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AVALIAÇÃO DE TOXICIDADE E POTENCIAL ANTIOXIDANTE *IN VITRO* E *IN VIVO* DE KOMBUCHA DE CHÁ VERDE PADRONIZADA

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PORTO ALEGRE

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Tese apresentada ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos como requisito parcial para obtenção do título de Doutora.

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RESUMO

Kombucha é a bebida obtida através da fermentação de infusão ou extrato de *Camellia sinensis* e açúcares por cultura simbiótica de bactérias e leveduras microbiologicamente ativas (SCOBY). Seu consumo popularizou-se devido às suas possíveis propriedades funcionais, associadas principalmente aos seus compostos fenólicos (CF). Por ser um produto fermentado, a produção da kombucha não é padronizada e pode apresentar grande variação na sua composição, dificultando a comprovação dos efeitos benéficos do seu consumo descritos na literatura. Este trabalho teve como objetivo avaliar o perfil fenólico e a atividade antioxidante *in vitro* e *in vivo* de uma kombucha de chá verde padronizada, com o intuito de sugerir uma dose diária segura de consumo desta bebida. Além disso, objetivamos verificar o efeito da fermentação do chá verde na produção de duas kombuchas (padronizada e comercial) no seu perfil fenólico e atividade antioxidante *in vitro* em meio *cell-free* e em células VERO. Primeiramente, foi reproduzida uma kombucha de chá verde usando uma cultura *starter* definida contendo a bactéria *Komagataeibacter saccharivorans* (10^7 UFC•mL^{-1}) e as leveduras *Brettanomyces anomala* (10^5 UFC•mL^{-1}) e *Kluyveromyces marxianus* (10^6 UFC•mL^{-1}). Três concentrações desta kombucha (25, 125 e 250 µL•mL^{-1}) foram avaliadas quanto a marcadores de estresse oxidativo *(in vitro*) e no modelo biológico *Caenorhabditis elegans* exposto ao indutor de estresse H_2O_2. Após, para analisar o efeito da fermentação, comparamos o chá verde e duas kombuchas padronizada e comercial através da identificação e quantificação dos seus CF por HPLC-MS. Foi possível observar que a kombucha de chá verde padronizada em todas as doses avaliadas não produziu toxicidade *in vivo* e reduziu a geração de espécies reativas e os níveis de peroxidação lipídica induzidos pelo H_2O_2. Ainda, as doses de 125 e 250 µL•mL^{-1} foram capazes de aumentar a fluorescência da enzima superóxido dismustase em *C. elegans*. Entretanto, nenhuma dose da kombucha atenuou a perda de sobrevivência de *C. elegans* causada pelo H_2O_2. Quanto ao efeito da fermentação, quando comparado o perfil penólico das três amotras por HPLC, estas demonstraram ser semelhantes. Contudo, o chá verde demonstrou uma quantificação significativamente maior de CF comparada às kombuchas padronizada e comercial. Ainda, o chá verde não fermentado demonstrou atividade antioxidante *in vitro* em meio *cell-free* superior as kombuchas. Contudo, nos ensaios *in vitro* com células VERO a kombucha de chá verde padronizada apresentou melhores resultados na sua ação protetora frente ao indutor de estresse H_2O_2. Ainda, os resultados deste trabalho sugerem que a dose de 125 µL•mL^{-1} de kombucha de chá verde padronizada é a dose que melhor desempenhou atividade antioxidante no modelo *in vivo* de *C. elegans*, sendo equivalente a 600 mL de kombucha para consumo humano. Esta dose é uma sugestão inicial de consumo para a kombucha desenvolvida neste trabalho. Mais estudos são necessários, principalmente em humanos, para comprovar os benefícios antioxidantes da ingestão desta kombucha.

Palavras-chave: Bebidas funcionais; Chá verde; Toxicidade; Estresse oxidativo; Compostos bioativos, Fitoquímicos.

ABSTRACT

Kombucha is a beverage obtained by the fermentation of *Camellia sinensis* infusion or extract and sugars by a symbiotic culture of microbiologically active bacteria and yeast (SCOBY). Its consumption has become popular due to its possible functional properties, mainly associated with its phenolic compounds (PC). As a fermented product, kombucha production is not standardized and can vary greatly in its composition, making it difficult to prove the beneficial effects of its consumption described in the literature. This work aimed to evaluate the phenolic profile and antioxidant activity *in vitro* and *in vivo* of a standardized green tea kombucha, to suggest a safe daily consumption dose of this beverage. Furthermore, we aimed at verifing the effect of green tea fermentation in the production of two kombuchas (standardized and commercial) on their phenolic profile and antioxidant activity *in vitro* in cell-free medium and in VERO cells. First, a green tea kombucha was produced using a defined starter culture containing the bacterium *Komagataeibacter saccharivorans* (10^7 CFU•mL^{-1}) and the yeasts *Brettanomyces anomala* (10^5 CFU•mL^{-1}) and *Kluyveromyces marxianus* (10^6 CFU•mL-^{1}). Three concentrations of this kombucha (25, 125, and 250 μL•mL^{-1}) were evaluated for oxidative stress markers (*in vitro*) and in the *Caenorhabditis elegans* biological model exposed to the stress inducer H_2O_2. Afterward, to analyze the effect of fermentation, we compared green tea and two standardized and commercial kombuchas through the identification and quantification of their PC by HPLC-MS. It was observed that standardized green tea kombucha at all doses evaluated did not produce toxicity *in vivo* and impaired the generation of reactive species and lipid peroxidation levels induced by H_2O_2. Moreover, 125 and 250 μL•mL^{-1} doses increased superoxide dismustase enzyme fluorescence in *C. elegans*. However, no dose of kombucha attenuated the loss of *C. elegans* survival caused by H_2O_2. Regarding the effect of fermentation, when comparing the phenolic profile of the three samples by HPLC, they were shown to be similar, but green tea had a significantly higher amount of PC compared to standardized and commercial kombuchas. Even so, unfermented green tea demonstrated *in vitro* antioxidant activity in a cell-free medium superior to kombuchas. The *in vitro* tests with VERO cells and standardized green tea kombucha showed better results in its protective action against the stress inducer H_2O_2. Furthermore, the results of this study suggest that the dose of 125 μL•mL^{-1} of standardized green tea kombucha is the dose that best performed antioxidant activity in the *in vivo* model of *C. elegans*, being equivalent to 600 mL of kombucha for human consumption. This dose is an initial suggested consumption for the kombucha developed in this study. Further studies are needed, especially in humans, to prove the antioxidant benefits of ingesting this kombucha.

Keywords: Functional beverages; Green tea; Toxicity; Oxidative stress; Bioactive compounds, Phytochemicals.

LISTA DE ABREVIATURAS

AAPH - Dicloridrato de 2,2-azobis (2-amidinopropano)
C. elegans - *Caenorhabditis elegans*
C. sinensis - *Camellia sinensis*
CDC - Centro de Controle e Prevenção de Doenças
CEUA - Comitê de Ética para o Uso de Animais
CF – Compostos Fenólicos
DCF - Diclorofluoresceína
DCNT - Doenças Crônicas NãoTransmissíveis
EAG - Equivalentes de Ácido Gálico
ECG – epigalocatequina
EGCG – epigalocatequina galato
ER – Espécies Reativas
GSH – Glutationa
H_2O_2 - Peróxido de hidrogênio
HPLC - Cromatografia Líquida de Alta Eficiência
IN – Instrução Normativa
LCC - Linha celular contínua
MAPA- Ministério da Agricultura, Pecuária e Abastecimento
MTT - 3-(4,5-Brometo de dimetil-2-tiazolil) -2,5-difenil-2H-tetrazólio
ORAC - Capacidade de absorção de radicais de oxigênio
RL - Radicais livres
$ROO^{\bullet}$ - Radical peroxil
SCOBY - Cultura simbiótica de bactérias e leveduras
SOD - Superoxido dismutase
TE - Trolox Equivalentes

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INTRODUÇÃO

Kombucha é uma bebida originada pela mistura do chá (*Camellia sinensis*) e açúcares, fermentada por cultura simbiótica de bactérias e leveduras microbiologicamente ativas (SCOBY) (BRASIL, 2019). Após seu processo de fermentação, a kombucha pode conter diversas substâncias como açúcares, etanol, aminoácidos, ácidos orgânicos (como ácido acético, glicônico e glicurônico), uma ampla gama de compostos fenólicos (CF), enzimas hidrolíticas e alguns micronutrientes, como vitaminas e minerais (zinco, cobre, ferro, manganês e cobalto) (HARDINSYAH et al., 2023; JAYABALAN et al., 2014).

O consumo da kombucha vem crescendo em diversos países, sendo considerada umas das bebidas com baixo teor alcoólico mais populares mundialmente, principalmente devido às suas prováveis propriedades funcionais (BATISTA et al., 2022; WATAWANA et al., 2015). Alimentos funcionais são produtos que além de possuírem constituintes essenciais para a nutrição humana, contém componentes que podem oferecer benefícios à saúde. Como exemplos de compostos funcionais podemos citar fibras dietéticas, probióticos, prebióticos e antioxidantes (HARDINSYAH et al., 2023; RAMÍREZ-MORENO et al., 2022).

Neste sentido, a kombucha devido a sua composição vem sendo relatada na literatura por exercer atividades antimicrobiana, antiobesogênica, antiinflamatória, estimulante dos sistemas imune, neuroprotetora, antioxidante entre outras ações benéficas (ABACI; SENOL DENIZ; ORHAN, 2022; IVANIŠOVÁ et al., 2020; KABIRI; SETORKI, 2016; MOREIRA et al., 2022; MULYANI et al., 2019). Os benefícios da kombucha primeiramente foram atribuídos ao chá verde, matéria prima mais utilizada na elaboração desta bebida, que apresenta alto conteúdo de antioxidantes, principalmente CF.

Os CF mais abundantes na kombucha são catequinas e flavonoides (teaflavinas e flavanois) (ABACI; SENOL DENIZ; ORHAN, 2022; CARDOSO et al., 2020; YUAN et al., 2022). Os

CF atuam como antioxidantes exógenos, neutralizando espécies reativas (ER), juntamente com as defesas endógenas do nosso organismo (SILVA et al., 2021). Além disso, os CF podem atuar indiretamente estimulando as defesas antioxidantes endógenas (AUGUSTI et al., 2021). Desta maneira, os CF contidos na kombucha, com destaque para seus majoritários como as catequinas, podem combater o estresse oxidativo e previnir danos a macromoléculas como proteínas, lipídios e DNA, auxiliando na redução das doenças crônicas não transmissíveis, como diabetes, doenças cardiovasculares e câncer (RAMÍREZ-MORENO et al., 2022; RUDRAPAL et al., 2022).

Contudo, alimentos fermentados como a kombucha podem apresentar muita diversidade em sua composição final, por conseguinte, em seu conteúdo de antioxidantes. O processo fermentativo da kombucha pode ocasionar mudanças no potencial antioxidante do chá verde, através do aumento ou diminuição de alguns CF específicos ou na biodisponibilidade destes (CHAKRAVORTY et al., 2016; KIM et al., 2023; VILLARREAL-SOTO et al., 2018). Isto se deve a fatores como a composição da cultura *starter*, quantidade e tipo de chá e açúcar utilizados, a temperatura, o pH e o tempo de fermentação (DARTORA et al., 2023; DE MIRANDA et al., 2022).

Apesar de o Brasil possuir uma legislação específica para a produção de kombucha, a Instrução Normativa Nº 41 de 2019 (IN Nº41/2019) do Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (BRASIL, 2019), os parâmetros contemplados na mesma não garantem uma padronização da bebida, dificultando assim a determinação de suas bioatividades e o estabelecimento de uma recomendação de ingestão diária segura. Adicionalmente, são escassos os estudos científicos toxicológicos, especialmente em humanos, que auxiliem na definição de quantidade de consumo seguro da kombucha. Embora esta bebida seja considerada um produto natural e saudável, existem alguns relatos de efeitos tóxicos após sua ingestão. Dois casos de acidose metabólica foram descritos devido ao consumo excessivo (> 360 mL por dia) desta bebida. Com isso, o Centro de Controle e Prevenção de Doenças dos EUA (CDC-USA) estabeleceu que até 120 mL de kombucha por dia não representaria risco à saúde do consumidor (JAYABALAN et al., 2014; MARTÍNEZ-LEAL; PONCE-GARCÍA; ESCALANTE-ABURTO, 2020). No Brasil, a IN Nº41/2019 do MAPA não aborda sobre questões de consumo de kombucha (BRASIL, 2019).

Além da importância de se conhecer um limite seguro de ingestão diário de kombucha, esta definição é necessária para correlacionar uma dose-efeito desta bebida às suas possíveis alegações de saudabilidade. Para isso, modelos *in vivo* de animais, como roedores e camundongos, são normalmente utilizados. Alternativamente a estes modelos tradicionais citados, outros modelos como a cultura de células e o nematóide *Caenorhabditis elegans* (*C. elegans*) vêm sendo empregados (WANG et al., 2022b). Ambos os modelos contribuem para o princípio bioético dos 3Rs, *(reduction*, *replacement* e *refinement*) redução, substituição e refinamento no uso de animais. Adicionalmente, o uso destes modelos não requer aprovação no Comitê de Ética para o Uso de Animais (CEUA).

O cultivo de células é uma ferramenta importante para uma avaliação inicial de bioatividades e possível toxicidade de uma série de compostos e fármacos. Entretanto, esse modelo não é capaz de mimetizar um sistema fisiológico como um todo. Além disso, a cultura de células requer ambiente esterilizado e altos custos para manutenção das linhagens. O nematóide *C. elegans* tem atraído atenção por sua capacidade de mimetizar estados fisiológicos de sistemas vivos, possuir uma facilidade de cultivo e manuseio em laboratório, curto ciclo de vida e metabolismo relativamente simples. Além disso, esse modelo possui genes e vias de sinalização homólogos aos humanos, especialmente em relação ao estresse oxidativo, garantindo assim eficácia na simulação de mecanismos de defesa antioxidante a nível molecular (HUNT, 2017; SCHMIDT et al., 2020). Todas estas características fazem deste modelo um organismo ideal para avaliar biologicamente e toxicologicamente novos produtos alimentares.

