results may explain the higher anti-inflammatory activity in coffee cascara beverages than to black tea beverages since coffee cascara has more chlorogenic acids and caffeine than black tea.

Studies evaluating the effect of oolong *C. sinensis* infusion and kombucha, as well as of its phenolic compound epigallocatechin gallate on NO production using this model have also reported similar effects to the present study (Novilla et al., 2017). In the study of Wang et al. (2021), kombucha improved the survival status in LPS-treated mice, effectively inhibiting the release of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α), restoring the levels of T cells and macrophages, and downregulating the NF-κB signaling pathway in mice with LPS-induced sepsis. The anti-inflammatory activity of hydro-alcoholic and aqueous coffee pulp extracts was studied by measurement of IL-8, one of the most important chemokines involved in gastric inflammation release in human gastric epithelial cells. Coffee pulp extracts similarly inhibited IL-8 release (Magoni et al., 2018). As aforementioned, no similar study using coffee fermented or unfermented cascara was found, but other polyphenols-rich plant extracts like cranberry, black raspberry, red raspberry, strawberry, blueberry, blackberry, and cocoa, citrus and beer production byproducts have exerted inhibition of NO production in LPS-stimulated RAW 264.7 cells (Xu et

al., 2017, Rebollo-Hernanz et al., 2019b, Gu et al., 2020, Fernandéz-Fernández et al., 2021, Gutierrez-Barrutia et al., 2022).

Concluding remarks

In summary, black tea and coffee cascara infusions and kombuchas suppressed the oxidative stress in HK-2 cells treated with IS and high glucose concentration and reduced uric acid concentration in the cells supernatant similarly. Anti-inflammatory activity was also observed in LPS-treated RAW 264.7 cells, after incubation with all tested beverages, with better results in coffee cascara beverages.

The phenolic compounds, including catechins and chlorogenic acids, and caffeine were probably the main components responsible for the beneficial effects of these beverages. Considering that phenolics are the main compounds responsible for the antioxidative and anti-inflammatory activities of black tea and cascara, it is worth noting that independently of being free, bound, or partly metabolized, these phenolics exerted similar activities in the cell models. When the free and bound compounds from the infusions are consumed, they are most probably released and broken down by the gut microbiota and then absorbed. It is possible, however, that the kombucha phenolic metabolites will be absorbed earlier than the infusions metabolites in the gut. The health outcomes of this fact should be investigated in bioavailability studies. Overall, the present results suggest that coffee cascara is a novel promising ingredient for infusions and kombuchas elaboration with potential health benefits comparable to black tea.

CONCLUSÕES FINAIS

A partir dos resultados obtidos nos estudos apresentados, pode-se concluir:

Estudo 1:

- A composição microbiológica da cultura simbiótica de bactérias e leveduras após 9 dias de fermentação mostrou que o gênero predominante de bactérias ácido acéticas for *Komagateibacter*, enquanto para leveduras o gênero predominante foi *Pichia*, sem diferenças entre os kombuchas de chá preto, cascas de café e folhas do cafeeiro;
- Em relação à composição físico-química dos kombuchas de chá preto, cascas de café e folhas do cafeeiro, houve redução de sólidos solúveis totais (variando de 12,2 °Brix no dia 0 a 9,0 °Brix no dia 9), redução de sacarose (de 10g/100mL no dia 0 a 7g/100mL no dia 9, em média), aumento na concentração de glicose (de 1,14 g/100mL no dia 0 a 0,62 g/100mL no dia 9, em média) e frutose (de 0,07 g/100mL no dia 0 a 1,19 g/100mL no dia 9, em média), redução de pH (de 3,9 no dia 0 a 3,4 no dia 9, em média), e aumento de acidez tituláveis (de 0,04 mEq/L no dia 0 a 0,3 mEq/L no dia 9, em média), durante a fermentação dos kombuchas, indicando a ação dos microorganismos durante a fermentação para produção das bebidas;
- Em relação à composição química dos kombuchas de chá preto, foram identificadas as catequinas epigalocatequina, galocatequina, epigalocatequina galato, epicatequina e catequina. Seus teores reduziram cerca de 15% do dia 0 ao dia 9 de fermentação. Foram identificados três ácidos clorogênicos (ácidos 3-cafeoilquínico, 4-cafeoilquínico, 5- cafeoilquínico), cuja concentração média aumentou cerca de 19% provavelmente devido à liberação de formas conjugadas.
- Em relação aos ácidos fenólicos, no chá preto, ácido gálico foi identificado na infusão e em todos os kombuchas, com aumento de 42%, em média, nos kombuchas. Ácido cafeico, ácido hipúrico, ácido 3,4-dihidroxifenilacético, ácido 4-hidroxifenilacético, ácido vanílico e ácido dihidrocafeico foram identificados a partir de 3 dias de fermentação, com aumento em seus teores (25%; 42%; 13%; 36%; 42%; 283%, respectivamente);

