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Desenvolvimento de kombucha com farinha de casca de manga: caracterização físico-química e sensorial e atividade antitumoral da bebida

Development of kombucha with mango peel flour: physicochemical and sensory characterization and antitumor activity of the beverage

Rio de Janeiro
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição (PPGAN), da Universidade Federal do Estado do Rio de Janeiro (UNIRIO), como requisito parcial para obtenção do título de Doutora em Alimentos e Nutrição.

Orientador: Prof.º. Dr.º. Anderson Junger Teodoro

Coorientadora: Prof.ª. Dr.ª. Rosana Bizon Vieira Carias

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Dedico este trabalho ao meu companheiro de vida, Vagner, às minhas filhas Maria Eduarda e Maria Gabriela e aos meus pais, Maria Helena e Vicente (in memorian), pelo constante incentivo, e por estarem ao meu lado sempre.

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“Mas os que esperam no Senhor renovarão as suas forças; subirão com asas como águias; correrão, e não se cansarão; andarão, e não se fatigarão”

Salmos 28:7-8

RESUMO

AZARA, C. R. P. **Desenvolvimento de kombuchá com farinha de casca de manga: caracterização físico-química e sensorial e atividade antitumoral da bebida** 2024. 153p. Tese (Doutorado em Alimentos e Nutrição) – Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, 2024.

Evidências científicas destacam a importância das escolhas dietéticas na preservação da saúde, especialmente na atualidade, onde a indústria alimentícia frequentemente oferece produtos práticos e de baixo custo, porém com valor nutricional insatisfatório e adição de aditivos químicos prejudiciais ao organismo humano. Neste contexto, alimentos fermentados, definidos como alimentos ou bebidas produzidas pelo crescimento microbiano controlado e conversão dos componentes desses alimentos através de ação enzimática, se tornam uma opção saudável. A kombucha é uma bebida fermentada originalmente oriental, feita com chá da *Cammelia sinensis*, caracterizada pelo baixo pH final, que permite sua conservação sem a utilização de aditivos e proporciona benefícios nutricionais próprios. A adição de outros ingredientes ricos em compostos bioativos pode enriquecer a kombucha e aumentar seu potencial benéfico. O objetivo deste estudo foi desenvolver e caracterizar uma bebida fermentada com farinha de casca de manga, avaliando sua atividade antioxidante e potencial antitumoral, em células humanas. Foi desenvolvida uma kombucha fermentada a 20ºC por 10 dias em chá verde (8g/L + 50g/L de açúcar) com adição de 20% de farinha de casca manga na segunda fermentação por mais 10 dias, acondicionada em garrafa de vidro e vedada. Foi feita a carcterização de antioxidantes e fenólicos totais, além da composição físico-química. A aceitação foi avaliada com teste sensorial afetivo e a atividade antitumoral foi avaliada por meio dos ensaios de cultivo celular *in vitro* para citotoxicidade, viabilidade celular, apoptose e senescência, com uso das linhagens de células tumorais MG-63 e Caco-2. Nossos resultados mostram que conseguimos desenvolver uma kombucha com adição de resíduo agroindustrial dentro dos padrões da bebida com aceitação geral satisfatória, porém com diferença significativa entre consumidores e não consumidores. Observamos que ocorre redução de pH e aumento de ácido acético em decorrência do processo fermentativo, sólidos solúveis e açúcares redutores foram reduzidos ao longo do processo de F1 e F2. Não houve aumento no conteúdo de fenólicos totais em F1 em comparação ao chá verde, mas na F2 com a adição da casca da manga em 10% e 20% tanto o conteúdo de fenólicos totais como a capacidade antioxidante (DPPH e ABTS) foi recuperada mesmo em baixa termperatura. Sobre os efeitos antitumorais, observamos que ambas as linhagens de células tumorais foram sensíveis ao tratamento com a kombucha de primeira e de segunda fermentação, causando morte celular. As células de adenocarcinoma colorretal (Caco-2) foram suscetíveis à morte por senescência, apesar de entrarem em apoptose, sendo o tratamento com F2 o que apresentou maior taxa de apoptose. As células de osteosarcoma (MG-63) foram mais susceptíveis à apoptose. Os ensaios de citotoxicidade MTT, de apoptose e de crescimento de colônias, mostraram que as células MG-63 foram mais sensíveis do que as Caco-2 ao tratamento com a kombucha. Concluímos que a temperatura mais baixa de fermentação é desfavorável para a atividade antioxidante da kombucha, mas que mesmo não aumentando ela fica igual ao do chá verde e que a adição de subproduto da manga exerce papel de aumento dessa atividade antioxidante, os ensaios *in vitro* mostraram que a kombucha exerce um papel importante na atividade antitumoral em células de adenocarcinoma colorretal e osteossarcoma.

Palavras-chaves: kombucha, compostos bioativos, fermentados

ABSTRACT

AZARA, C. R. P. **Development of kombucha with mango peel flour: physicochemical and sensory characterization and antitumor activity of the beverage** 2024. 153p. Thesis (Doctorate in Food and Nutrition) – Federal University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro, 2024.

Scientific evidence highlights the importance of dietary choices in preserving health, especially today, where the food industry often offers practical and low-cost products, but with unsatisfactory nutritional value and the addition of chemical additives that are harmful to the human body. In this context, fermented foods, defined as foods or beverages produced by controlled microbial growth and conversion of the components of these foods through enzymatic action, become a healthy option. Kombucha is an originally oriental fermented beverage, made with Cammelia sinensis tea, characterized by a low final pH, which allows its preservation without the use of additives and provides its own nutritional benefits. The addition of other ingredients rich in bioactive compounds can enrich kombucha and increase its beneficial potential. The aim of this study was to develop and characterize a fermented beverage with mango peel flour, evaluating its antioxidant activity and antitumor potential in human cells. A kombucha fermented at 20ºC for 10 days in green tea (8g/L + 50g/L sugar) was developed with the addition of 20% mango peel flour in the second fermentation for another 10 days, packaged in a glass bottle and sealed. The characterization of antioxidants and total phenolics, in addition to the physicochemical composition, was performed. Acceptance was evaluated with an affective sensory test and the antitumor activity was evaluated through in vitro cell culture assays for cytotoxicity, cell viability, apoptosis and senescence, using the MG-63 and Caco-2 tumor cell lines. Our results show that we were able to develop a kombucha with the addition of agro-industrial waste within the beverage standards with satisfactory general acceptance, but with a significant difference between consumers and non-consumers. We observed a reduction in pH and an increase in acetic acid as a result of the fermentation process, soluble solids and reducing sugars were reduced throughout the F1 and F2 processes. There was no increase in the total phenolic content in F1 compared to green tea, but in F2 with the addition of mango peel at 10% and 20%, both the total phenolic content and the antioxidant capacity (DPPH and ABTS) were recovered even at low temperatures. Regarding the antitumor effects, we observed that both tumor cell lines were sensitive to treatment with first and second fermentation kombucha, causing cell death. Colorectal adenocarcinoma cells (Caco-2) were susceptible to death by senescence, despite undergoing apoptosis, with treatment with F2 showing the highest apoptosis rate. Osteosarcoma cells (MG-63) were more susceptible to apoptosis. MTT cytotoxicity, apoptosis and colony growth assays showed that MG-63 cells were more sensitive than Caco-2 cells to treatment with kombucha. We conclude that the lower fermentation temperature is unfavorable for the antioxidant activity of kombucha, but that even without increasing it, it remains the same as that of green tea and that the addition of mango byproduct plays a role in increasing this antioxidant activity. In vitro assays showed that kombucha plays an important role in the antitumor activity of colorectal adenocarcinoma and osteosarcoma cells.