Muitos autores descrevem estudos *in vivo* sobre doses-efeitos de kombucha atreladas a seus efeitos a saúde e/ou possível toxicidade (MURUGESAN et al., 2009; PAULINE, 2001; SILVA et al., 2021; VIJAYARAGHAVAN, 2000). No entanto, como não são bebidas padronizadas, de composição conhecida, não são passíveis de reprodutibilidade e portanto não pode-se afirmar que o consumo contínuo de kombuchas de diferentes origens demonstrarão os mesmos efeitos benéficos.

Diante deste contexto, é necessário avaliar o potencial antioxidante de kombucha de chá verde com cultura *starter* padronizada, utilizando quantidades específicas de microrganismos conhecidos, a fim de sugerir uma recomendação de consumo segura para os consumidores.

OBJETIVOS

Objetivo geral

Avaliar a atividade antioxidante *in vitro* e *in vivo* e possível toxicidade de uma kombucha de chá verde padronizada contendo os microrganismos *Komagataeibacter saccharivorans*, *Kluyveromyces marxianus* e *Brettanomyces anomala*.

Objetivos específicos

- Escrever um artigo de revisão sobre os benefícios à saúde e os possíveis efeitos probióticos e prebióticos do consumo da kombucha;

- Separar, identificar e quantificar os CF majoritários da kombucha de chá verde padronizada e comparar com sua matriz não fermentada (chá verde) e uma kombucha industrializada;

- Avaliar a possível toxicidade de diferentes doses de kombucha padronizada em *C. elegans;*

- Verificar o potencial antioxidante de diferentes doses de kombucha de chá verde padronizada *in vitro* (meio *cell-free*) e sobre marcadores de estresse oxidativo em modelo *C. elegans*;

- Avaliar o potencial antioxidante *in vitro* (meio *cell-free* e em cultura de células) da kombucha de chá verde padronizada e comparar com sua matriz não fermentada (chá verde) e uma kombucha industrializada;

CAPÍTULO I - REVISÃO BIBLIOGRÁFICA

1 Kombucha: definição, origem e composição

Acredita-se que a kombucha, uma bebida de chá levemente fermentada, tenha se originado na China, na região da Manchúria, sendo consumida pela primeira vez há mais de 2000 anos (DOROTHY et al., 2020; KAPP; SUMNER, 2019). Em 414 d.C., o médico Kombu trouxe o fungo do chá para o Japão, por isso sua denominação de kombucha (JAYABALAN et al., 2014). Tradicionalmente, a kombucha era elaborada de forma caseira. A preparação desta bebida é simples: adiciona-se à água fervente as folhas de chá e açúcar, deixando a mistura em repouso por alguns minutos. Após resfriamento da solução adiciona-se a SCOBY, responsável por fermentar o chá adoçado, sendo que esta etapa normalmente demora de oito a 14 dias. Depois de fermentada, a SCOBY é retirada da kombucha e separa-se também um pequeno volume da bebida pronta, ambos utilizados como cultura *starter* para as próximas fermentações (DUFRESNE; FARNWORTH, 2000; VILLARREAL-SOTO et al., 2018).

Atualmente, a kombucha está disponível comercialmente. Seu consumo vem crescendo em diversos países, sendo considerada umas das bebidas com baixo teor alcoólico mais populares mundialmente, principalmente devido seu apelo de saudabilidade (BASCHALI et al., 2017; BATISTA et al., 2022). Com a disseminação do seu consumo, o preparo da kombucha foi sendo modificado e seu processo produtivo, até o presente, não é padronizado. Mesmo quando sua elaboração se restringe ao uso da espécie *Camellia sinensis* (ou conhecida simplesmente como chá), esta engloba as variantes chás branco, verde, oolong e preto (JAKUBCZYK et al., 2020). Cada um desses tipos de chá possuem um grau de fermentação diferente, e por isso, apresentam características físico-químicas distintas (DARTORA et al., 2023). Porém, os tipos de chá mais utilizados para a kombucha são os chás verde e preto

(COTON et al., 2017; FU et al., 2014).

Ainda, podem ser utilizados outros substratos na produção da kombucha, como vinho, leite, sucos de frutas (JAYABALAN et al., 2014), arroz e cevada (AHMED; HIKAL; ABOU-TALEB, 2020) e outros tipos de chá, como mil em rama (VITAS et al., 2018), *roiboss* (GAGGÌA et al., 2019) e folhas de mostarda (RAHMANI et al., 2019). No entanto, na legislação brasileira são permitidos apenas o uso de outras espécies vegetais em associação a *C. sinensis* (BRASIL, 2019).

Além da variabilidade da matriz vegetal, o resultado final do produto kombucha vai ser influenciado também pelos parâmetros na sua produção, como concentração do chá (SHAHBAZI et al., 2018), tipo e concentração do açúcar (REVA et al., 2015; WATAWANA et al., 2017), tempo e temperatura de fermentação (DE FILIPPIS et al., 2018; TRAN et al., 2020) e a composição da cultura *starter* (KIM et al., 2023; NGUYEN et al., 2015; VILLARREAL-SOTO et al., 2018).

Quanto a cultura *starter*, normalmente são encontradas bactérias ácido acéticas e diferentes tipos de leveduras. Alguns estudos demonstram que a comunidade de microrganismos contidos na SCOBY da kombucha podem se modificar entre as fermentações (CHAKRAVORTY et al., 2016; COTON et al., 2017). Porém, há algumas espécies que parecem permanecer na maioria das culturas *starters*, como *Gluconobacter* e *Acetobacter* para as bactérias e *Saccharomyces cerevisiae*, *Zygosaccharomyces*, *Brettanomyces/Dekkera* e *Candida* para leveduras (JAYABALAN et al., 2014). Kim et al. (2023) realizaram uma triagem dos microrganismos contidos em diversas kombuchas de chá preto e encontraram que a maioria destes se tratava de bactérias ácido acéticas do gênero *Komagataebacter* spp., dominantes no SCOBY. Arikan et al. (2020) investigando os microrganismos de duas kombuchas caseiras, produzidas na Turquia, também identificaram como gêneros dominantes três espécies de *Komagataebacter* para as bactérias e *Zygosaccharomyces* para fungos, constatando uma baixa diversidade relativa no ecossistema destas bebidas. Adicionalmente, bactérias ácido láticas, tais como *Lactobacillus* e *Lactococcus* também podem estar presentes em algumas kombuchas. Todavia, devido sua ocorrência facultativa, estas não parecem ser essenciais no consórcio microbial da kombucha (FU et al., 2014; LAUREYS; BRITTON; DE CLIPPELEER, 2020; MURPHY; WALIA; FARBER, 2018).

O conjunto dos gêneros microbianos inclusos na kombucha são encarregados pela redução do potencial hidrogeniônico (pH) inicial da bebida, inibição da proliferação de possíveis bactérias contaminantes e também por realizarem o processo fermentativo (NEFFE-SKOCIŃSKA et al., 2017). A fermentação da kombucha é composta por duas fases. A primeira fase é a líquida do chá e a segunda é a SCOBY, uma película semelhante a um biofilme bacteriano, que fica flutuante no chá (VILLARREAL-SOTO et al., 2018), como demonstra a Figura 1.

Figura 1. Fermentação da kombucha em 2 fases. (A) Camada de película de celulose microbiana flutuante e (B) fase de chá líquido. Fonte: Próprio autor.

A fermentação é iniciada pelas leveduras que hidrolisam a sacarose convertendo-a em glicose e frutose, produzindo ao fim etanol, glicerol e dióxido de carbono. Na sequência, as bactérias oxidam o etanol produzindo ácido acético e transformam a glicose em ácido glicurônico (GAGGÌA et al., 2019; MAY et al., 2019). Inclusive, a glicose é utilizada por algumas bactérias, principalmente da espécie *Komagataeibacter*, para a produção de celulose. Com isso, a cada fermentação forma-se uma nova SCOBY acima da SCOBY primária adicionada (CHAKRAVORTY et al., 2016; COTON et al., 2017).

Após processo fermentativo é originada uma bebida refrescante, levemente ácida e gaseificada e com um conteúdo diversificado de componentes, tais como açúcares (sacarose, glicose e frutose), etanol, aminoácidos essenciais e não essenciais (isoleucina, leucina, lisina, metionina, fenilalanina, valina, alanina, cisteína, ácido aspártico, ácido glutâmico, glicina, histidina, prolina, entre outros), dióxido de carbono, ácidos orgânicos (principalmente acético, glucônico e glicurônico), CF, enzimas hidrolíticas e até mesmo micronutrientes, como vitaminas do complexo B e minerais (cobre, ferro e manganês, cobalto) (JAYABALAN et al., 2014; KITWETCHAROEN et al., 2023; TRAN et al., 2020).

Ivanišová et al. (2020) identificaram na kombucha de chá preto principalmente os ácidos acéticos, tartárico e cítrico. Já Dartora et al. (2023) comparando kombuchas de chá preto e verde, verificaram que a de chá verde apresentou maiores valores de ácido acético, menor conteúdo de etanol e uma melhor atividade anti-hipertensiva. Importante ressaltar que os resultados obtidos na caracterização de cada kombucha são de acordo com suas condições de preparo, podendo facilmente serem alterados. De toda forma, a kombucha é conhecida por conter numerosos compostos bioativos e por isso diversas atividades biológicas são atribuídas a esta bebida (DIEZ-OZAETA; ASTIAZARAN, 2022; KITWETCHAROEN et al., 2023).

2 Atividades biológicas da kombucha

Nos últimos anos, a kombucha tornou-se um dos produtos fermentados mais promissores devido às suas potenciais características benéficas (DIEZ-OZAETA; ASTIAZARAN, 2022). Primeiramente, as atividades biológicas desempenhadas pela kombucha foram atribuídas a composição do próprio chá utilizado na elaboração da mesma. O chá é historicamente reconhecido como um alimento saudável por apresentar um alto conteúdo de antioxidantes (LI et al., 2020). Em um segundo momento, percebeu-se que os possíveis efeitos benéficos da kombucha poderiam ser provenientes do seu processo fermentativo (BASCHALI et al., 2017; DUFRESNE; FARNWORTH, 2000; MORALES, 2020). A fermentação causa muitas alterações bioquímicas nos alimentos. Podemos citar desde modificações na digestibilidade dos nutrientes, melhora nas características sensoriais dos produtos e, até mesmo, alterações na composição química dos alimentos (ALFKE; KAMPERMANN; ESSELEN, 2022; VILLARREAL-SOTO et al., 2018).

As supostas propriedades funcionais da kombucha são atribuídas ao seu processo fermentativo, devido a sinergia de sua diversificada comunidade microbiana com a formação de alguns subprodutos metabólicos (SANWAL et al., 2023). Podemos citar como exemplo o ácido glicurônico, um dos principais ácidos orgânicos provenientes da oxidação da glicose na fermentação da kombucha (NEFFE-SKOCIŃSKA et al., 2017). Esse ácido possui propriedades desintoxicantes e é produzido naturalmente no fígado humano, realizando a excreção de xenobióticos e fenóis através dos rins (JAYABALAN et al., 2014; LEAL et al., 2018; NGUYEN et al., 2015). Vina et al. (2014) relataram em seu trabalho alguns benefícios fisiológicos relacionadas ao consumo de kombucha, como propriedades antioxidante, desintoxicante, promoção da imunidade e propriedades energizantes, sendo esta última relacionada ao aumento do nível de hemoglobina no sangue devido a liberação de ferro do chá durante o processo fermentativo. Com isso, a oxigenação dos tecidos é favorecida, estimulando a síntese de ATP no organismo (VINA et al., 2014). Diversos estudos demonstram os benefícios a saúde através da ingestão da kombucha. No Quadro 1 estão apresentadas algumas das propriedades biológicas já relatas pelo consumo da mesma.

Quadro 1. Propriedades biológicas relacionadas a kombucha

Tipo de ensaio	**Propriedade biológica**	**Referência**
In vitro	Antimicrobiana	(BATTIKH; BAKHROUF; AMMAR, 2012; IVANIŠOVÁ et al., 2020; MULYANI et al., 2019; SREERAMULU; ZHU; KNOL, 2000; VALIYAN; KOOHSARI; FADAVI, 2020).
	Anti-carcinogênica	(JAYABALAN; HSIEH; YUN, 2011)
	Antiinflamatória	(VÁZQUEZ-CABRAL et al., 2017)
	Antioxidante	(HOON et al., 2014; MULYANI et al., 2019; VÁZQUEZ-CABRAL et al., 2017)
	Radioprotetora (radiação ionizante)	(CAVUSOGLU; GULER, 2010)
	Modulação de células linfocitárias	(MRDANOVIĆ et al., 2007)

In vivo	Anti-hipercolesterolêmica	(ALAEI; DOUDI; SETORKI, 2020; ALOULOU et al., 2012; BELLASSOUED et al., 2015)
	Anti-hiperglicêmica	(ALOULOU et al., 2012; SRIHARI et al., 2013)
	Radioprotetora (radiação eletromagnética)	(GHARIB, 2014)
	Anti-diabética	(BHATTACHARYA; GACHHUI; SIL, 2013)
	Anti-hiperuricemica	(SUKRAMA, 2015)
	Antioxidante	(KABIRI; SETORKI, 2016; SAI RAM et al., 2000; YANG et al., 2009)
	Modulação do microbioma	(JUNG et al., 2019)

Das propriedades biológicas da kombucha mostradas no Quadro 1, destaca-se a antimicrobiana por ser uma das mais descritas na literatura. Esta atividade está relacionada à comunidade microbiana da cultura *starter* da kombucha, que age de forma simbiótica para evitar possíveis contaminações, bem como, aos metabólitos formados durante a fermentação da kombucha, como catequinas, etanol e ácidos orgânicos, principalmente o acético (LOCHYŃSKI, 2014; WATAWANA et al., 2015). O ácido acético é relatado como controlador do crescimento de bactérias patogênicas devido à redução do pH da kombucha, evitando a contaminação da mesma. O ácido acético também já demonstrou algumas propriedades antifúngicas e antivirais (MOUSAVI et al., 2020; WATAWANA et al., 2015). A ação antimicrobiana da kombucha já foi referida para vários microorganismos, incluindo bactérias Gram-negativas e Gram-positivas (DUFRESNE; FARNWORTH, 2000), sendo os mais frequentemente relatados *Escherichia coli*, *Staphylococcus aureus* e *Helicobacter pylori*, esta última sendo a causa de úlceras gástricas e outras complicações do aparelho digestivo (LOCHYŃSKI, 2014).