- Rutina, quercetina e cafeína também foram identificados na infusão e kombuchas de chá preto, com aumento em seus teores no decorrer na fermentação (34%; 23% e 33% , respectivamente), denotando também conjugação com outros compostos ou degradação de estruturas mais complexas.
- Nas infusões e kombuchas elaborados com cascas dos frutos do café oito ácidos clorogênicos (ácidos 3-cafeoilquínico, 4- cafeoilquínico, 5- cafeoilquínico, 4-feruloilquínico; 5-feruloilquínico, 3,4-dicafeoilquínico, 3,5- dicafeoilquínico e 4,5-dicafeoilquínico), foram identificados com aumento no decorrer da fermentação, em média, de 66% nos kombuchas elaborados com cascas do Brasil e 22% com cascas da Nicarágua). Traços de catequinas foram identificados (< 0,2 - < 0,08 mg/100mL).
- Nas bebidas de cascas de café, os ácidos cafeico, ferúlico e benzóico foram identificados nas infusões e nos kombuchas, com aumento nas suas concentrações (75%; 75% e 40%, em média). Nas bebidas elaboradas com cascas de frutos de café do Brasil, alguns ácidos fenólicos foram identificados após 3 dias de fermentação, com aumento em suas concentrações: ácido dihidrocafeico (45%), ácido hipúrico (75%), ácido p-cumárico (50%), ácido 4-hidroxifenilacético (40%), ácido vanílico (20%), ácido 3,4-hidroxifenilacético (33%) e ácido 3,4-dihidrobenzóico (46%). Nas bebidas elaboradas com cascas de café da Nicarágua, os ácidos 3,4-dihidroxibenzóico, hipúrico, vanílico e dihidrocafeico foram observados nas infusões até o dia 9 de fermentação, com aumento de 75%, 121%, 83% e 118%, respectivamente, enquanto os ácidos p-cumárico (sem diferenças na concentração) e 4-hidroxifenilacético (aumento de 36%) foram identificadas a partir de 3 dias de fermentação. Também foram identificados rutina e cafeína, com aumento, em média de 64% e 52%, respectivamente, ao longo da fermentação de 9 dias. Não houve diferenças nos teores de trigonelina;
- Nos kombuchas com folhas do cafeeiro foram identificados oito ácidos clorogênicos (ácidos 3-cafeoilquínico, 4- cafeoilquínico, 5- cafeoilquínico, 4-feruloilquínico; 5-feruloilquínico, 3,4-dicafeoilquínico, 3,5- dicafeoilquínico e 4,5- dicafeoilquínico), com aumento de 31% (em média) ao longo de 9 dias de fermentação.
- Também foram identificados os ácidos gálico, cafeico, ferúlico na infusão e nos kombuchas de folhas do cafeeiro, com aumento de 116%, 43%, respectivamente,

enquanto o ácido ferúlico reduziu 6% durante a fermentação. O ácido sinápico também foi identificado nas infusões e kombuchas, com aumento de 18% ao longo de 9 dias de fermentação. Os ácidos p-cumárico, hipúrico, vanílico e dihidrocafeico foram identificados após 3 dias de fermentação, com aumento de 29%; 27%; 9%; e 35%, respectivamente e os ácidos 3,4-dihidroxifenilacético e 4-hidroxifenilacético após 6 dias de fermentação, com aumento de 71% e 50%, respectivamente, indicando produtos de degradação dos compostos fenólicos. Não houve modificação nos teores de mangiferina das folhas de cafeeiro ao longo da fermentação. Traços de catequinas foram identificados (< 0,01 - < 0,09 mg/100mL)