Keywords: kombucha, bioactive compounds, fermented

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LISTA DE SIGLAS E ACRÔNIMOS

°C – Graus Célsius;
µg – micrograma;
ABTS - 2,2 acid '-azino-bis 3-6 ethylbenzothiazolinsulfonic acid;
AG – ácido gálico;
AICR - /Instituto Americano de Pesquisa do Câncer
ANOVA – Análise de variância;
ANVISA – Agência Nacional de Vigilância Sanitária;
ATT – Acidez total titulável;
BAA - bactérias ácido acéticas;
BAL - bactérias ácido lácticas;
CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior;
CFT – Compostos fenólicos totais;
CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico;
DPPH - 2,2-diphenyl-1-picrylhydrazyl;
EGCG - Epigalocatequina-3-galato;
EAG – Equivalente de ácido gálico;
EROS - espécies reativas de oxigênio
FAO - Organização das Nações Unidas para Agricultura e a Alimentação;
FAPERJ - Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro;
KEAP1 - kelch como proteína 1 associada a ECH
Keap1-Nrf2-ARE - *Kelch-like ECH associated with protein 1 - Nuclear factor 2 related factor erythroid-2 – Antioxidant response element.*
LAAF – Laboratório de Alimentos Funcionais;
LABAL – Laboratório de Análise de Alimentos;
mg – Miligramas;
mm – Milímetros;
MS – Ministério da Saúde;
ONU – Organização das Nações Unidas;
ORAC - Oxygen Radical Absorption Capacity Test
pH – Potencial Hidrogeniônico;
PPGAN – Programa de Pós graduação e
Scoby - – Cultura Simbiótica de Bactérias e Leveduras
TE – Equivalente de Trolox;

TSS – Sólidos Solúveis Totais;
U% - Umidade;
Ueq – Umidade equivalente;
UFC - unidade formadora de colônia;
UFF – Universidade Federal Fluminense;
UNIFASE – Centro Universitário Arthur Sá Earp Neto;
UNIRIO – Universidade Federal do Estado do Rio de Janeiro;
UR% - Umidade Relativa;
$\lambda_{máx}$ – Absorção máxima do comprimento de ondas;
WCRF - World Cancer Research Fund / Fundo Mundial de Pesquisa sobre o Câncer
16s rRNA – RNA ribossomal 16S
ITS – Espaçador interno transcrito

SUMÁRIO

4

Introdução Geral

Uma em cada cinco pessoas no mundo desenvolverá câncer ao longo da vida. O aumento previsto da população e seu envelhecimento nos próximos anos são fatores que contribuirão para o aumento da incidência e mortalidade associados à doença (Bray *et al.*, 2024; Ferlay *et al*., 2024). A prevenção do câncer se tornou um dos desafios de saúde pública mais importantes do século, principalmente pelo fato de que 40% de todos os casos de câncer poderiam ser evitados de forma eficaz com a prevenção primária (Mourouti *et al*., 2015; Ferlay *et al*., 2024).

Fatores ambientais e de estilo de vida já foram estabelecidos e identificados como contribuintes para cerca de 50% da carga global de câncer, destes, 35% podem estar relacionados à alimentação. Grande parte desses fatores é modificável, fato que torna o seu conhecimento fundamental para que a prevenção efetiva do câncer seja atingida (OMS, 2020; Poorolajal, *et al*., 2021; Wang *et al*., 2023; Bray *et al*., 2024; Murthy *et al*., 2024).

Dos fatores de risco modificáveis, a ingestão dietética é um dos mais significativos e, também, uma das principais vias de exposição à diferentes compostos, fornecendo uma mistura complexa de substâncias químicas, sendo que algumas podem ter efeitos mutagênicos e/ou carcinogênicos e outras podem atenuar ou anular estes efeitos (Vaiserman *et al*., 2016; Kirkwood *et al*., 2017; Beetch *et al*., 2020; WCRF., 2024). As evidências científicas apontam para a importância das escolhas dietéticas para a preservação da saúde, principalmente na atualidade, onde a indústria alimentícia oferece produtos práticos para o consumo, com baixo custo, porém com pobre valor nutricional e ainda com níveis elevados de aditivos químicos conhecidamente lesivos ao organismo humano e ao meio ambiente (Vaiserman *et al*., 2016; WCRF, 2024; WHOC, 2024).

Na última década, nota-se um movimento de destaque para o papel da alimentação tanto na prevenção quanto no prognóstico do câncer. Diante da divulgação constante deste importante fator de risco modificável, uma parcela crescente da população tem apresentado maior preocupação na escolha dos alimentos ingeridos. Fato que torna necessário o desenvolvimento de produtos que apresentem alta qualidade nutricional, além de características sensoriais satisfatórias para que a indústria de alimentos possa atender à essa demanda (Katzke *et al*., 2015; Kaur & Singh, 2017; Nucci *et al*., 2021; Zheng *et al*., 2021; OMS, 2022)

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Nesse sentido, alimentos fermentados, definidos como alimentos ou bebidas produzidas através do crescimento microbiano controlado e a conversão dos componentes desses alimentos através de ação enzimática, se tornam uma opção no cenário de alimentos saudáveis. Das bebidas obtidas por processos fermentativos, a kombucha é uma bebida originalmente oriental, feita a partir do chá da *Cammelia sinensis*, que apresenta baixo pH final, o que permite sua conservação sem a utilização de aditivos e ainda apresenta os benefícios nutricionais do chá desta erva.

O consumo desta bebida vem crescendo no Brasil e em outros países da Europa, no Canadá e nos EUA, não só pelos benefícios associados à saúde provenientes da fermentação, mas também pelas próprias características da bebida, como a gaseificação e as diversas possibilidades de saborização. Características que permitem, além de oferecer uma bebida com diferentes sabores, a agregação de propriedade antioxidante, antiinflamatória e antitumoral, podendo ainda aumentar e/ou diversificar essas propriedades com a adição de resíduos agroindustriais que na maioria das vezes são descartados incorretamente, poluindo o meio ambiente. Portanto, esta técnica antiga de elaboração e conservação de bebidas resulta em um produto de excelente qualidade nutricional e ainda contribui para a sustentabilidade (Jayabalan *et al*., 2011; Jayabalan *et al*., 2014; Bellassoued *et al*., 2015; Coton *et al*., 2017; Gaggia *et al*., 2018; Jung *et al*., 2019; Cardoso *et al*., 2020).