Além da propriedade antimicrobiana, uma das ações mais conhecidas da kombucha é a antioxidante (MASSOUD et al., 2022). Diferentes compostos presentes da kombucha podem exercer atividade antioxidante, como vitaminas, ácidos orgânicos e compostos fenólicas (KITWETCHAROEN et al., 2023; VILLARREAL-SOTO et al., 2019). Sai Ram et al. (2000) constataram em um estudo *in vivo* que compostos bioativos presentes na kombucha podem reduzir o estresse oxidativo e a imunossupressão causada por essa condição. No entanto, destacamos a colaboração dos CF na atividade antioxidante da kombucha, pois são substâncias que possuem um conhecido papel na melhora da imunidade, na redução de focos inflamatórios e danos celulares, evitando o desenvolvimento de doenças crônicas não transmissíveis (DCNT) (KAPP; SUMNER, 2019; RAMÍREZ-MORENO et al., 2022; VÁZQUEZ-CABRAL et al., 2017; VINA et al., 2014)

2.1 CF da kombucha e seus benefícios à saúde

Mais de 8.000 compostos bioativos já foram encontrados na natureza, sendo os CF os fitoquímicos mais abundantes em matrizes vegetais. Os CF são metabólitos secundários das plantas, tendo sua síntese modulada sob condições de estresse e são conhecidos pela sua ação antioxidante. Os CF possuem em sua estrutura química um hidrocarboneto aromático ligado diretamente a pelo menos um grupamento hidroxila. São um amplo grupo de substâncias que incluem os flavonoides, estilbenos, lignanas e ácidos fenólicos e são considerados antioxidantes exógenos por serem provenientes da dieta (RAMÍREZ-MORENO et al., 2022; XU et al., 2017).

O chá (*C. sinensis*), substrato utilizado para preparar a kombucha, já possui uma alta atividade antioxidante, como mencionado anteriormente. Isto porque quase 30 % do peso seco de suas folhas contém diversos CF, como catequina (epicatequina, epigalocatequina, epicatequina galato, epigalocatequina galato), teaflavinas e tearubiginas (ABACI; SENOL DENIZ; ORHAN, 2022; CARDOSO et al., 2020; MASSOUD et al., 2022; YANG et al., 2022). Dentre estes, a epigalocatequina-3-galato (EGCG) é o principal CF do chá verde, com atividade antioxidante de 25 a 100 vezes maior que as vitaminas C e E (ALFKE; KAMPERMANN; ESSELEN, 2022; KITWETCHAROEN et al., 2023).

A fermentação do chá verde até kombucha pode alterar o perfil de CF e, desta maneira, as propriedades benéficas da bebida. Isto ocorre porque as bactérias e leveduras presentes na fermentação são capazes de liberar enzimas que degradam os CF em moléculas menores, modificando sua estrutura molecular, dando origem a novos metabólitos (JAKUBCZYK et al., 2020; SHI et al., 2023). Adicionalmente, além do perfil, a fermentação pode influenciar também a biodisponibilidade dos CF, fator crucial para os efeitos benéficos desempenhados por esses compostos. Normalmente, na fermentação os CF são convertidos em substâncias mais bioativas do que seus compostos precursores, aumentando assim sua atividade antioxidante (LEONARD et al., 2021).

Chakravorty et al. (2016) demonstraram que o processo fermentativo do chá preto para produção de sua kombucha aumentou gradualmente sua capacidade de eliminação de radicais in vitro, com um incremento máximo no sétimo dia de fermentação. Também utilizando chá preto como matriz vegetal, Kim et al. (2023) verificaram um acréscimo de 2,88 vezes na atividade antioxidante *in vitro* de sua kombucha quando comparado ao chá não fermentado, com destaque para o aumento dos CF catequina, epicatequina, cafeína, ácido caféico, ácido gálico e rutina. Hoon et al. (2014) perceberam um crescimento no teor de CF de kombuchas fermentadas com cinco diferentes tipos de chás. Na cultura *starter* das kombuchas, estavam presentes a bactéria *Acetobacter aceti* e as leveduras *Zygosaccharomyces bailii* e *Brettanomyces claussenii*. Dos cinco chás estudados, os chás de rooibos, chá preto do Sri Lanka e chá verde tiveram aumentos estatisticamente significativos também em suas atividades antioxidantes *in vitro*, mensuradas pelos ensaios de ORAC, DPPH e capacidade de eliminação do radical superóxido, a partir do primeiro dia de fermentação. Já Cardoso et al. (2020) relataram que a kombucha de chá verde demonstrou ter maior atividade antioxidante *in vitro* que a kombucha de chá preto, devido à maior concentração de catequinas e à presença de verbascosídeo. Igualmente, Jakubczyk et al. (2020) verificaram em seu estudo que os chás vermelho e verde são maiores fontes de CF do que o chá preto, tornando-os alternativas potencialmente mais desejáveis para a produção da kombucha. Contudo, Teixeira Oliveira et al. (2023) utilizando o chá verde para produzir kombucha, demonstraram que o processo fermentativo do chá não alterou a quantificação de seus CF através da análise de Folin-Ciocalteau. Apesar de os estudos mostrarem relação entre as alterações de perfil de CF durante a elaboração da kombucha, a maioria dos estudos avaliam atividade antioxidante utilizando metodologias *in vitro* em sistemas livres de células (*cell-free*), o que torna difícil a extrapolação dos resultados para os sistemas biológicos (TEIXEIRA OLIVEIRA et al., 2023).

A kombucha possui alta concentração de CF, como ácido gálico, catequinas, ácido p-cumárico, rutina, vitexina, entre outros, garantindo assim um bom aporte de antioxidantes (IVANIŠOVÁ et al., 2020; SANWAL et al., 2023). Estes compostos podem neutralizar radicais livres (RL) e espécies reativas (ER), principalmente de oxigênio, como o peróxido de hidrogênio (H_2O_2) radicais ânion superóxido ($^{\bullet}O2$) e hidroxila ($^{\bullet}OH$) (HALLIWELL, 2024). Estas espécies são produzidas durante o metabolismo normal do organismo e desempenham importantes funções. Suas concentrações são reguladas por antioxidantes endógenos como as enzimas antioxidantes superóxido dismutase (SOD), catalase e glutationa peroxidase e pelo tripeptídeo glutationa (GSH) (ERUSLANOV; KUSMARTSEV, 2010). Quando há um desequilíbrio nessa relação, estabelece-se uma condição de estresse oxidativo, podendo levar ao dano à importantes biomoléculas como lipídios, proteínas e DNA. Tais danos oxidativos têm sido associados à patogênese de doenças crônicas não transmissíveis, como diabetes, doenças cardiovasculares e neurodegenerativas, bem como o câncer (MUSCOLO et al., 2024). Os mecanismos envolvidos na ação antioxidante do CF ocorrem através da doação direta de elétrons, quelação de metais e/ou modulação de enzimas antioxidantes (CHAKRAVORTY et al., 2019; IVANIŠOVÁ et al., 2020). Assim, os CF da kombucha agem juntamente com as defesas antioxidantes endógenas e exógenas, como vitaminas C e E, para garantir o bom funcionamento do organismo e evitar situações de estresse oxidativo (ALFKE; KAMPERMANN; ESSELEN, 2022; SILVA et al., 2021).

Além da atividade antioxidante, os CF também estão sendo associadas a modulação da microbiota intestinal, sendo reconhecidos como agentes tipo prebióticos (*prebiotics-like*) (DUEÑAS et al., 2015; TOMÁS-BARBERÁN; SELMA; ESPÍN, 2016). Prebióticos são componentes que servem como substrato para os microrganismos benéficos, nutrindo linhagens probióticas e estimulando seletivamente o crescimento e/ou atividade da microbiota residente no intestino humano. Após ingestão, a maioria dos CF passam pelo intestino delgado sem serem absorvidos, chegando intactos no cólon, onde são usados como substrato pela microbiota intestinal (GIBSON et al., 2017; HEINEN; AHNEN; SLAVIN, 2020). Desta maneira, os efeitos biológicos dos CF são tanto devido à sua fração absorvida, quanto aos metabólitos gerados pela ação da microbiota.

Apesar das propriedades biológicas da kombucha apresentadas até agora, não é possível afirmar que todas as bebidas produzidas desempenharão esses benefícios através de seu consumo contínuo. Isto ocorre pela falta de padronização em seu processo produtivo, podendo o produto final conter um perfil de CF muito variável, o que também modificará suas bioatividades. Por isso, atenta-se a importância do desenvolvimento de uma kombucha padronizada, com espécies e concentrações conhecidas de microrganismos.

3 Avaliação de possíveis efeitos tóxicos da kombucha

Embora a kombucha seja considerada um produto natural e saudável, é importante relatar também que esta bebida pode trazer alguns efeitos indesejáveis pelo seu consumo (MARTINI, 2018; MOUSAVI et al., 2020). Existem poucos relatos na literatura sobre efeitos tóxicos após ingestão da kombucha. Hartmann et al. (2000) avaliaram efeitos da ingestão crônica de kombucha em camundongos. Durante 21 dias, os animais que consumiram kombucha de chá preto *ad libitum* demonstraram reduções no apetite e peso corporal e maior longevidade em comparação com o grupo controle. Contudo, os animais que consumiram kombucha apresentaram hepatomegalia e alterações histológicas no fígado e baço. Os autores sugerem que análises bioquímicas extensas devem ser realizadas para compreender completamente a relação do consumo da kombucha com a esplenomegalia e hepatomegalia presenciada nos camundongos.

Alguns casos de efeitos adversos de consumo de kombucha em humanos foram associados a doenças pré-existentes ou imunocompromentimento, e/ou devido a um consumo excessivo de kombucha (BATISTA et al., 2022). Os casos descrevendo hepatoxicidade tratam-se de uma possível acidose metabólica devido ao consumo excessivo (> 360 mL por dia) de kombucha (SFINIVASAN; SMOTINSKE; GREENBAUM, 1997). Outro relato de acidose e insuficiência renal aguda foi observado em um paciente com HIV, imunocomprome tido, que ingeriu cerca de um litro de kombucha (KOLE et al., 2008). Com isso, o Centro de Controle e Prevenção de Doenças (CDC-USA) estabeleceu que até 120 mL desta bebida por dia não representaria risco à saúde do consumidor (JAYABALAN et al., 2014; MARTÍNEZ-LEAL;

PONCE-GARCÍA; ESCALANTE-ABURTO, 2020).

O consumo da kombucha não pode ocorrer de maneira indiscriminada. Isto porque em sua composição pode conter certa quantidade de álcool, além de concentrações consideráveis de açúcar. Estas questões limitam o consumo desta bebida para mulheres grávidas e lactantes e pessoas com diabetes. Ainda, são escassos estudos científicos toxicológicos, especialmente em humanos, que auxiliem na definição de quantidade de consumo seguro da kombucha (DIEZ-OZAETA; ASTIAZARAN, 2022; MARTINI, 2018).

4 Modelos alternativos para avaliação de bioatividades e/ou toxicidade de compostos alimentares

A definição de uma dose segura de kombucha é necessária para correlacionar uma dose-efeito desta bebida às suas alegações de saudabilidade. Para isso, modelos *in vivo* de animais, como roedores e camundongos, são normalmente utilizados. Alternativamente a estes modelos citados, estão sendo empregados modelos alternativos de experimentação, como o cultivo celular da linhagem VERO e o nematóide *C. elegans*, visando seguir os princípios dos 3R's no uso de animais, como já citado (HUBRECHT; CARTER, 2019). Tanto a cultura de células quanto o uso de *C. elegans* não requerem aprovação no Comitê de Ética para o Uso de Animais (CEUA), uma vez que os ne matóides são invertebrados.

A linhagem VERO, mostrada na Figura 2, foi extraída em 1962 de células epiteliais renais de um macaco verde africano (*Cercopithecus aethiops*) e é uma linha celular contínua (LCC), ou seja, pode ser passada indefinidamente permitindo extensa caracterização celular e a criação de grandes bancos de células (MARIE; WIJAYANTI, 2020).