- Nas infusões e kombuchas de folhas do cafeeiro, foram identificados rutina, quercetina, cafeína, com aumento de 26%, 77% e 21%, respectivamente. Não houve alteração nos teores de trigonelina.
- As infusões de chá preto, cascas dos frutos e folhas do cafeeiro apesentaram maior atividade inibitória contra *Listeria monocytogenes* ATCC 19117. Não houve inibição de *Lactiplantibacillus plantarum* ATCC 8414.
- Kombuchas elaborados com cascas dos frutos e folhas do cafeeiro apresentaram maior halo de inibição frente aos patógenos *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076 quando comprado com as respectivas infusões. Não houve diferenças na atividade inibitória quando os kombuchas foram neutralizados (pH = 7). Não houve ação inibitória sobre o *Lactiplantibacillus plantarum* ATCC 8414.

Estudos 2 e 3:

- Na composição de compostos orgânicos voláteis das bebidas elaboradas a partir de chá preto, cascas de café, folhas do cafeeiro e folha do cafeeiro com erva-mate, durante a fermentação, foram identificados aumento no número de ácidos e ésteres, e redução no número de álcoois e aldeídos.
- Nos kombuchas elaborados com chá preto, utilizado como controle, foram identificadas as classes químicas aldeídos, ácidos, álcoois, esteres, cetonas, monoterpenos, monoterpenos de álcool, pirróis, furanos e fenóis. Foram identificados

os compostos de impacto (Z)-3-hexenol, 1-octen-3-ol, álcool benzílico, trans-óxido de linalool, linalool, álcool de feniletila, 1-octen-3-one, 2-nonanona, hexanal, nonanal, diidroactinidiolida, teaspirano e geranil acetona;

- Nas bebidas de cascas de frutos do cafeeiro, foram identificadas as classes aldeídos, ácidos, álcoois, esteres, cetonas, monoterpenos, monoterpenos de álcool, furanos e fenóis. Nas infusões de cascas dos frutos, foram identificados compostos voláteis previamente descritos: decanal, nonanal, palmitaldeído, undecanal, 1-dodecanol, palmitato de etila, γ-nonalactona e furfural.
- Nos kombuchas de cascas de frutos do cafeeiro, além dos compostos nonanal, undecanal, 1-dodecanol, γ-nonalactona e furfural, novos compostos voláteis foram identificados nos kombuchas como α-hexilcinamaldeído, 1-dodecanol, 1-heptanol, hexadecanol, isopulegol, butirato de etila, hexanoato de etila, acetato de isoamila, 2-propanona, e γ-nonalactona.
- Considerando as infusões de folhas de café, foi observado similaridades com o chá de *C. sinensis* na composição volátil, compartilhando os compostos de impacto previamente identificados em chá preo: hexanal, nonanal, linalool, α-ionona, β-ionona and β-damascenona e dihidroactinidiolida. Na infusão de folha de café com erva mate, além destes compostos, foram identficado o composto de impacto 2,4-heptadienal.
- Considerando os compostos voláteis presentes nos kombuchas de folhas do cafeeiro e folha do cafeeiro com erva mate, foram identificadas as classes aldeídos, ácidos, álcoois, esteres, cetonas, monoterpenos, monoterpenos de álcool, furanos e fenóis. Novos compostos voláteis foram identificados nos kombuchas: 2,4-heptadienal, tetradecanal, tridecanal, undecanal, (S)-2-heptanol, (z)-3-hexanoato de etila, dihidrojasmonato de metila, 3,2-octadien-2-one, (-)-carvona, isoforona, mentona, 3-δ-carene, mirceno e canfeno.
- A bebida elaborada com cascas dos frutos pré-fermentados e de natureza frutada fermentada por 3 dias, obteve a maior média de aceitação (7,0 ± 0,19, em escala hedônica de 9 pontos). Nesta amostra, foram percebidos pelos avaliadores maiores intensidades de aroma de mel, frutas vermelhas e fruta passa; gosto doce e sabor de fruta passa e fruta em calda, e sensação encorpada;