A inserção de subprodutos obtidos da agroindústria, ricos em compostos bioativos e nutrientes, como vitaminas e minerais, pode enriquecer a kombucha e aumentar seu potencial benéfico à saúde. Essa inserção têm ajudado a dar impulso para o desenvolvimento de processos com a obtenção de alimentos mais saudáveis. Na perspectiva da agroindústria, a manga é a sexta fruta mais importante do Brasil em termos de área colhida, com 75 mil hectares. Parte dessa produção é utilizada pela indústria de polpas, onde a casca normalmente é descartada, representando 16% do peso total da fruta. Atualmente, a geração de resíduos agroindustriais é um problema com impactos ambientais negativos e importantes em todo o mundo, uma vez que, na maioria dos casos, esses resíduos não são processados e destinados adequadamente. O principal problema encontrado durante o gerenciamento de resíduos com alto teor de umidade, como a casca da manga, é o baixo uso por outros setores industriais devido a problemas de manuseio, armazenamento, transporte e conservação. ((Carvalho *et al*., 2004); Vargas &Peres, 2018; Sharmila *et al*., 2020).

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Comentado [5]: CARVALHO, C. R. L.; ROSSETTO, C. J.; MANTOVANI, D. M. B.; MORGANO, M. A.; CASTRO, J. V.; BORTOLETTO, N. Avaliação de cultivares de mangueira selecionadas pelo Instituto Agronômico de Campinas comparadas a outras de importância comercial. Revista Brasileira de Fruticultura, Jaboticabal, v. 26, n. 2, p. 264-271, 2004.

Comentado [6]: Sharmila G, Muthukumaran C, Manoj N, et al.: Current Developments in Biotechnology and Bioengineering. Chapter 12, Food waste valorization for biopolymer production. 2020;233–249. 10.1016/B978-0-444-64321-6.00012-4

Diante do alto volume de produção nacional, do descarte significativo da casca da fruta e seu potencial benéfico à saúde, a farinha da casca de manga se apresenta como ingrediente promissor a ser utilizado para enriquecimento de alimentos e bebidas. Considerando que a kombucha permite a incorporação de outros ingredientes, como os resíduos agroindustriais, essa bebida se torna um ótimo veículo para uso destes resíduos (Jaiswal *et al*., 2015).

Levando em consideração os objetivos de desenvolvimento sustentável (ODS) aprovados em 2015 pela ONU, com prazo para que sejam atingidos até 2030, a pesquisa na área de alimentos e saúde precisa ser integrada para apoiar a indústria de alimentos na oferta de produtos sustentáveis e saudáveis, atendendo dessa forma os ODS números 3, 9 e 12 relacionados à saúde e bem-estar, indústria sustentável e o consumo consciente e sustentável.

Nesse contexto, os métodos de produção e a eficácia do produto devem ser estudados para que a base científica brasileira seja incrementada para a industrialização e introdução de produtos inovadores que permitam a inserção de compostos bioativos com o objetivo de promoção da qualidade de vida e prevenção de doenças, contribuindo para estreitar a relação entre ciência e consumidor, para de fato levar benefícios à saúde da população.

O presente trabalho segue as normas de elaboração de teses no formato de artigo científico, definido e aprovado pelo Programa de Pós-Graduação em Alimentos e Nutrição (PPGAN) da Universidade Federal do Estado do Rio de Janeiro em 14 de maio de 2019. Assim, esta dissertação está seccionada em 5 capítulos, sendo eles:

Capítulo 1: Revisão Bibliográfica

Descrição do capítulo: Neste capítulo é apresentada uma breve revisão da literatura com relação à temática geral abordada no trabalho, com objetivo da criação de uma estrutura teórica para melhor delimitar e concretizar as ideias subsequentes.

Capítulo 2: Artigo de revisão da literatura: *"Kombucha technology: production and legal aspects"*

Descrição do capítulo: Buscando criar um arcabouço teórico acerca da temática do projeto de pesquisa e considerando o panorama supracitado, esta revisão visa trazer informações atualizadas sobre o processo de produção da kombucha e suas variações possíveis na composição da bebida final.

(Concilium - Qualis A2 – publicado em maio/2024)

Capítulo 3: Resultados - Artigo Original 1*: "Physicochemical, microbiology and sensory characteristics of kombucha prepared with tommy mango peel flour"*

Descrição do capítulo: Este estudo contempla a maior parte dos resultados e discussão dos experimentos realizados, onde o objetivo central foi, avaliar a composição físico-química, atividade antioxidante, microbioma e a aceitação da kombucha desenvolvida.

(Food Science and Technology International - Qualis A3 – artigo submetido em junho/2024)

Capítulo 4: Resultados - Artigo Original 2*: "Effects of kombucha added with tommy mango peel flour on cytotoxicity and in vitro antitumor activity of colorectal adenocarcinoma (Caco-2) and human osteosarcoma (MG-63) strains"*

Descrição do capítulo: Este estudo contempla os resultados e discussão dos ensaios *in vitro*, onde o objetivo central foi avaliar a atividade antitumoral da kombucha desenvolvida. Foram utilizadas duas linhagens de células tumorais humanas de origem e morfologia diferentes; adenocarcinoma colorretal e osteossarcoma.

(ACS omega - Qualis A4 – artigo submetido – agosto/2024)

Capítulo 5: Conclusão, considerações finais e perspectivas futuras.

Descrição do capítulo: Neste capítulo são apresentadas as conclusões gerais do projeto de pesquisa, as considerações sobre limitações do estudo e novos horizontes de pesquisa e ainda perspectivas futuras para a continuidade da pesquisa com parcerias já firmadas.

Esboço da pesquisa

A figura 1 mostra as etapas consideradas para a elaboração do projeto de pesquisa.