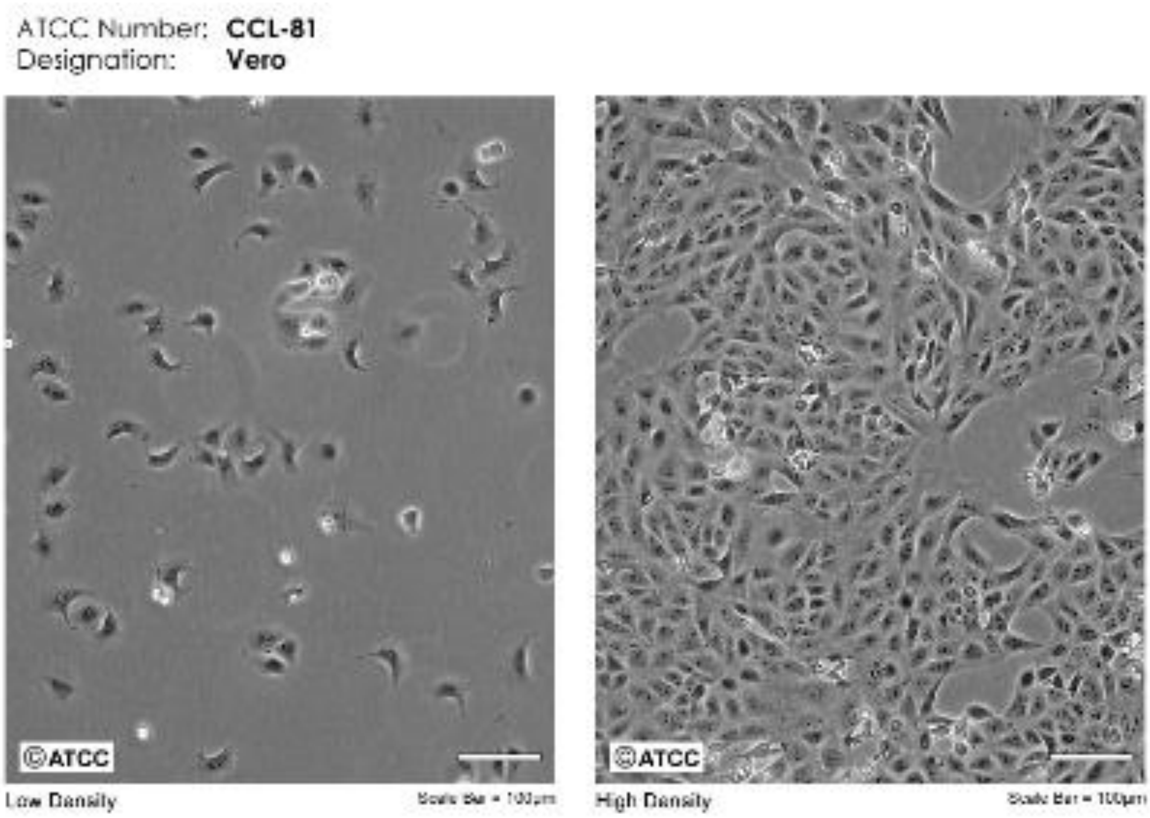

Figura 2. Linhagem celular VERO. Fonte: ATCC Org. Disponível em: <https://www.atcc.org/products/ccl-81>

A VERO é uma LCC de mamíferos mais comumente empregada em pesquisas em microbiologia, biologia molecular e celular. Historicamente, esta é a primeira linhagem celular aprovada pela OMS para a produção de vacinas humanas (KIESSLICHAND; KAMEN, 2020). No entanto, a linhagem VERO possui muitas outras aplicações, como detecção de toxinas, testes de eficácia e propagação, até mesmo, avaliação de toxicidade, antioxidante e de bioatividade de diferentes fitoquímicos (ELHAJ et al., 2021; CHOWDHURY et al. 2020; SUJANA; WIJAYANTI, 2022). Inclusive esta linhagem já foi utilizada para verificar citotoxicidade e atividade antiviral de kombuchas (NADY et al. 2023; ELASHMAWY et al., 2023). A aplicação de cultivos celulares em sistemas de estresse oxidativo é interessante pois as células sob condições de estresse podem sofrer oxidação de macromoléculas, peroxidação lipídica, danos a proteínas e ao DNA, facilmente sendo detectadas neste modelo *in vitro*

(MARIE; WIJAYANTI, 2020).

Outro modelo alterativo utilizado neste trabalho é o *C. elegans*, um nematoide bacterívoro de 1 mm de comprimento encontrado naturalmente no solo, mas atualmente está adaptado e presente em diversos ambientes. O modelo *C. elegans* surgiu no início do ano de 1960 com o pesquisador Sydney Brenner, que desejava encontrar um modelo *in vivo* adequado para avaliar mecanismos moleculares de desenvolvimento e função do sistema nervoso. O *C. elegans* foi o primeiro organismo multicelular a ter seu genoma inteiramente sequenciado (*Caenorharditis elegans* Sequencing Consortium, 1998). Isto permitiu que existam hoje muitas linhagens mutantes e transgênicas deste modelo *in vivo* (STRANGE, 2006). Com isso, o *C. elegans* vem ganhando espaço como alternativa a outros modelos experimentais, possibilitando a redução do uso de animais na pesquisa (KALETTA; HENGARTNER, 2006; WANG et al., 2022b).

O *C. elegans* é um organismo modelo que se tem recebido atenção científica pois apresenta diversas facilidades de manutenção, fácil cultivo em laboratório, não oneroso, além de possuir uma anatomia relativamente simples e ciclo de vida curto com quatro estágios larvais (L1-L4), como apresentado na Figura 3. A maioria dos vermes adultos de *C. elegans* são hermafroditas e podem produzir muitos descendentes, de 300 a 1000 ovos, com a fecundação por vermes machos (STRANGE, 2006; WANG et al., 2022b).

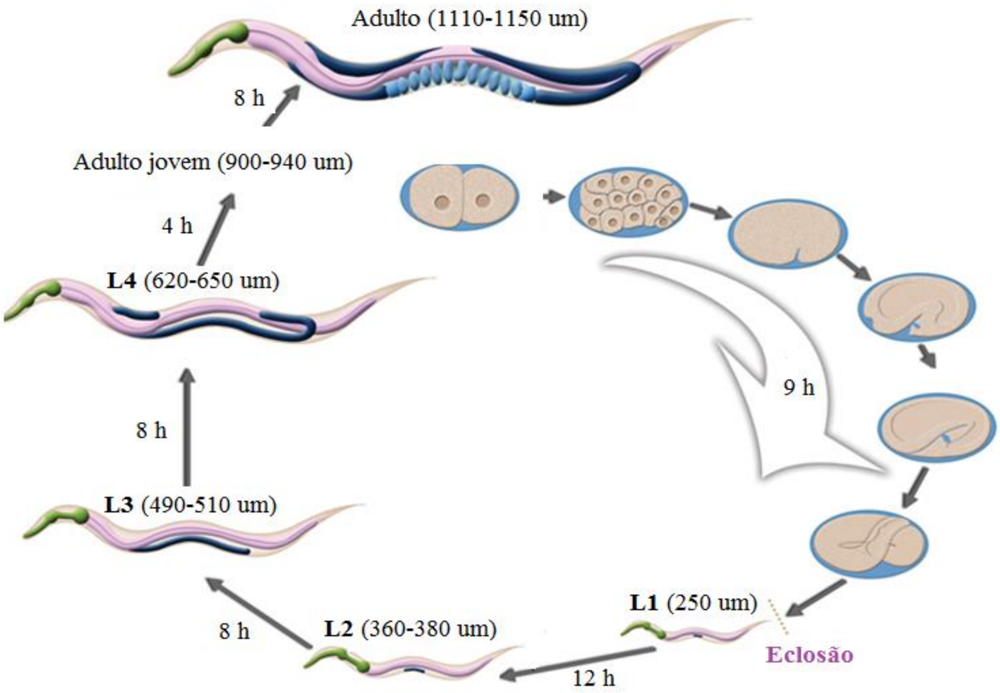

Figura 3. Ciclo de de vida do *C. elegans*, apresentando seus estágios larvais (L1-L4), o tempo de duração de cada estágio e o tamanho dos vermes em cada estágio. Fonte: adaptado de Worm Atlas. Disponível em: <https://www.wormatlas.org/aging/introduction/mainframe.htm>.

Atualmente, o *C. elegans* é amplamente utilizado para pesquisar processos biológicos, como sinalização celular, ciclo celular, regulação gênica, metabolismo, apoptose e mecanismos celulares de envelhecimento. O *C. elegans* possui muitos genes e vias de sinalização similares aos mamíferos, com aproximadamente de 60 a 80 % dos genes homólogos aos humanos (KALETTA; HENGARTNER, 2006). Essas semelhanças se dão a nível celular e molecular, especialmente em relação ao estresse oxidativo, garantindo assim eficácia na simulação de mecanismos de defesa antioxidante (HUNT, 2017; LI et al., 2023). Todas estas características fazem do *C. elegans* um modelo *in vivo* ideal para avaliar bioatividades e toxicidade de compostos alimentares (WANG et al., 2022b).

CAPÍTULO II – HEALTH EFFECTS AND PROBIOTIC AND PREBIOTIC POTENTIAL OF KOMBUCHA: A BIBLIOMETRIC AND SYSTEMATIC REVIEW

O capítulo II, intitulado “Health effects and probiotic and prebiotic potential of kombucha: a bibliometric and systematic review”, está apresentado na forma de artigo científico, publicado na revista Food Bioscience (https://doi.org/10.1016/j.fbio.2021.101332).

O artigo explora as informações disponíveis na literatura sobre a kombucha e seus benefícios à saúde, como atividades antimicrobiana, antioxidante, desintoxicante, hepatoprotetora, entre outras. Uma revisão sistemática também foi realizada para discutir especificamente os potenciais efeitos probióticos e prebióticos da kombucha.

Health effects and probiotic and prebiotic potential of Kombucha: a bibliometric and systematic review

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Abstract

Kombucha is a fermented beverage composed of a range of natural compounds such as sugars, ethanol, organic acids, and complex microbial communities of bacteria and yeasts. Based on this several biological properties are attributed to this drink. However, the production of kombucha is not standardized and the final composition of the beverage is highly dependent on the raw materials used and the physicochemical parameters adopted in the process. As a consequence, kombuchas not only vary from one producer to another but also from different batches of the same producer, making the assumptions of quality and properties questionable. In this review, we explore the largely unchecked relations between kombucha and its claimed health benefits. A systematic review was also performed to specifically discuss the potential probiotic and prebiotic effects of kombucha. Although several studies report that kombucha present antimicrobial, antioxidant, detoxifying, and hepatoprotective activities, among others, whereas others classify kombucha as a probiotic drink, there is a lack of scientific evidence about the content of probiotics in this drink and its possible role in the intestinal microbiota. These facts highlight the opportunities in researching and modifying the microbiome composition of kombucha, possibly improving the general qualities of this so-called functional drink.

Keywords: *Bibliometrix*; functional beverages; fermented tea; symbiotic product; gut microbiota modulation; post-biotics compounds.

1 Introduction

Kombucha is an ancient beverage obtained by the infusion or extract of *Camellia sinensis* and sugars fermented by a symbiotic culture of microbiologically active bacteria and yeasts (SCOBY) (Greenwalt et al., 2000). Kombucha is believed to have originated in China, in the Manchuria region, being consumed for more than 2,000 years (Dorothy et al., 2020; Jayabalan et al., 2014). Traditionally, kombucha was homemade, based on a very simple preparation. Initially, the tea leaves and sugar are added to boiling water, leaving the mixture to rest for a few minutes. After the cooling of this solution, a starter culture, consisting of a cellulosic pellicle (known as SCOBY) and a percentage of the previously fermented batch of kombucha is added to perform the fermentation of the sweetened tea. In this way, kombucha tea fermentation comprises two distinct portions, as demonstrated in Figure 1: a liquid tea phase and a floating microbial cellulose pellicle layer (Chen and Liu, 2000).

Figure 1. Two portions of kombucha fermentation process. (A) Floating microbial cellulose pellicle layer and (B) liquid tea phase.

This fermentation stage usually takes from 7 to 14 days; at the end of it, the SCOBY is removed from the kombucha along with a small volume of the ready drink to be used as a starter culture in the next fermentations (Dufresne and Farnworth, 2000). Currently, kombucha is commercially available, its consumption has been growing in several countries and it is considered one of the most popular low-alcohol beverages worldwide, mainly because of claims on its functional properties and health appeals (Watawana et al., 2015).

The production process of kombucha has not been standardized and the final composition of the beverage will heavily depend on the parameters adopted, such as type and concentration of tea and sugar (Shahbazi et al., 2018; Reva et al., 2015b; Watawana et al., 2016), fermentation time, and temperature (De Filippis et al., 2018; Tran et al., 2020), and in particular on the composition of the starter culture (Nguyen et al., 2015; Villarreal-Soto et al., 2018). The latter is a complex multi-species microbial ecosystem, mostly composed of acetic acid bacteria and yeasts. Several studies show that this community of microorganisms varies in great stance based on the geographical origin (Chakravorty et al., 2016; Coton et al., 2017; De Filippis et al., 2018) and also over time between batch fermentations of the same producer (Marsh et al., 2014). However, some genera prevalently appear in most starter cultures, such as *Gluconacetobacter*, *Komagataeibacter*, and *Acetobacter*, among bacteria, and *Saccharomyces*, *Zygosaccharomyces*, *Brettanomyces*/*Dekkera*, and *Candida* for yeasts (Jayabalan et al., 2014; Arıkan et al., 2020). It is important to note that in some kombuchas, lactic acid bacteria (LAB) may also be present, such as *Lactobacillus* and *Lactococcus*. However, due to their optional and occasional occurrences, these LAB do not seem to be essential in the microbial consortium of kombucha (Laureys et al., 2020; Morales, 2020; Murphy et al., 2018).