- Na análise sensorial dos kombuchas elaborados com folhas do cafeeiro a aceitação melhorou quando foi introduzida a erva mate, sendo a maior média de aceitação (6,6 ± 2,0) a dos kombuchas elaborados com folhas do cafeeiro e erva mate fermentados por 3 dias. Nestas amostras, foram relatadas maiores intensidades de aroma de ervas e folha tostada, gosto doce, sabor de folha tostada e de ervas e sensação refrescante;

Estudo 4:

- Infusões e kombuchas elaborados com cascas dos frutos e chá preto foram capazes de reduzir o estresse oxidativo intracelular, induzido por indoxil sulfato e altas concentrações de glicose em células tubulares proximais (HK-2), além de reduzir as concentrações de ácido úrico no sobrenadante celular na mesma linhagem de células e exercer atividade antiinflamatória em macrófagos (RAW 264.7)

Os resultados apresentados mostram que as cascas dos frutos e as folhas do cafeeiro são matérias-primas adequadas na produção de kombuchas, por possuírem elevados teores de compostos bioativos, boa aceitação sensorial e potenciais benefícios à saúde. A utilização desses subprodutos descartados durante o manejo do cafeeiro e no processamento pós-colheita contribuirá para a sustentabilidade no setor cafeeiro, reduzindo e evitando problemas ambientais.

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ANEXOS

Anexo 1: aprovação no Comitê de Ética em Pesquisa

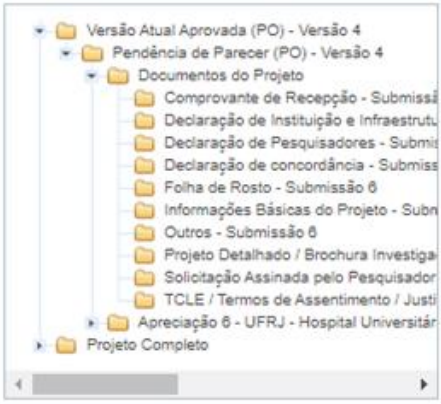
DETALHAR PROJETO DE PESQUISA

DADOS DA VERSÃO DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização sensorial de bebidas fermentadas (kombucha) elaboradas a partir de casca e folha do cafeeiro
Pesquisador Responsável: AMANDA LUISA SALES
Área Temática:
Versão: 4
CAAE: 39037320.3.0000.5257
Submetido em: 14/01/2021
Instituição Proponente: Instituto de Nutrição Josué de Castro
Situação da Versão do Projeto: Aprovado
Localização atual da Versão do Projeto: Pesquisador Responsável
Patrocinador Principal: UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

Comprovante de Recepção: PB_COMPROVANTE_RECEPCAO_1618692

DOCUMENTOS DO PROJETO DE PESQUISA



Tipo de Documento	Situação	Arquivo	Postagem	Ações

LISTA DE APRECIAÇÕES DO PROJETO

Apreciação	Pesquisador Responsável	Versão	Submissão	Modificação	Situação	Exclusiva do Centro Coord.	Ações
PO	AMANDA LUISA SALES	4	14/01/2021	28/01/2021	Aprovado	Não	

Anexo 2: artigo intitulado "Intracellular antioxidant and anti-inflammatory effects and bioactive profiles of coffee cascara and black tea kombucha beverages", publicado na revista Foods. Disponível em: https://doi.org/10.3390/foods12091905

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Article

Intracellular Antioxidant and Anti-Inflammatory Effects and Bioactive Profiles of Coffee Cascara and Black Tea Kombucha Beverages

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