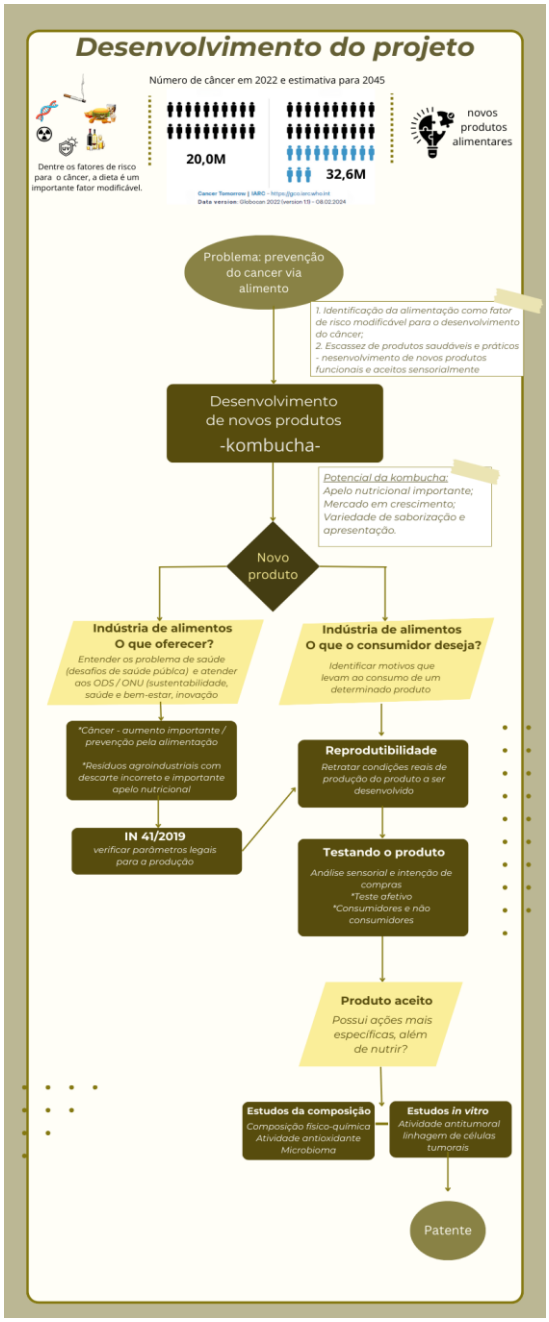

Figura1. Representação gráfica do projeto de doutorado.

A figura 2 apresenta todo o processo de produção/fermentação da kombucha desenvolvida para -pç 0aaa este projeto.

o

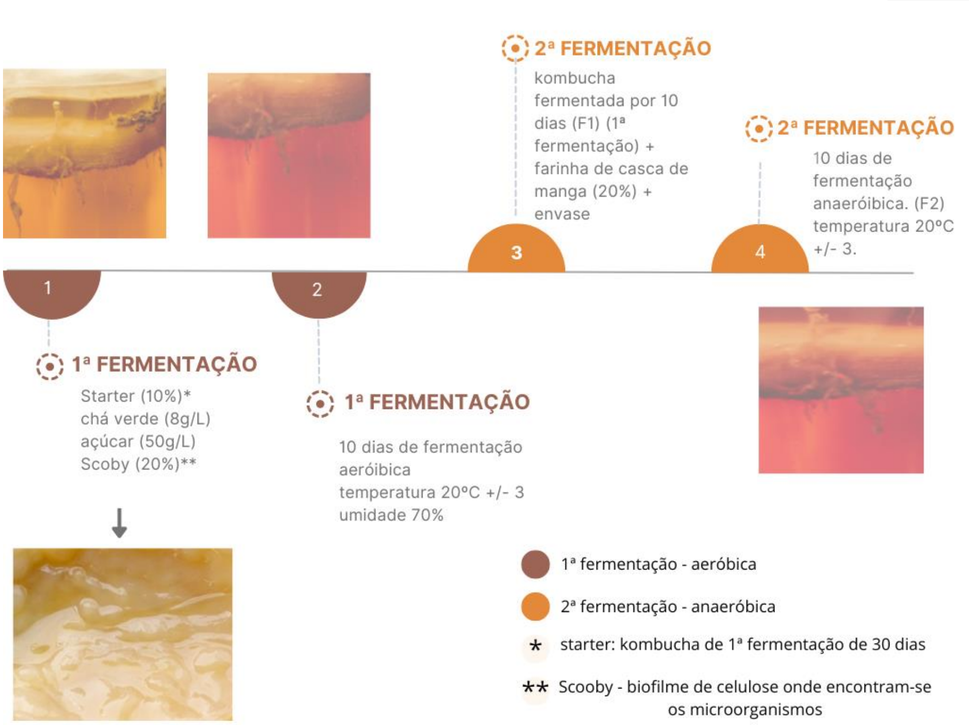

Figura 2. Representação gráfica do processo de produção da kombucha usada em todo o projeto.

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Capítulo 1
Revisão de literatura

1. Panorama do câncer

O câncer é um grande problema de saúde pública e econômico deste século, responsável por 22,8% das mortes por doenças crônicas não transmissíveis (DCNTs) em todo o mundo. A doença causa 30% das mortes prematuras por DCNTs em pessoas com idade entre 30 e 69 anos e está entre as três principais causas de morte nessa faixa etária em 177 de 183 países estudados. Além de ser uma barreira importante para o aumento da expectativa de vida, o câncer está associado a custos sociais e macroeconômicos substanciais que variam entre os tipos de câncer, geografia e gênero (Bray *et al*., 2021; Chen *et al.*, 2023; Ferlay *et al*., 2023).

O número de indivíduos com incapacidades devido ao câncer também vem aumentando e impactando nos custos com sistemas de saúde do mundo todo. O estudo sobre a Carga Global de Doenças, Lesões e Fatores de Risco de 2019 (GBD 2019) mostra dados de incidência, morbidade e mortalidade com o objetivo de fornecer dados para que estratégias governamentais sejam estipuladas para reduzir a carga do câncer no mundo. A contribuição dos cânceres para o total de anos perdidos por incapacidade aumentou durante a última década, subindo do terceiro lugar em 2010 para o segundo lugar em 2019, ficando atrás apenas das doenças cardiovasculares (Kocarmik *et al*., 2022).

O câncer é uma doença caracterizada principalmente pela alteração do crescimento e proliferação celular. Portanto, é uma doença genética, mas os fatores que originam o processo da doença são principalmente (40-45%) atribuíveis a fatores ambientais e de estilo de vida. Estes incluem consumo de tabaco, dieta, excesso de peso corporal, consumo de álcool, inatividade física, exposição ao sol, entre outros, o que significa que há um grande potencial para a prevenção do câncer. Em relação aos fatores modificáveis do estilo de vida, há evidências de que a dieta apresenta importante impacto no risco de câncer, podendo ser mais representativa do que fumar, assim, mudanças no comportamento alimentar podem reduzir significativamente a carga do câncer (Stein & Colditz, 2004; Anand *et al*., 2008; Gonzalez *et al*., 2010; WCRF, 2018; Morze *et al*., 2020; Hardt *et al.*, 2022).