The set of microbial genera included in the kombucha are responsible for reducing the initial pH of the beverage, inhibiting the proliferation of possible contaminating bacteria, and also for carrying out the fermentation process (Neffe-Skocińska et al., 2017). Fermentation is initiated by yeasts that hydrolyze sucrose, converting it into glucose and fructose, ultimately producing ethanol, glycerol, and carbon dioxide. In the sequence, the bacteria oxidize ethanol, producing acetic acid and transforming glucose into glucuronic acid (Gaggìa et al., 2018). After the fermentation process, a refreshing, slightly acidic, and carbonated drink is produced, containing a diversified content of components, such as sugars (sucrose, glucose, and fructose remaining from fermentation), ethanol, amino acids, organic acids (mainly acetic, gluconic, and glucuronic acids), polyphenols, hydrolytic enzymes, and micronutrients, such as B-complex and C vitamins, and minerals (zinc, copper, iron, manganese, and cobalt) (Jayabalan et al., 2014; Kaczmarczyk and Lochyński, 2014). This nonetheless interesting composition is perhaps the basis for claims of several biological properties attributed to kombucha (Kapp and Sumner, 2019).

This article reviews the information available in the literature on the relationship between kombucha composition, consumption, and the health effects and probiotic potentials attributed to it. The contribution of this bibliometric and systematic review is to gather data about the biological properties of kombucha, improving knowledge about this so-called functional beverage.

2 Methodology

This study was performed to present a review of health effects associated with kombucha consumption and employed methods such as a bibliometric and systematic review, according to Figure 2.

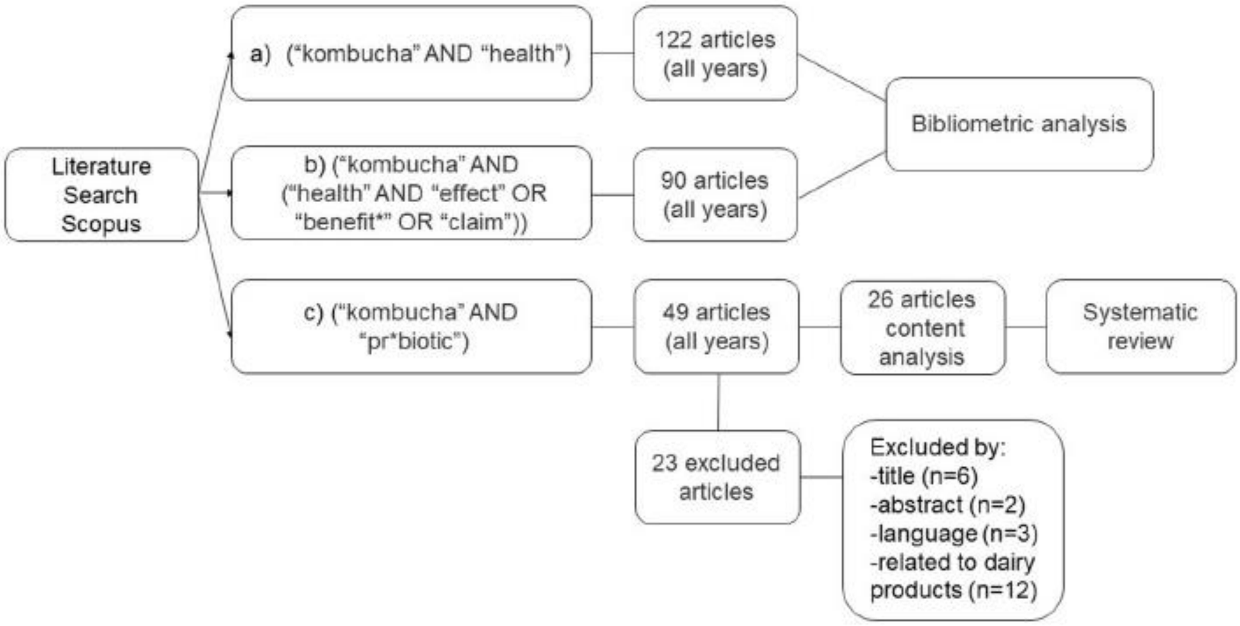

Figure 2. Methodological flowchart performed in this work.

The review was organized through a search for studies published to date in the Scopus (scopus.com) database using the following terms: (a) "kombucha" AND "health"; (b) "kombucha" AND (health" AND "effect" OR "benefit*" OR "claim"); (c) "kombucha" AND "pr*biotic", according to the supplementary materials. Boolean Operators were used to find the publications containing the terms of interest on the title, abstract, or keywords of the articles available on Scopus in the years.

The bibliometric review was conducted to present an overview of scientific production on the topic *kombucha* and *health* over the years and to identify the 50 most incident words between title, keywords, and summary among all the articles found. All publications found with the terms "a" and "b" in the English language until March of 2021 were exported in BibTex format and imported into Rstudio software (RStudio Team 2010) through the Bibliometrix package (bibliometrix.org). Figure 3 and Figure 4 were generated by bibliometric analysis.

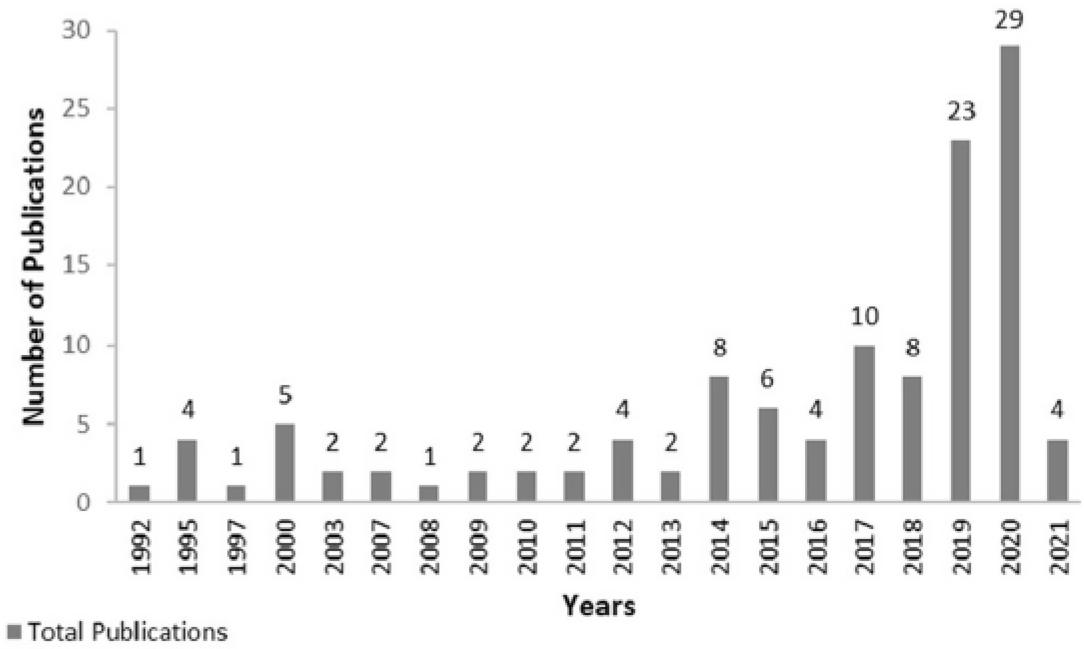

Figure 3. Scientific production over the years from articles found on Scopus with the terms "kombucha" AND "health".

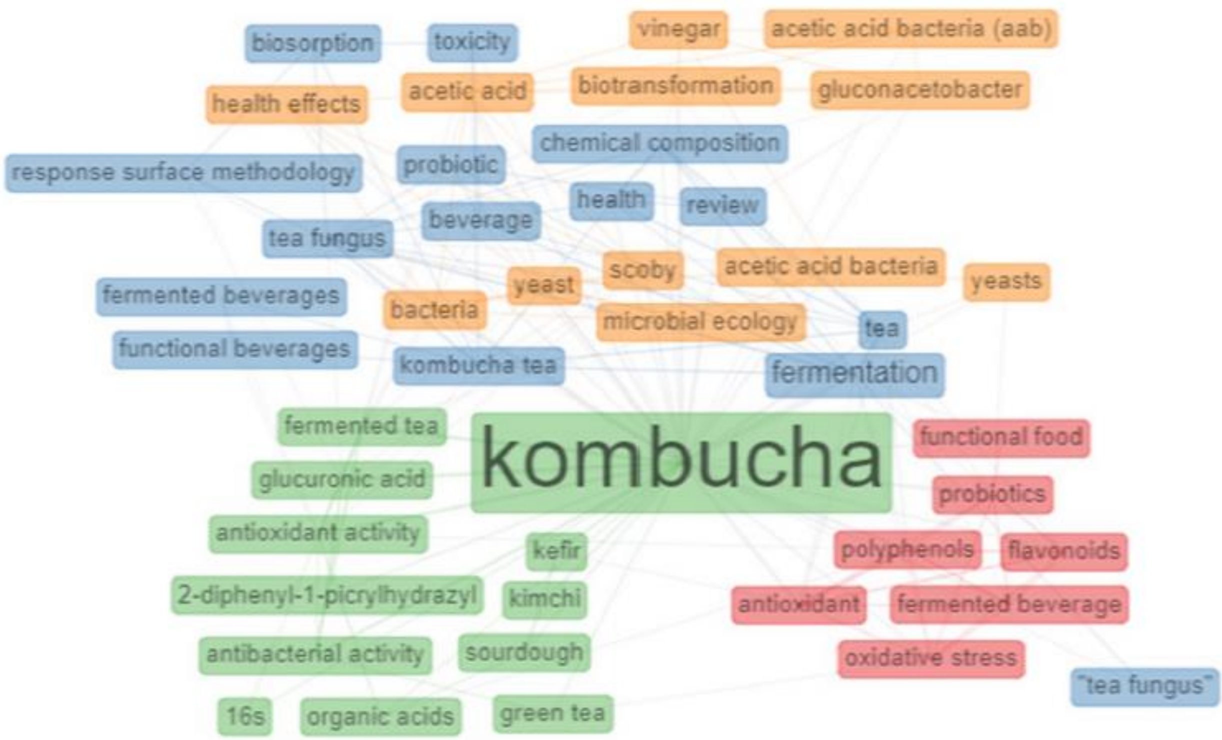

Figure 4. Thematic map generated on *Bibliometrix* from articles found on Scopus with the terms "kombucha" AND ("health" AND "effect" OR "benefit*" OR "claim").

Figure 3 was elaborated through 122 articles found using the term “a" analyzed according to the year of publication and Figure 4 was developed using thematic maps (Aria and Cuccurullo, 2017), generated through the 90 articles found in the term “b”. The systematic review was performed to explore, specifically, the general aspects in the context of the probiotics and prebiotics effects of kombucha and to respond to these questions:

- Is kombucha a probiotic beverage in the technical definition of the term?
- What is the evidence of this possible probiotic and/or prebiotic effect?

A content analysis of all articles found in the database from terms “c” was performed to discuss and deeply explore this topic.

3 Results and discussion

3.1 Kombucha and its health effects

To guide the discussion about kombucha and its health effects, Figure 4 was generated through bibliometric analysis and presents the thematic map from the analysis and grouping of the 50 most recurrent words found in the literature with the term “c” (Cobo et al., 2011). It is possible to observe in Figure 4 the occurrence of four main clusters, among which we can find the words “tea”, “kombucha tea” and “fermented tea” in two of these clusters. This results from the important association of tea in the effects of kombucha.

The biological activities performed by kombucha were primarily attributed to the composition of the tea itself used in the preparation of this beverage. *Camellia sinensis* is a species in the Theaceae family, which includes pu-erh, jasmine, white, oolong, yellow, green, and black tea (Chakravorty et al., 2019). Tea is historically recognized as a healthy beverage due to the presence of antioxidants (Wang et al., 2020). The distinction between these types of tea of the species *Camellia sinensis* is related to the degree of oxidation in the processing of tea leaves, which can significantly impact on its content and health benefits (Almeida Souza et al., 2020).

Traditionally, black tea is the most used for preparing kombucha. Along with the refined white sugar (sucrose), are considered the best ingredients to the proper content of the kombucha (Jakubczyk et al., 2020; Kaczmarczyk and Lochyński, 2014). Black tea is the most processed and susceptible to oxidation. The major compounds found in this type of tea are alkaloids (caffeine, theobromine, and theophylline), and polyphenols, such as catechins, theaflavins, and thearubigins (Anal, 2019). On the other hand, green tea undergoes little to no processing and does not suffer oxidation reactions. The term *green tea* can be found in the thematic map (Figure 4), configuring it as one of the most used in the articles. Gaggìa et al. (2018) in their study comparing black, green, and rooibos teas found that green tea presented the most significant antioxidant properties, whereas black tea showed the lowest values. In addition, green tea kombucha showed less degradation of epicatechin isomers compared to black tea kombucha (Jayabalan, et al., 2007). A great number of studies evaluated the choices of other types of tea for fermentation, instead of the traditional black tea, in order to enhance the functional properties of kombucha (Jayabalan et al., 2007; Gaggìa et al., 2018; Jakubczyk et al., 2020).

Over the years, investigations began to be carried out to understand the impact of tea fermentation by the microorganisms of the kombucha and the influence of this process on its beneficial health effects (Baschali et al., 2017; Dufresne and Farnworth, 2000). This can be seen in Figure 4 where, among the most cited words in the title, keywords, and summary of all articles analyzed, are the terms “fermentation”, “fermented tea”, “fermented drink” and “tea fungus”, also known as SCOBY, appearing in 3 of the 4 clusters on the thematic map.

The production of fermented foods is an ancient practice among many cultures and remains as the earliest biological method of food processing and preservation (Anal, 2019; Marco et al., 2017). Fermentation is considered one of the first known applications of biotechnology (de Almeida Souza et al., 2020). The fermentative process in kombucha drink has several advantages. We can cite initially the reduced risk of contamination, which are prevented by compounds formed during fermentation, such as organic acids, ethanol, and bacteriocins (Marco et al., 2017). Notwithstanding, the fermentation process is also responsible for many biochemical modifications of the drink (Chakravorty et al., 2016). Some of these improve the sensorial profile of the beverage, developing new and complex tastes and desirable flavors, characterizing kombucha, and differentiating it from the initial vegetable matrix (Marco et al., 2017).

Besides, fermented foods and beverages are an important nutritional source. This is because these products carry potential beneficial microorganisms and their metabolites. Some of these can be genetically similar to probiotics strains, providing microbial stability and changes in the digestibility of nutrients, enhancing the nutritional content (de Almeida Souza et al., 2020; Marco et al., 2017; Villarreal-Soto et al., 2018). Fermented products have been attracting the attention of consumers mainly because of the health benefits associated with their consumption (Marco et al., 2017). Today, Kombucha is a ready-to-drink beverage easily found in several markets and has been related to various functional effects such as helping to establish the balance of the organism and avoiding the appearance of chronic non-communicable diseases like obesity and diabetes. The claims of "functional beverages" and "functional food" are related to kombucha in two clusters in the thematic map (Figure 4). In view of this growing consumer demand, food industries have been exploiting the fermentation process for the development of new functional products (Baschali et al., 2017).

Along with the growing interest of the population and the food industry in fermented foods, research in this area has also been expanding, especially concerning kombucha. The potential therapeutic properties of kombucha have become a field of interest (Murugesan et al., 2009), as shown in Figure 3, which presents the increase in articles published with the terms “kombucha” and “health” over years. It can be noticed that, in the Scopus database, the publications in 2019 exceeded the number of 20 articles, increasing in 2020 to almost 30 published works. Until March 2021, 4 articles were published on the same base. One of the first articles found using these terms, by Hartmann et al. (2000), investigated the effects of chronic kombucha ingestion during an *in vivo* pilot study using mice. They found positive differences in appetitive behaviors, gross body weight, and greater longevity in animals receiving kombucha compared to the control group. Moreover, the consumption-group had longer spleens and enlarged livers. These adverse effects need more biochemical analysis for a full understanding. The hepatomegaly effect could be associated with some hepatotoxicity cases in humans, but the authors stated that comparable effects and mechanisms in humans were uncertain at the time. This shows kombucha may bring health or some undesirable outcomes by its consumption. Regarding positive outcomes, several health benefits make Kombucha popular as a functional beverage or food (Mousavi et al., 2020; Villarreal-Soto et al., 2018). Table 1 presents some *in vitro* and *in vivo* studies showing the possible biological properties associated with the consumption or administration of kombucha.

Table 1. *In vivo* and *in vitro* biological properties associated with kombucha.

Type of Assay	Biological property	Method/Experimental model	References
In vitro	Antibacterial	*S.epidermidis, S. aureus, Mi. luteus, E. coli, P. aeruginosa, S. typhimurium, L. monocytogenes*	(Battikh, Bakhrouf, and Ammar 2012)
		E. coli, H. influenzae	(Ivanišová et al. 2020)
		E. coli	(Mulyani et al. 2019)
		E. coli, S. sonnei, S. typhimurium, S. enteritidis, C. jejuni	(Sreeramulu, Zhu, and Knol 2000)

	E. coli, S. aureus. B. cereus, S. dysenteriae	(Valiyan, Koohsari, and Fadavi 2021)
	E. coli, Salmonella	(Ma et al. 2019)
	S. aureus, K. pneumoniae, E. coli, P.vulgaris, P. mirabilis, B. Subtilis	(Vitas et al. 2018)
	E. coli, S. typhimurium, M. luteus, S. epidermidis	(Deghrigue et al. 2013)
	E. coli, Salmonella, S. aureus, L. Monocytogenes	(Cardoso et al. 2020)
Antifungal	*C. glabrata, C. tropicalis, C. sake, C. dubliniensis, C. Albicans*	(Battikh, Bakhrouf, and Ammar 2012)
	C. krusei C. glabrata, C. albicans, C. tropicalis	(Ivanišová et al. 2020)
	C. albicans, A. niger	(Vitas et al. 2018)
Anti-carcinogenic	Human cell lines A549 (lung carcinoma), U2OS (osteosarcoma), 786-O (renal carcinoma)	(Jayabalan et al. 2011)
	Human cell lines A549 (lung carcinoma), Hep-2 (epidermoid carcinoma)	(Deghrigue et al. 2013)
	Human cell lines RD (rhabdomyosarcoma), Hep2c (cervix carcinoma-HeLa derivative) and murine cell line L2OB (fibroblast)	(Vitas et al. 2018)
	Human cell lines HCT-116 (colon cancer) and MCF7 (breast cancer)	(Villarreal-Soto et al. 2019; Villarreal-Soto et al. 2020)
	Human cell line PC-3 (prostate cancer)	(Srihari et al. 2013)
	Cell lines A549 (lung adenocarcinoma epithelial), HCT8 (ileocecal colorectal adenocarcinoma), CACO-2	(Cardoso et al. 2020)

	(colorectal adenocarcinoma epithelial)	
Anti-inflammatory	Human monocytic cells (THP-1) differentiated to macrophages	(Vázquez-Cabral et al. 2017)
	5- lipoxygenase enzyme	(Villarreal-Soto et al. 2019)
	15-lipoxygenase enzyme	(Villarreal-Soto et al. 2020)
Antioxidant	ABTS radical scavenging	(Ivanišová et al. 2019; Cardoso et al. 2020; Jafari et al. 2020; Aung and Eun 2021; Gamboa-Gómez et al. 2017)
	DPPH (1,1-diphenyl 2-picrylhydrazyl) radical scavenging	(Ivanišová et al. 2020; Mulyani et al. 2019; Villarreal-Soto et al. 2019; Villarreal-Soto et al. 2020; Gamboa-Gómez et al. 2017; Gaggìa et al. 2018; Hoon et al. 2014; Yang et al. 2009; Aung and Eun 2021; Jakubczyk et al. 2020; Bhattacharya, Gachhui, and Sil 2013)
	Oxygen Radical Absorbance Capacity (ORAC)	(Vitas et al. 2018; Gamboa-Gómez et al. 2017)
	Ferric reducing antioxidant power (FRAP)	(Aung and Eun 2021; Gaggìa et al. 2018; Jakubczyk et al. 2020)
	Reactive oxygen species induced by H_2O_2	(Yang et al. 2009; Vázquez-Cabral et al. 2017)
	Superoxide radical (O_2^-) scavenging ability	(Hoon et al. 2014; Yang et al. 2009; Bhattacharya, Gachhui, and Sil 2013)
	Cupric ion reducing antioxidant capacity (CUPRAC)	(Jafari et al. 2020)
	Nitric oxide scavenging (NO)	(Gamboa-Gómez et al. 2017)

	Anti-hyperglicemic	Glucose diffusion	(Gamboa-Gómez et al. 2017)
	Radioprotective (ionizing radiation)	Human peripheral lymphocytes	(Cavusoglu and Guler 2010)
	Cytotoxicity	Human peripheral blood lymphocytes	(Mrdanović et al. 2007)
		Human cell IMR90 (normal lung cell)	(Cardoso et al. 2020)
In vivo	Antihypercholestero lemic	White rabbits	(Alaei, Doudi, and Setorki 2020)
		Alloxan diabetic rats	(Aloulou et al. 2012)
		Wistar rats	(Bellassoued et al. 2015)
		Mice	(Yang et al. 2009)
	Anti-hyperglycemic	Alloxan diabetic rats	(Aloulou et al. 2012)
		Female C57BL/6 mice	(Gamboa-Gómez et al. 2017)
		Swiss albino male rats	(Bhattacharya, Gachhui, and Sil 2013)
		Streptozotocin-induced diabetic rats	(Zubaidah et al. 2019)
		Normal and alloxan-induced diabetic mice	(Shenoy 2000)
	Radioprotective (electromagnetic radiation)	Male albino rats	(Gharib 2014)
	Brain damage prevention	Ischemic rats	(Kabiri and Setorki 2016)
	Anti-hyperuricemic	Rats	(Sukrama 2015)
	Hepatoprotective	Mice	(Wang et al. 2014)
		Male albino rats	(Jayabalan, Baskaran, et al. 2010)
		Mice	(Lee et al. 2019)
	Microbiome modulation	Male C57BLKS db/db mice	(Jung et al. 2018)
	Longevity	Mice	(Hartmann et al. 2000)

Antioxidant	Male albino rats	(Sai Ram et al. 2000)
	Mice	(Yang et al. 2009)
Prevention of myocardial injury caused by isoproterenol	Male albino Wistar rats	(Lobo, Chandrasekhar Sagar, and Shenoy 2017)
Antiviral	Swine	(Fu et al. 2015)

Vīna et al., (2013) reviewed the main physiological properties related to kombucha consumption, listing, among others, antioxidant reaction, detoxifying properties, promoting immunity, and energizing capacity. The latter may be related to the release of iron from the kombucha tea, increasing the hemoglobin level. As a result, tissue oxygenation is favored, stimulating ATP synthesis in the body.

Regarding the antioxidant capacity, several studies show the association of kombucha consumption with the improvement of antioxidant protection to the cells, helping in the reduction of the damage caused by oxidative stress to the body (de Almeida Souza et al., 2020). The terms "antioxidant activity", "antioxidant" and "oxidative stress" appear in two clusters of the thematic map (Figure 4) associated with the words "polyphenols" and "flavonoids". Antioxidant capacity is one of the best-known actions performed by phenolic compounds, and kombucha is a beverage rich in these components. For instance, Cardoso et al. (2020) screened the phenolic profile of green and black tea and their resulting kombuchas and observed that black tea presented a higher abundance of phenolic compounds. Also, this kombucha showed a greater diversity of these components after the fermentative process at 25 °C for 10 days.

Differences in the profile of phenolic compounds were observed by ultra-performance liquid chromatography coupled with an electrospray ionization quadrupole time-of-flight mass spectrometry operating in MSE mode (UPLC-QTOF-MSE) analysis wherein 127 phenolics were detected in the green and black tea kombuchas (70.2 % flavonoids, 18.3 % phenolic acids, 8.4 % other polyphenols, 2.3 % lignans, and 0.8 % stilbenes). At least 103 of these phenolic components were detected for the first time in kombuchas. Among them are pelargonidin 3-O-glucoside, gardenin B, lithospermic acid, and oleuropein, found exclusively in black tea kombucha. These bioactive substances are known for their activities, such as antitumoral and hypotensive effects and positive gut microbiota modulation (Cardoso et al., 2020). Ivanišová et al., (2019) also compared the phenolic profile of black tea and its kombucha after fermentation at 22 °C for 7 days. They found higher content of total polyphenols in kombucha (0.42 mg gallic acid equivalent (GAE)/ml), flavonoids (0.13 mg quercetin equivalent (QE)/ml), and phenolic acids (0.19 mg caffeic acid equivalent (CAE)/ml) than in black tea (0.18 mg GAE/ml; 0.02 mg QE/ml; 0.05 mg CAE/ml, respectively). The same author further demonstrated that black tea kombucha has a significantly higher value in total polyphenol content (412.25 mg (GAE)/l) compared to black tea (180.17 mg GAE/l) (Ivanišová et al. 2020). Similarly, Wang et al. (2020) reported an increase in the concentrations of total phenol and flavonoids of kombucha during fermentation, boosting its health benefits. Sai Ram et al. (2000) carried out an *in vivo* study, showing that phenolic compounds in kombucha, in addition to reducing oxidative stress, could decrease the immunosuppression caused by this stress condition. Based on these studies, kombucha would contribute to immunity and reduce inflammatory foci and cell damage, preventing the development of chronic non-communicable diseases (Vīna et al., 2013). Despite that, the phenolic content of kombucha is variable and changes according to the chemical composition of the tea before fermentation. It will also depend on the concentration of carbon source, pH, temperature, inoculum size, and microbial community of kombucha (Jafari et al., 2020). The bacteria and yeasts present in kombuchas are responsible for releasing enzymes that determine the metabolites generated during its fermentation, as well as degrading the polyphenols in smaller molecules (de Almeida Souza et al., 2020; Jakubczyk et al., 2020).

Ivanišová et al., (2019) revealed kombucha has a high concentration of phenolic metabolites, such as gallic acid, chlorogenic acid, protocatechuic acid, p-coumaric acid, ellagic acid, rutin, vitexin, and resveratrol. Other compounds, such as catechins, epicatechin, and flavonoids are also produced by the microorganisms through biotransformation (Villarreal-Soto et al., 2018; Jayabalan, Malini, et al., 2010; Ivanišová et al., 2020; Mousavi et al., 2020).

When analyzing the thematic map (Figure 4), it can be identified as a cluster that reunites the words “bacteria”, “acid acetic bacteria”, “yeast”, “SCOBY”, “microbial ecology”. In the same cluster, the terms “biotransformation” and “health effects" appear. Thereby, it can be noticed the importance of the microbial community of kombucha in the generation of products with better biological action than in unfermented tea (Bhattacharya et al., 2013).

As demonstrated by many authors, the fermentative process is responsible for the increase of the bioactivity of these phenolic components. Chakravorty et al., (2016) demonstrated that fermentation of kombucha gradually enhanced its ability to eliminate radicals, peaking on the seventh day of fermentation. Ivanišová et al., (2020) measured the antioxidant activity of kombucha and observed significantly higher values (1318.56 mg Trolox equivalent antioxidant capacity (TEAC)/l) when compared with unfermented black tea (345.59 mg TEAC/l). Similarly, Bhattacharya et al. (2013) found that despite black tea shows potent antioxidant power itself, the scavenging activities of fermented black tea on parameters such as 2,2-diphenyl-1-picryl hydrazyl (DPPH), hydroxyl, and superoxide radicals increased 18.9 %, 17.2 % and 14.97 % respectively. Ivanišová et al. (2019) also observed that kombucha after 7 days of fermentation at 22 °C showed higher antioxidant capacity by ABTS and phosphomolybdenum method (1.16 mg TEAC/ml and 2.04 mg TEAC/ml, respectively) when compared to black tea (0.67 and 0.81 mg TEAC/ml, respectively).