Um estudo de coorte prospectivo feito na Europa (*European Prospective Investigation into Cancer and Nutrition* - EPIC) sobre câncer e nutrição, apontou

Comentado [7]: Global Burden of Disease 2019 Cancer Collaboration, Kocarnik, J. M., Compton, K., Dean, F. E., Fu, W., Gaw, B. L., Harvey, J. D., Henrikson, H. J., Lu, D., Pennini, A., Xu, R., Ababneh, E., Abbasi-Kangevari, M., Abbastabar, H., Abd-Elsalam, S. M., Abdoli, A., Abedi, A., Abidi, H., Abolhassani, H., Adedeji, I. A., … Force, L. M. (2022). Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. JAMA oncology, 8(3), 420–444. https://doi.org/10.1001/jamaoncol.2021.6987

Comentado [8]: Anand P., Kunnumakara A.B., Sundaram C., Harikumar K.B., Tharakan S.T., Lai O.S., Sung B., Aggarwal B.B. Cancer Is a Preventable Disease That Requires Major Lifestyle Changes. Pharm. Res. 2008;25:2097–2116. doi: 10.1007/s11095-008-9661-9. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
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9. World Cancer Research Fund/American Institute for Cancer Research . Diet, Nutrition, Physical Activity and Cancer: A Global Perspective. World Cancer Research Fund/American Institute for Cancer Research; London, UK: 2018. [Google Scholar]
10. Riboli E., Kaaks R. The EPIC Project: Rationale and Study Design. Int. J. Epidemiol. 1997;26:S6–S14. doi: 10.1093/ije/26.suppl_1.S6. [PubMed] [CrossRef] [Google Scholar]
11. Riboli E., Hunt K.J., Slimani N., Ferrari P., Norat T., Fahey M., Charrondière U.R., Hémon B., Casagrande C., Vignat J., et al. European Prospective Investigation into Cancer and Nutrition (EPIC): Study Populations and Data Collection. Public Health Nutr. 2002;5:1113–1124. doi: 10.1079/PHN2002394. [PubMed] [CrossRef] [Google Scholar]

resultados significativos relacionando o consumo de alimentos ultra-processados e seu impacto no aumento do risco de câncer, especialmente de cabeça e pescoço e trato gastrointestinal. O estudo incluiu 266.666 mil participantes de 7 países que foram acompanhados por 11 anos, e enfatizou a contribuição dos hábitos alimentares no risco de desenvolver a doença (Cordova *et al*., 2023). Outro aspecto importante quando se relaciona dieta e risco de câncer é a inflamação crônica, alguns estudos vêm descrevendo que o índice inflamatório da dieta tem um papel importante no risco de câncer e na piora daqueles indivíduos já acometidos pela doença (Tabung *et al*., 2016; Hodge *et al*., 2016; Wirth *et al*., 2016; Zahedi *et al*., 2020; Ali *et al*., 2022).

Comentado [9]: Cordova, R., Viallon, V., Fontvieille, E., Peruchet-Noray, L., Jansana, A., Wagner, K. H., Kyrø, C., Tjønneland, A., Katzke, V., Bajracharya, R., Schulze, M. B., Masala, G., Sieri, S., Panico, S., Ricceri, F., Tumino, R., Boer, J. M. A., Verschuren, W. M. M., van der Schouw, Y. T., Jakszyn, P., … Freisling, H. (2023). Consumption of ultra-processed foods and risk of multimorbidity of cancer and cardiometabolic diseases: a multinational cohort study. The Lancet regional health. Europe, 35, 100771. https://doi.org/10.1016/j.lanepe.2023.100771

2. O papel da dieta na prevenção do câncer

A literatura científica descreve que alguns compostos naturalmente presentes na dieta, modulam o risco de câncer e retardam sua progressão, por outro lado aponta que a frequente ingestão de alimentos ultraprocessados contribui como fator de risco (2009; Kaewkod *et al*., 2019; OMS, 2020; Cordova *et al*., 2023). O Fundo Mundial de Pesquisa do Câncer/Instituto Americano de Pesquisa do Câncer (WCRF/AICR) aponta algumas evidências para direcionar diretrizes nutricionais em escala global com o objetivo de reduzir o risco de câncer (WCRF, 2018; WCRF 2023).

Comentado [10]: International Agency for Research on Cancer . World cancer report: cancer research for cancer prevention. Lyon: World Health Organization; 2020. pp. 1–5.

Comentado [11]: World Cancer Research Fund International Dieta, nutrição, atividade física e câncer: uma perspectiva global. [citado em 1º de maio de 2023]. Disponível em: https://www.wcrf.org/diet-activity-and-cancer/global-cancer-update-programme/resources-and-toolkits/ [Lista de referências

Ao analisarmos a literatura, observamos um consenso de que, alimentos ultraprocessados devem ser evitados e frutas e vegetais devem ser incluídos na dieta em maiores quantidades para prevenir o câncer. Nesse sentido as bebidas se mostram uma boa opção para atender a essa recomendação, considerando que habitualmente bebidas não alcoólicas consumidas pela população, em sua maioria, estão no grupo de alimentos ultra-processados, assim, o desenvolvimento de bebidas saudáveis é essencial e urgente para melhorar a oferta desses nutrientes benéficos.

Comentado [12R11]: Jideani AI, Silungwe H, Takalani T, Omolola AO, Udeh HO, Anyasi TA. Antioxidant-rich natural fruit and vegetable products and human health. Int J Food Prop. 2021;24:41–67.

World Cancer Research Fund International. Wholegrains, vegetables and fruit on risk of cancer. Disponível em: dietandcancerreport.org

A variabilidade de escolhas dietéticas pode aumentar sinergicamente os efeitos dos nutrientes e dos compostos bioativos em alimentos nos mecanismos moleculares por meio de suas propriedades antioxidantes e anti-inflamatórias, que desencadeiam diversas vias de sinalização para prevenir ou retardar a progressão do câncer (Jideani *et al*., 2021; Ganesan & Chen, 2022). Um dos compostos

Comentado [13]: Ganesan K, Du B, Chen J. Effects and mechanisms of dietary bioactive compounds on breast cancer prevention. Pharmacol Res. 2022;178:105974.

Comentado [14R13]: Jideani AI, Silungwe H, Takalani T, Omolola AO, Udeh HO, Anyasi TA. Antioxidant-rich natural fruit and vegetable products and human health. Int J Food Prop. 2021;24:41–67.

bioativos, naturalmente presentes em alimentos, bastante descrito na literatura pelo seu importante potencial antioxidante e antiinflamatório são os polifenóis (Kaewkod *et al*., 2019; Yang *et al*., 2020; Ali *et al*., 2022). Na figura 1 podemos visualizar a classificação dos compostos bioativos em alimentos e a grande variedade dos polifenóis.

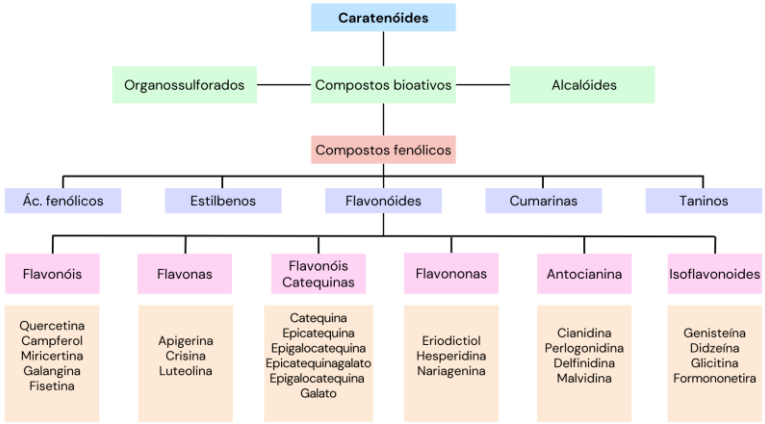

Figura 1: Classificação dos compostos bioativos em alimentos.
Fonte: Muscolo *et al.*, 2024.