Abuduaibifu and Tamer (2019) verified that along with the enhancement of the protective effect against oxidative stress of three kombuchas samples (black tea, black goji berry, and red goji berry kombucha), the bioaccessibility properties also increased. The total phenolic content of all samples was higher in post-digestion (gastric, intestinal) than in pre-digestion. In the same way, Aloulou et al., (2012) demonstrated by histological analysis that kombucha tea had better protective actions on the pancreas and liver-kidney functions on rats, compared to black tea. These *in vivo* improvements and the greater protective capacity of the kombucha is due to the large number of phenolic compounds and its products, which are responsible for inhibiting the formation of reactive species and strengthening endogenous enzyme defenses (Murugesan et al., 2009; Sai Ram et al., 2000; Vázquez-Cabral et al., 2017; Mulyani et al., 2019). Furthermore, this characteristic may even promote curative effects, for example, in conditions of hyperglycemia and hypercholesterolemia (Aloulou et al., 2012; Bellassoued et al., 2015; de Almeida Souza et al., 2020).

In relation to the fermentation process, the products synthesized by microorganisms in kombucha may play an important function in human health caused by their detoxifying action (Anal, 2019). As for the products that are formed during the fermentative process, the most relevant and often reported is glucuronic acid, the main organic acid resulting from the oxidation of glucose in kombucha (Neffe-Skocińska et al., 2017). This acid is naturally synthesized in the human liver and has detoxifying properties from its potential of binding toxin molecules and by carrying out the excretion of xenobiotics and phenols through the kidneys or intestines. Thus, kombucha assists in the elimination of excess metabolites and may bring relief in situations related to the accumulation of toxins in the body, such as kidney stones, rheumatism, or arthritis (Dufresne and Farnworth, 2000; Kaczmarczyk and Lochyński, 2014). The relevance of this organic acid can be noted by its presence on the thematic map (Figure 4). The production of this important organic acid is directly related to predominant bacteria in the starter culture, which can vary largely depending on the variables of the process, such as pH and temperature. De Filippis et al. (2018) characterized bacterial populations in kombucha fermented at 20 °C and 30 °C and evaluated glucuronic acid production, finding substantial differences between the final products. Fermentation at 20 °C favored *Gluconacetobacter xylinus* growth and resulted in approximately 45 mg.L^{-1} of glucuronic acid, whereas fermentation at 30 °C favored G*luconacetobacter saccharivorans*, resulting in a production of 28 times higher glucuronic acid concentrations (approx. 1300 mg.L^{-1}). These results empathize the importance of not generalizing information about kombucha, since one product may be completely different from another, even when using the same starter culture.

Other compounds with health-modulating potential may result from the fermentation, such as B vitamins, including folate, riboflavin, and B_{12} (Chamlagain et al., 2015; Martínez Leal et al., 2018). Nevertheless, these microbial-derived products are strain dependent and are synthesized by certain bacteria in plant and dairy foods (Russo et al., 2014). Therefore, as the microbial community of the kombucha is variable, not all kombuchas will necessarily produce these vitamins. In this sense, Wang et al. (2014) identified the functional strains and quantified the functional components with hepatoprotective effects in kombucha tea (KT). The authors concluded that *Gluconacetobacter* sp. A4 was the microorganism able to produce significantly high values of the D-saccharic acid-1,4-lactone (DSL) in the modified KT (fermented by single *G. sp*. A4) when compared to original kombuchas, fermented by tea fungus. DSL is believed to be the compound responsible for hepatoprotection conferred by kombucha by assisting the glucuronic acid to exert some properties like anti-tumor, detoxifying, and antioxidant (Baschali et al., 2017). These results encourage the standardization of inocula in the production of kombucha, with microorganisms selected in the starter culture, in order to synthesize compounds of interest.

Finally, antibacterial activity also stands out on the thematic map (Figure 4), being one of the most reported effects in the literature in relation to kombucha. The antimicrobial action of this drink has already been reported for several microorganisms, including Gram-negative and Gram-positive bacteria (Dufresne and Farnworth, 2000), the most frequently reported being *Escherichia coli*, *Staphylococcus aureus*, and *Helicobacter pylori*. The latter being the cause of gastric ulcers and other complications of the digestive system (Kaczmarczyk and Lochyński, 2014). The antibacterial activity of kombucha is related to the microbial community of its starter culture, acting in a symbiotic way to prevent potential contaminations, and also to the metabolites formed during the fermentation, such as catechins, ethanol, and organic acids, especially acetic acid (Kaczmarczyk and Lochyński, 2014; Watawana et al., 2015). This acid is reported to control the growth of pathogenic bacteria, avoiding contamination in the production of kombucha and having antifungal and antiviral properties as well (Watawana et al., 2015; Mousavi et al., 2020; Fu et al., 2015). It should also be recalled that low pH values contribute to this antimicrobial action.

Although several benefits are regarded to the consumption of kombucha, it is important to highlight that it may be harmful when not safely brewed and also may lead to metabolic acidosis in some individuals when consumed in excess (Martini, 2018). Adverse effects from kombucha consumption have been reported, such as allergies, jaundice, nausea, head and neck pain (Srinivasan et al., 1997). Also, kombucha should be avoided by lactating and pregnant women (Jayabalan et al., 2014).

Nevertheless, kombucha fulfills the consumer's demand for healthy beverages, reflecting in a market growth that is partially based on widespread information of health-promoting effects (Kapp and Sumner, 2019; Kim and Adhikari, 2020). Recently, some scientific studies are using kombucha as a vehicle to reduce the pathogens associated with human illnesses (Mousavi et al., 2020), while many researchers have reported the different activities of kombucha and demonstrated its potential health benefits. This wide range of advantages has led to investigating the role of the microbiome on health, where it is proposed that kombucha may have the ability to act as a probiotic agent (Kapp and Sumner, 2019; Kim and Adhikari, 2020; Martínez Leal et al., 2018; Mann et al., 2017).

3.2 **Probiotic and prebiotic potential**

Recent studies associate the consumption of kombucha as influencing and modifying the gastrointestinal microbiota. The human gut microbiota is complex and colonized by approximately $1 \cdot 10^{13}$ to $1 \cdot 10^{14}$ microorganisms (Li et al., 2016). Studies report that the phyla most identified in the colon are: *Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria,* and *Proteobacteria* (Zmora et al., 2019). When this microbial community is in dysbiosis, that is, pathogenic species are in greater concentration, an imbalance situation arises, and different pathologies can originate in individuals. Because of the association between the deregulated intestinal microbiome and the genesis of illnesses, there is a growing interest in the modulation of microbial genera (Morales, 2020).

In this context, with a currently strong link between intestinal health and its direct role in human health, new treatment approaches and pharmaceutical interventions for disease prevention are being investigated. Thus, one of the main therapies that has been implemented is the use of beneficial microbes, such as probiotics in functional foods (de Almeida Souza et al., 2020; Heinen et al., 2020). According to the World Health Organization (WHO), probiotics are "*live microorganisms which when administered in adequate amounts confer a health benefit on the host*" (FAO/WHO, 2006; Gibson et al., 2017; Hill et al., 2014). Probiotics are said to maintain the intestinal barrier integrity, improve digestion and modify and select the host-microbiota (Watawana et al., 2015; Kozyrovska et al., 2012). There are different mechanisms in that these microorganisms perform these actions. First, they stimulate the growth of beneficial resident gut microorganisms by supplying some substances generated by their metabolism. Second, directly inhibit the growth of pathogenic genera through niche competition, bacteriocins, or a decrease in pH. Finally, some microorganisms can indirectly interact with the host epithelium and the epithelial immune system. As a result, they impact the host-microbiota by controlling pro-inflammatory cytokines (Derrien and van Hylckama Vlieg, 2015; Marco et al., 2017). The agents most associated with the probiotic function are lactic acid bacteria, especially *Lactobacillus* and *Bifidobacterium*, non-lactic acid species such as *Bacillus cereus* and *Propionibacterium freudenreichii*, and yeasts of the genus *Saccharomyces* (*boulardii* and *cerevisiae*) (Kozyrovska et al., 2012; Holzapfel et al., 2001). These microorganisms can assist in the generation of a healthy microbiome, restoring homeostasis in the body (Kozyrovska et al., 2012).

Human diets are capable of changing the composition of gut microbiota. Modifications in the food intake pattern, even for a short period of time, may increase the amount of some bacteria, while reducing the number of others, altering the whole microbial community (Heinen et al., 2020). In this sense, it is observed that a typical western diet, with high fat and sugar intakes, is associated with increased Firmicutes and reduced Bacteroides. On the other hand, plant-based diets, with high consumption of fibers such as fruits and vegetables, leads to a higher abundance of Bacteroidetes. Thereby, the consumption of a diversified diet is the key to microbial homeostasis (Million et al., 2018; Zmora et al., 2019).

The delivery of beneficial microorganisms to the gut microbiota is dependent on the food matrix. Many fermented foods and beverages are related to the modification in the indigenous microbiota. The regular ingestion of these fermented products possibly enhances up to $1 \bullet 10^4$ times the microorganisms' resident in the colon (Lang et al., 2014). Although fermented products demonstrate being a potential vehicle to provide probiotic strains, some of these are processed in a way that those beneficial microorganisms are absent when consumed (Marco et al., 2017).

Kombucha is known worldwide as a probiotic drink, as it contains live microorganisms in its composition. This beverage represents the fastest growing product in the probiotics market. It is possible to find kombucha labels in several countries with the appeals of “natural”, “organic”, “raw”, “living culture”, “non-dairy probiotics”, “healthy for your gut”, among other claims (Kim and Adhikari, 2020). However, some of these statements are mostly unfounded or at least unproved. So much so, that in most parts of Europe the use of claims such as "probiotics" or “contain probiotics” is not allowed on labels (Marco et al., 2017). The presence of probiotic strains is inconsistent in kombuchas. Normally, they are not present or remain in low concentrations, mainly after storage (Matei et al., 2018; Coton et al., 2017; Fu et al., 2014).

Preparations or products that contain probiotic microorganisms must have an adequate quantity, making them capable of altering the intestinal microbiota through implantation or colonization (Schrezenmeir and de Vrese, 2001; Kapp and Sumner, 2019). So far, there is no evidence that these probiotic microorganisms are in sufficient quantity in kombucha and bring some specific health benefit (Martini, 2018). Furthermore, products that use probiotic microorganisms in order to confer health benefits to the consumer must indicate the dosage and the time needed to obtain the desired effect, based on scientific evidence obtained from studies carried out *in vitro* and *in vivo* (in animals and humans) (FAO/WHO, 2006). Moreover, legislation from the country of sale must be observed and may vary from place to place (FAO/WHO, 2006).

In this research, many articles mentioned kombucha as a “probiotic-containing food”, without presenting scientific evidence that the microbial community indeed delivers the benefits to the host (Dikeocha et al., 2020; Fu et al., 2014; Onur et al., 2019; Salafzoon et al., 2018; Ranjan et al., 2020; Vilela, 2019; Chandrakala et al., 2019).This possibly inadequate use of the term *probiotic* frequently appears and may be related to the fact that several articles have reported the benefits of kombucha to health and also owing to the presence of live microorganisms in the drink (Kapp and Sumner, 2019; Martínez Leal et al., 2018). However, the studies suggest that the benefits come from the tea and metabolites from fermentation, such as organic acids, polyphenols, and vitamins (Kapp and Sumner, 2019). To the best of our knowledge, no studies have reported health benefits from the whole microbial community of kombucha. Notwithstanding, the microbial culture of kombucha has been used in other raw materials in order to develop new *probiotic* drinks (Soares et al., 2021). Mulyani et al. (2019), in their study, aimed to produce an allegedly probiotic beverage made from beat (Beta vulgaris L.) fermented with kombucha culture to prevent digestive infections. The results showed that the drink had antibacterial activity against *E. coli*. The authors, however, did not identify or quantify the probiotic strains in the drink, and regarding antibacterial activity, it is not known to what extent this effect would be observed *in vivo* gastrointestinal tract (Lavefve et al., 2019). Fermented probiotic products are produced from a complex and unstable set of microorganisms, which can be modified because they remain in open environments and are susceptible to variations according to the growing conditions and substrates available for their development (Reva et al., 2015a). The studies reporting yeasts and bacteria communities in kombucha show that there exists a huge variability between products, although there are some genera that appear more frequently. In the case of bacteria, the most predominant genera are *Komagataeibacter* (Arıkan et al., 2020; Chakravorty et al., 2016; Gaggìa et al., 2018; Reva et al., 2015a), *Gluconacetobacter* (Marsh et al., 2014; Villarreal-Soto et al., 2020) and *Acetobacter* (Coton et al., 2017). In relation to its potential probiotic capacity, it is assumed that the set of central microorganisms of the kombucha could recruit some environmental bacteria, particularly of the genus *Lactobacillus*. Possibly, this whole microbial community favors the fermentation of kombucha for the development of a probiotic characteristic (Reva et al., 2015a). However, only a few genera of LAB may be found in this beverage, such as *Lactobacillus* (Chakravorty et al., 2016; Coton et al., 2017; Marsh et al., 2014; Reva et al., 2015a), *Bifidobacterium* (Chakravorty et al., 2016; Marsh et al., 2014), and *Lactococcus* (Marsh et al., 2014), all appearing to a lesser amount than acetic acid bacteria. Yeast genera are more variable than bacteria, with

Brettanomyces/*Dekkera* being the prevalent species (Coton et al., 2017; Gaggìa et al., 2018; Reva et al., 2015a; Villarreal-Soto et al., 2020), followed by *Zygosaccharomyces* (Arıkan et al., 2020; Coton et al., 2017; Marsh et al., 2014; Villarreal-Soto et al., 2020), *Candida* (Chakravorty et al., 2016; Villarreal-Soto et al., 2020), and *Hanseniaspora* (Coton et al., 2017).

Among the most predominant bacteria and yeasts found in kombucha, some have been studied regarding their potential probiotic properties. Matei et al., (2018) isolated nine yeast species from pollen fermented kombucha and identified them by PCR-ITS RFLP technique, aiming isolation of prospective probiotic yeasts. It was possible to identify the species *Dekkera bruxellensis*, but no studies on probiotic potential were performed. The same research group has studied the possible probiotic effect of five LAB isolated from kombucha (Matei et al., 2018). The isolates of *Pediococcus pentosaceus* species were subjected to on-plate screening for bacteriocin production, wherein three of five strains were positive. Furthermore, isolates were tested for bile-salt resistance, with three strains showing tolerance to 3 % and 6 % of bile salts. Among the five isolates, the "L5" strain showed higher potential as a probiotic because it was the only bacterium that showed both bacteriocin production and bile salt resistance (Matei et al., 2018). This strain was further studied regarding the potential of adhesivity to Caco-2 intestinal cells and authors suggested a probiotic effect, since L5 adhered to intestinal cells in a time-dependent manner (Utoiu et al., 2018). *Pediococcus* is not a common genus found in kombucha and it has not been identified in studies that used next-sequencing generation (Arıkan et al., 2020; Bueno et al., 2021; Chakravorty et al., 2016; Coton et al., 2017; Gaggìa et al., 2018; Marsh et al., 2014; Villarreal-Soto et al., 2020).

The variability in the kombucha microbial community demonstrates how complex is the process of this fermented beverage. Although some other fermentations contain only a few dominant taxa, the fluctuations in the microorganisms of kombucha may affect the quality of this drink (Reva et al., 2015a; Marco et al., 2017). With the increase in kombucha consumption, consequently, its homemade production evolved to an industrial scale (Soares et al., 2021). While SCOBY can be useful as a starter culture for homemade kombucha, in the industrial sector this type of inoculum is not suitable because of the unrepeatable and uncontrollable qualities of the process, leading to variations in the organoleptic properties, affecting the final product (Marco et al., 2017; Wang et al., 2020). The beverage industries are investing in food safety and improvements in the biotechnological properties of kombucha production. Standard process conditions and a stable set of microorganisms throughout fermentations result in kombuchas showing stable characteristics. The consistency of end products is a primordial factor to produce high-quality food (Marco et al., 2017; Mousavi et al., 2020). Thus, efforts are being made to characterize the essential microbiome of kombucha in an attempt to standardize its production and also to identify the best mixture of microorganisms to improve kombuchas yield (Mousavi et al., 2020; Wang et al., 2020). Wang et al., (2020) developed a symbiotic microbial community for kombucha with three main microorganisms, *Acetobacter pasteurianus, Gluconacetobacter xylinus,* and *Zygosaccharomyces bailii*. The authors verified that this microbial set could accelerate the fermentation process of kombucha and generated a beverage with desirable qualities. Furthermore, the ability to select species of bacteria and yeasts is also an important approach to determine the metabolites generated through the fermentation process. Thereby, the modification of the kombucha inoculum can also be fundamental in improving the functional properties of this drink (Kozyrovska et al., 2012). Wang et al., (2020) also concluded in their study that the designed starter culture was capable of enhancing the antioxidant content of kombucha. Similarly, Nguyen et al. (2015) carried out a screening of ideal strains for high production of glucuronic acid, in order to improve the antioxidant, antimicrobial and detoxifying activities of the kombucha. The authors found that the best ratio would be an initial combination of *Dekkera bruxellensis* KN89 and *Gluconacetobacter intermedius* KN89 in a 4:6 parts, respectively.

Another alternative strategy to obtain a probiotic kombucha could be the addition of well-known and studied probiotic strains into the fermentation process, as was shown in a few studies reported in this systematic review. Al-Dulaimi et al. (2018) developed two kombuchas using black tea and skim milk, adding in each formulation a probiotic culture starter, composed of two species of *Lactobacillus* (*delbruekii* and *fermentum*). Investigating the beneficial effects of both kombuchas on some physiological and biochemical parameters in rats, the authors verified that animals that were treated with probiotic kombuchas had decreased concentrations of total lipid profile, ALT, AST, ALP hepatic enzymes, and serum glucose, when compared to the control group. The reduced serum glucose levels can be addressed to the activity of probiotic bacteria since they use glucan compounds to carry out fermentation. Thus, this beverage could present some beneficial actions on the liver and human body health in general. In another study using the incorporation of probiotics Bueno et al. (2021) developed a coffee kombucha added of two probiotic strains: *Lactobacillus rhamnosus* (LR) and *Lactobacillus casei* (LC). After fermentation and removal of the biofilm, the kombucha fermented from coffee was added with LR or LC, or no probiotics (control) and stored for 15 days at 4 °C. The authors evaluated the survival of probiotics in the kombuchas and the microbial viabilities under simulated gastric and intestinal conditions. The authors also identified the microbial diversity through next-sequencing generation on the fifteenth day of storage. Results were very interesting and showed LC viability in coffee kombucha remained without significant changes at the end of storage, whereas LG presented a slight reduction of viable counts, allowing to suggest that LC and LG survive in the gastric and intestinal environment. Microbial community analysis revealed that the addition of *Lactobacillus* shifted the predominance of *Acetobacteraceae* family to *Lactobacillaceae* and, although the species added was *L. rhamnosus* and *L. casei*, the predominant genus in each kombucha was *Lactobacillus zeae* for LR and *Lactobacillus paracasei* for LG, both strains documented as probiotics (Bueno et al., 2021).

On the other hand, Fu et al. (2014) evaluating the survival of specific strains in kombucha after 14 days of refrigerated storage did not obtain results as positive as in the previous upper study. The authors developed a low-cost green tea kombucha (LGTK) inoculated with 5 % of starter culture composed of *Saccharomyces cerevisiae Meyen ex Hansen* (10^8 CFU/mL), *Gluconacetobacter* sp. (10^8 CFU.mL^{-1}), and *Lactobacillus plantarum* (10^8 CFU.mL^{-1}), in a ratio of 1:1:1. At the end of the study they verified that, under refrigeration at 4 °C, acetic acid bacteria moderately decreased up to ten days of storage, whereas the survival rate of lactic acid bacteria was only 0.98 % on the eighth day of storage. These results reinforce the importance of the choice of the microorganisms contained in the starter inoculum and their influence on the final probiotic characteristics of the beverage.

Some beverage companies do not pasteurize the industrial scale kombuchas in order to maintain the drink's natural microbiome, in such a way that keeping the product raw would allow the development of a prebiotic or probiotic drink. In another approach, other companies proceed with the pasteurization process in their production and subsequently add recognized probiotic strains, such as *Bacillus coagulans*, *Saccharomyces boulardii* and *Lactobacillus rhamnosus*, to the final kombucha formulation (Kim and Adhikari, 2020). It is important to note that this strategy of adding probiotics is useful depending on the country of sale regulation. In the case of Brazil, for instance, the Normative Instruction for Quality and Identity Standards for Kombucha does not allow the addition of strains after fermentation, as it must be added at the beginning of the process (BRAZIL, 2019).

The criteria for probiotic strain selection involve functional, safety, technological and physiological aspects (Terpou et al., 2019). Among these aspects, the kombucha microbial community presents high potential as probiotic because it is non-pathogenic (Arıkan et al., 2020; Chakravorty et al., 2016; Gaggìa et al., 2018), presents competitive inhibition of undesirable microbiota (Ayed et al., 2017; Kaewkod et al., 2019; Sreeramulu et al., 2000), and tolerance to acidic environments (Villarreal-Soto et al., 2018). It is important to note that most probiotic products are of dairy origin. Plant-based probiotic foods and beverages are less available in the market (Muhialdin et al., 2021). However, with the growing knowledge about their health properties, including their supposed role in boosting immunity, taking into account the recent Covid-19 pandemic, the demand for plant-based probiotics has increased (Antunes et al., 2020). Kombucha has this advantage because it is a non-dairy drink. Its vegetable matrix used in its preparation guarantees that it can be consumed by vegans, people with lactose intolerance, or by people who are reducing the consumption of products of animal origin (Panghal et al., 2018; Reva et al., 2015a).

Furthermore, some authors have stated that kombucha not only can be a probiotic beverage but also acts as a prebiotic (Dufresne and Farnworth, 2000; Kozyrovska et al., 2012). Prebiotics are food-containing components that serve as a substrate for the beneficial microorganisms (Gibson et al., 2017; Hill et al., 2014). These prebiotic agents are responsible for nourishing the probiotic strains, thus selectively stimulating the growth and/or activity of the microbiota resident in the human gut (Marco et al., 2017; Heinen et al., 2020).

Lavefve et al., (2021) studying the microbiota modulation demonstrated that kombucha and especially the kombucha supernatant were able to significantly enhance the *in vitro* growth of *Bifidobacterium* and *Collinsella*. These preliminary results showed that kombucha stimulates these beneficial microorganisms, acting in a prebiotic manner.

Among the possible prebiotic components present in kombucha are phenolic compounds. Some studies describe these phytochemicals as substances that exert prebiotic-like effects, being a class of bioactive most associated with the promotion of a healthy microbiome (Million et al., 2018; Dueñas et al., 2015; Tomás-Barberánet al., 2016). This association occurs because most of the ingested polyphenols pass intact through the small intestine, reaching the large intestine where they will be used as a substrate by beneficial genera of the microbiota. In a reciprocal interaction, these microorganisms increase the bioavailability of the polyphenols metabolizing them into smaller molecules (Ozdal et al., 2016; Hollister et al., 2014). Therefore, there is an enhancement of the detoxifying enzymes and improvement in the antioxidant response of fermented products, protecting the organism from oxidative stress and other possible chemical damages (Marco et al., 2017; Senger et al., 2016).

Other classes of food components showing prebiotic properties are some polysaccharides. The cellulosic pellicle of kombucha, present in the SCOBY, could help in the growth and maintenance of the beneficial microbes in the beverage by the presence of insoluble fibers in its composition (Kozyrovska et al., 2012; Marco et al., 2017). These substances could be fermented by the microbial community of kombucha and then release some secondary metabolites, mainly short-chain fatty acids (SCFAs), such as acetate, propionate, butyrate, lactate, and succinate (Salazar et al., 2016; Sonnenburg and Fischbach, 2011). These compounds are known to provide some health benefits to the host. Particularly, butyrate is known to strengthen the intestinal epithelial barrier and to interact with inflammatory pathways, promoting the differentiation and maintenance of the defense cells, boosting immunity (Kozyrovska et al., 2012; Kim and de La Serre, 2018). Lactate, in turn, is said to reduce the reactive oxygen species in intestinal enterocytes (Kahlert et al., 2016). Therefore, if a portion of the lactic acid present in fermented kombucha reaches human metabolism, that organic acid may provide this health protection. Additionally, some ingested microorganisms produced in fermented foods might synthesize some vitamins, amino acids, and growth factors, such as exopolysaccharides, which could also compete as antioxidants, stimulating the immune system and preventing the adhesion of pathogens to the intestinal mucosa (Marco et al., 2017; Gerritsen et al., 2011; Thomas et al., 2017; Makino et al., 2016).

All these metabolites produced by the intestinal microbiota, such as SCFA, polyphenols, vitamins, and other functional compounds derived from the diet have recently been called post-biotics (Lavefve et al., 2021). Post-biotic is a new term used in the area of food and therefore there is no consensus on this concept yet. However, post-biotics can be defined in a wide spectrum as products or by-products generated or released by microorganisms during growth and fermentation in complex microbiological culture, food, or intestine (Moradi et al., 2021). These metabolites could resolve the intestinal imbalance, acting in the reestablishment of the microbiota symbiosis. Thus, post-biotics can offer positive physiological effects to the host, helping to promote their health (Klemashevich et al., 2014; Cuevas-González et al., 2020).

All things considered, it is known that the claim of any artisanal kombucha as a probiotic is inaccurate since the composition and concentration of the microorganisms present in the drink are unknown and change among batches depending on the fermentation variables (De Filippis et al., 2018). However, kombucha has been proven to be a potential source for isolation of probiotic bacteria in industrial or pharmaceutical applications (Matei et al., 2018; Sinir et al., 2019; Utoiu et al., 2018). Also, prebiotic potential and post-biotics compounds in kombucha have recently become research topics. In order to have a stated functional kombucha beverage, further studies are needed through the development of a defined starter culture for the fermentation of kombucha, as it happens for other fermented products such as yogurt, wine, or beer. Still, more studies are needed concerning the possible prebiotic and probiotic effects of kombucha.

4 Final considerations

Kombucha is an important source of bioactive compounds arising from raw material and increased by the fermentation process. These beneficial compounds change between kombuchas and depend on several variables, such as type of raw material, time of infusion, starter culture microbial community, and fermentation parameters. Therefore, kombucha health-promoting properties should not be generalized since there are so many variables affecting final product composition. So far, artisanal kombucha is produced through an uncontrolled fermentation process and the microbial community is complex, diverse, and variable. Also, interactions between yeasts, bacteria and their impact on human microbiota have not been understood or properly and thoroughly investigated. For this reason, kombucha cannot be considered a probiotic or symbiotic drink, unless documented probiotic strains have been added in the process. As the demand for this product is increasing, scientific studies are necessary to standardize the starter culture with well-known microorganisms, in order to control the metabolites produced, standardize the final product, and develop further studies on biological activities of kombucha, establishing it as a functional symbiotic beverage.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2021.101332.

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