2.1 Polifenóis.

Os polifenóis são amplamente distribuídos na natureza como metabólitos secundários de plantas e são pigmentos presentes em alimentos vegetais. Eles representam o maior grupo de fitoquímicos, com mais de 8.000 compostos já identificados. A quantidade desses compostos no alimento pode variar significativamente em função da cultivar, sazonalidade, altitude dentre outras condições ambientais (Jan *et al*., 2021). A característica estrutural comum do polifenol é caracterizada por uma ou mais hidroxilas ligadas a um ou mais anéis aromáticos (Fresco *et al*., 2006; Yahfoufi *et al*., 2018; Yan *et al*., 2020). Como estes compostos estão distribuídos amplamente nos alimentos vegetais, eles são

Comentado [15]: Muscolo, A., Mariateresa, O., Giulio, T., & Mariateresa, R. (2024). Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. International journal of molecular sciences, 25(6), 3264. https://doi.org/10.3390/ijms25063264

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Comentado [17]: Fresco P., Borges F., Diniz C., Marques M.P.M. New Insights on the Anticancer Properties of Dietary Polyphenols. Med. Res. Rev. 2006;26:747–766. doi: 10.1002/med.20060.

Yahfoufi N., Alsadi N., Jambi M., Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. Nutrients. 2018;10:1618. doi: 10.3390/nu10111618.

Comentado [18R17]: Yan Z., Zhong Y., Duan Y., Chen Q., Li F. Antioxidant Mechanism of Tea Polyphenols and Its Impact on Health Benefits. Anim. Nutr. 2020;6:115–123. doi: 10.1016/j.aninu.2020.01.001.

apontados como os compostos bioativos mais importantes na prevenção e reversão do câncer a partir da sua grande capacidade antioxidante e antiinflamatória. Estudos apontam ainda que os polifenóis estão superando a resistência a múltiplos medicamentos do câncer e aumentando inclusive a eficácia imuno terapêutica (Wang *et al*., 2021; Ali *et al*., 2022; Feng *et al*., 2023).

Os polifenóis vegetais são antioxidantes potentes que complementam a ação das vitaminas e enzimas antioxidantes na defesa contra o estresse oxidativo no organismo humano. O estresse oxidativo é uma condição resultante do excesso de espécies reativas de oxigênio (EROS) e está associado a várias condições patológicas crônicas e relacionadas ao envelhecimento, incluindo o câncer (Di Meo *et al*., 2016; Yang *et al*., 2020; Rana *et al*., 2022).

Antioxidantes são definidos como substâncias que, quando presentes em baixas concentrações em relação a um substrato oxidável, atrasam ou previnem significativamente a oxidação desse substrato. No corpo humano, os antioxidantes neutralizam os radicais livres e as espécies reativas geradas durante o metabolismo celular normal, protegendo diferentes biomoléculas dos danos oxidativos. Esses compostos possuem a capacidade de eliminar essas EROS, geradas tanto por fontes exógenas como endógenas, prevenindo assim o estresse oxidativo e seus efeitos deletérios (Fraga *et al*., 2019; Zhang *et al*., 2021).

A figura 2 ilustra a ação antioxidante de compostos bioativos em alimentos através da via Keap1-Nrf2, que é o principal regulador da defesa celular contra estímulos oxidativos extrínsecos e intrínsecos. A via Keap1-Nrf2 é uma das vias oncogênicas centrais em cânceres com um forte componente ambiental. Esta interação inicia uma série de eventos que ativam mecanismos de defesa celular contra o estresse oxidativo. A principal resposta fisiológica ao desequilíbrio redox é a ativação da via de sinalização celular Keap1-Nrf2-ARE (*Kelch-like ECH associated with protein 1 - Nuclear factor 2 related factor erythroid-2 – Antioxidant response element)*, na qual o fator de transcrição Nrf2 é um importante regulador da expressão de genes que codificam proteínas com ação antioxidante. Em condições basais a proteína Keap1 atua como um repressor endógeno de Nrf2, promovendo sua degradação pela via ubiquitina-proteassoma e em condições de estresse oxidativo ela se dissocia de Nrf2 para que seja translocada para o núcleo, funcionando como um sensor do ambiente redox celular. Em resposta ao estresse oxidativo, Nrf2 induz a transcrição de diversas

Comentado [19]: Feng, C., Chen, B., Fan, R., Zou, B., Han, B., & Guo, G. (2023). Polyphenol-Based Nanosystems for Next-Generation Cancer Therapy: Multifunctionality, Design, and Challenges. Macromolecular bioscience, 23(11), e2300167. https://doi.org/10.1002/mabi.202300167

Comentado [20]: Wang, T., Fan, Q., Hong, J., Chen, Z., Zhou, X., Zhang, J., Dai, Y., Jiang, H., Gu, Z., Cheng, Y., & Li, Y. (2021). Therapeutic Nanoparticles from Grape Seed for Modulating Oxidative Stress. Small (Weinheim an der Bergstrasse, Germany), 17(45), e2102485. https://doi.org/10.1002/smll.202102485

Comentado [21]: Di Meo S., Reed T.T., Venditti P., Victor V.M. Role of ROS and RNS Sources in Physiological and Pathological Conditions. Oxid. Med. Cell Longev. 2016;2016:e1245049. doi: 10.1155/2016/1245049.

Comentado [22]: Yang S., Lian G. ROS and Diseases: Role in Metabolism and Energy Supply. Mol. Cell. Biochem. 2020;467:1–12. doi: 10.1007/s11010-019-03667-9.

Comentado [23]: Rana, A., Samtiya, M., Dhewa, T., Mishra, V., & Aluko, R. E. (2022). Health benefits of polyphenols: A concise review. Journal of food biochemistry, 46(10), e14264. https://doi.org/10.1111/jfbc.14264

Comentado [24]: Fraga, C. G., , Croft, K. D., , Kennedy, D. O., , & Tomás-Barberán, F. A., (2019). The effects of polyphenols and other bioactives on human health. Food & function, 10(2), 514–528. https://doi.org/10.1039/c8fo01997e

Comentado [25R24]: Singla R.K., Dubey A.K., Garg A., Sharma R., Fiorino M., Ameen S. et al. Natural polyphenols: chemical classification, definition of classes, subcategories, and structures // J. AOAC Int. 2019. Vol. 102, N 5. P. 1397-1400. DOI: https://doi.org/10.5740/jaoacint.19-0133

Comentado [26R24]: Zhang L., Han Z., Granato D. Polyphenols in foods: Classification, methods of identification, and nutritional aspects in human health // Adv. Food Nutr. Res. 2021. Vol. 98. P. 1-33. DOI: https://doi.org/10.1016/bs.afnr.2021.02.004

enzimas envolvidas nas reações de biotransformação de fase I, II e III e mecanismos antioxidantes. A ativação dessa via de sinalização permite que organismos eucarióticos neutralizem os efeitos nocivos de agentes oxidantes, tornando-se um alvo atraente para prevenção e tratamento de doenças relacionadas ao estresse oxidativo, como é o caso do câncer (Kwak *et al*., 2002; Taguchi & Yamamoto, 2017; Oh & Jun, 2018; Mazdak *et al*., 2020).

Comentado [27]: Kwak MK, Itoh K, Yamamoto M, et al. Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cell Biol 2002;22:2883-92.

Oh, Y. S., & Jun, H. S. (2018). Effects of glucagon-like peptide-1 on oxidative stress and Nrf2 signaling. International journal of molecular sciences, 19(1), 26

Comentado [28R27]: Taguchi, K., & Yamamoto, M. (2017). The KEAP1–NRF2 system in cancer. Frontiers in oncology, 7, 85.

Mazdak H, Gholampour M, Tolou Ghamri Z. A Quick Review of Redox State in Cancer: Focus to Bladder The Gulf Journal of Oncology, 2020, 1:59-62

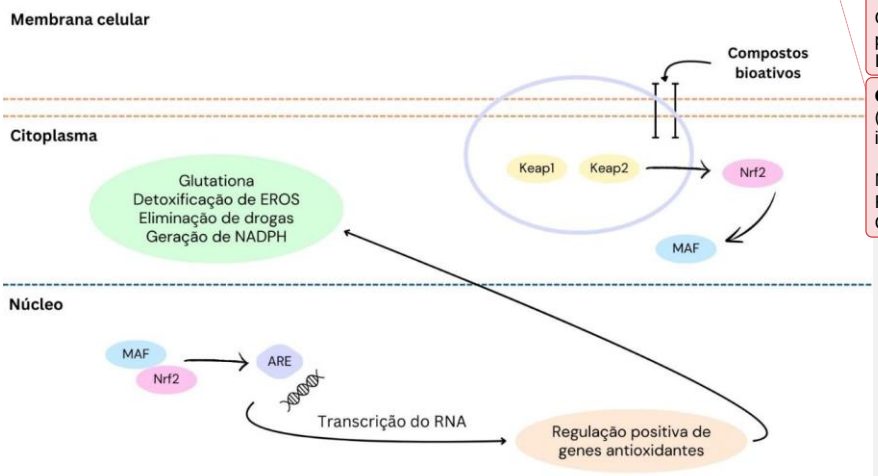

Figura 2: Via de sinalização 'Nrf2 - Keap1- Resposta antioxidante.. Além de proteger as células normais, esse complexo também pode proteger as células cancerígenas expostas ao estresse e prolongar a sobrevivência das células cancerígenas. Quando a célula é exposta ao estresse oxidativo, o NRF-2 é separado do Keap-1 e translocado para o núcleo e fornece a ativação transcricional de enzimas de desintoxicação e genes antioxidantes.
Fonte: Adaptado de Mazdak *et al*., 2020.

Polifenóis podem ser classificados em diferentes grupos em função do número de anéis fenólicos que eles contêm e dos elementos estruturais que ligam esses anéis uns aos outros (Botten *et al*., 2015). O conteúdo de polifenóis nos extratos de diversos vegetais está diretamente associado à sua atividade antioxidante total. Os flavonóides, que compartilham uma estrutura comum composta por 2 anéis aromáticos que são unidos por 3 átomos de carbono formando um heterociclo oxigenado, podem ser divididos em 6 subclasses em função do tipo de heterociclo envolvido: flavonóis , flavonas , isoflavonas ,flavanonas, antocianidinas e flavon óis (catequinas e proantocianidinas). Os flavonóis existem tanto na forma de

monômero (catequinas) quanto na forma de polímero (proantocianidinas) O chá verde e o chocolate são as fontes mais ricas, uma infusão de chá verde contém até 200 mg de catequinas. Catequina e epicatequina são os principais flavonóis nas frutas, enquanto galocatequina , epigalocatequina e galato de epigalocatequina são encontrados em certas sementes de plantas leguminosas, em uvas e, em maior quantidade no chá.

Diversos polifenóis foram estudados em função da sua atuação na carcinogênese, nesta revisão vamos dar ênfase às catequinas por serem os maiores constituintes bioativos do chá verde, que é a principal matéria prima da kombucha.

Uma das classes de polifenóis mais difundidas e estruturalmente variadas é chamada de flavonóides, com fortes atividades antioxidantes atribuídas. A catequina é o principal metabólito secundário da *Camellia sinensis* (L.), representando em torno de 12% a 24% do peso seco do chá, a EGCG é o conteúdo primário, representando aproximadamente 50–80% da quantidade total de catequinas. O EGCG é um derivado do 2-fenil benzo, que consiste em três anéis necessários (A, B e C) e um grupo acila gálico contendo um anel D. Encontramos grupos hidroxila fenólicos distribuídos em seus anéis A, B e D e, mais importante, três grupos hidroxila orto-fenólicos ocorrem nos anéis B e D, permitindo a forte capacidade antioxidante e a capacidade de eliminação de radicais livres. A estrutura di/tri-hidroxila dos anéis B e D, bem como o grupo meta-5,7-di-hidroxila no anel A tornam possível que ocorra a quelação de íons de metais de transição (figura 3). O número e a localização da fração acila gálico e do grupo hidroxila no anel catequina afetam suas propriedades farmacológicas (Tagashira *et al*., 2012; He *et al*., 2018).

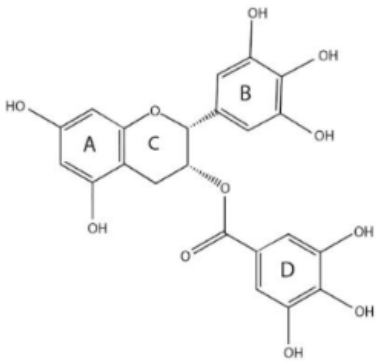

Figura 3: Estrutura química da epigalocatequina-3-galato (EGCG).

Inúmeros estudos avaliando a atividade antitumoral das catequinas, especialmente da Epigalocatequina-3-galato (EGCG) são encontrados na literatura tanto *in vitro* como *in vivo*. Estudos epidemiológicos ainda não sustentam consistência, fato que pode ser explicado pela existência de fatores de confundimento que não foram eliminados em muitos estudos, e ainda às diversas questões relacionadas à bioacessibilidade dos polifenóis, como matriz alimentar, fatores ambientais que impactam na quantidade de compostos bioativos e até a microbiota intestinal do indivíduo (Kumar *et al*., 2015; Kumar *et al*., 2016; Mao *et al*., 2019; Zhang *et al*., 2021; Yang *et al*., 2023).

No estudo *in vitro* de Wu *et al*. (2019), onde foram feitas exposições de EGCG com doses entre 10-200 μM/mL, foi verificado que ela inibe a proliferação celular, a viabilidade celular e causa parada do ciclo celular, além de aumentar o índice de apoptose. Em diversos estudos com catequinas, que identificam as vias de sinalização do seu efeito antitumoral, mostraram aumento da razão bax/bcl-2 e dos níveis de expressão da caspase-3 clivada e da PARP clivada em diversos tipos de câncer como colorretal, gástrico, mama, próstata, pulmão e osteossarcoma (Khan *et al*., 2009; Zhu *et al*., 2016; Wu *et al*., 2019; Cardoso *et al*., 2020; Almatroodi *et al*., 2020; Hayakawa *et al*., 2020; Sharma *et al*., 2022). A figura 4 mostra o mecanismo de ação do EGCG no câncer por meio da modulação de diversas vias de sinalização celular.

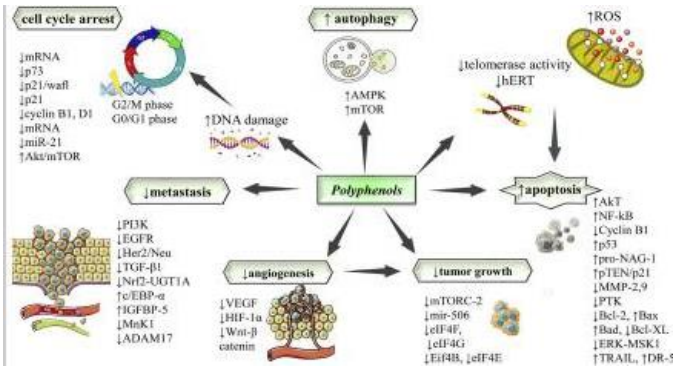

Figura 4: Alvos moleculares potenciais e vias de sinalização para o efeito antitumoral de polifenóis. Símbolos: ↑aumento, ↓diminuição. Abreviações: ADAM17, domínio 17 da metalopeptidase ADAM; Akt/mTOR, proteína quinase B/alvo mamífero

Comentado [29]: Khan N., Adhami V.M., Mukhtar H. Review: Green Tea Polyphenols in Chemoprevention of Prostate Cancer: Preclinical and Clinical Studies. Nutr. Cancer. 2009;61:836–841. doi: 10.1080/01635580903285056.

Comentado [30]: Enkhbat T., Nishi M., Yoshikawa K., Jun H., Tokunaga T., Takasu C., Kashihara H., Ishikawa D., Tominaga M., Shimada M. Epigallocatechin-3-Gallate Enhances Radiation Sensitivity in Colorectal Cancer Cells through Nrf2 Activation and Autophagy. Anticancer. Res. 2018;38:6247–6252. doi: 10.21873/anticanres.12980

Comentado [31]: Almatroodi, S. A., Almatroudi, A., Khan, A. A., Alhumaydhi, F. A., Alsahli, M. A., & Rahmani, A. H. (2020). Potential Therapeutic Targets of Epigallocatechin Gallate (EGCG), the Most Abundant Catechin in Green Tea, and Its Role in the Therapy of Various Types of Cancer. Molecules (Basel, Switzerland), 25(14), 3146. https://doi.org/10.3390/molecules25143146

da rapamicina; AMPK, proteína quinase ativada por monofosfato de adenosina; BAD, antagonista Bcl-2 da morte celular; Bax, proteína X associada a Bcl2; Bcl-2, proteína 2 de leucemia/linfoma de células B; Bcl-xL, linfoma de células B-extra grande; c/ebp-α, CCAAT/proteína de ligação ao intensificador-alfa; DR-5, receptor de morte 5; EGFR, receptor do fator de crescimento epidérmico; ERK/MSK, quinase regulada por sinal extracelular/quinase 1 ativada por mitógeno e estresse; Her2/Neu, receptor 2 do fator de crescimento epidérmico humano/neutrophills; HIF-1-alfa, fator 1-alfa induzível por hipóxia; IGFBP-5, proteína-5 de ligação ao fator de crescimento semelhante à insulina; hTERT, transcriptase reversa da telomerase humana; miR, microRNA; MMP, metaloproteinase da matriz; MnK-1, uma família de serina/treonina cinases; mRNA, ácido ribonucleico mensageiro; mTOR, alvo mamífero da rapamicina; mTORC2, complexo mTOR 2; NF-κB, fator nuclear kappa B; Nrf1, fator respiratório nuclear 1; PI3K: fosfatidilinositol 3-cinase; pro-NAG-1, gene-1 ativado por pró-anti-inflamatório não esteroide; pTEN, homólogo de fosfatase e TENsina deletado no cromossomo 10; PTK, proteína tirosina cinase; TGF-β1, fator de crescimento transformador-β1; TRAIL, ligante indutor de apoptose relacionado ao fator de necrose tumoral; UGT1A, família UDP glucuronosiltransferase 1; VEGF, receptor do fator de crescimento endotelial vascular; Wnt, sítio de integração relacionado ao wingless.

O potencial antiinflamatório dos polifenóis é outro ponto chave na ação destes compostos no desenvolvimento/tratamento do câncer, uma vez que a inflamação crônica representa uma das marcas registradas do câncer. Os componentes do chá (*Camellia sinensis*), especialmente o chá verde e mais especificamente a EGCG são alvos de vários estudos relacionados ao câncer e potenciais fontes antiinflamatórias como alvos terapêuticos (Sharma & Kanneganti, 2021; Zhang *et al*., 2021b; Zhang *et al.*, 2023; He *et al.*, 2023)

O inflamossomo NLRP3 é um complexo proteico citosólico multimérico que se reúne em resposta a perturbações celulares. Essa ação leva à ativação da caspase-1, que promove a maturação e liberação de citocinas inflamatórias, interleucina (IL)-1β e IL-18, e morte celular inflamatória, piroptose. As citocinas inflamatórias contribuem para o desenvolvimento de inflamação sistêmica de baixo grau, e a ativação do NLRP3 pode levar a um estado inflamatório crônico. O direcionamento do NLRP3, assim como outras moléculas de sinalização, como caspase-1, IL-1β ou IL-18, pode apresentar grande benefício terapêutico (Kelley *et al*., 2019; Castejón-Vega *et al.*, 2020). O tratamento *in vitro* de células aderentes com 2,5, 5 e 10 µM de epigalocatequina-3-galato mostrou uma diminuição dependente da dose na caspase-1, IL-1β e ROS. Os mesmos resultados foram observados em modelos *in vivo* e *in vitro* de inflamação microglial, em que a administração epigalocatequina-3-galato reduziu a expressão dos fatores do inflamossomo NLRP3 (Wang *et al*., 2020).

Também foram observadas respostas positivas em estudos *in vitro* de osteossarcomas, este tipo de tumor é agressivo e responde mal à quimioterapia citotóxica convencional, sendo urgente a busca por novas abordagens terapêuticas. O anti-inflamatório IL-1Ra foi testado sozinho e em combinação com a EGCG na produção de fatores tumorigênicos induzidos por IL-1 em células de osteossarcoma humano (U-2 OS). Um tratamento combinado resultou em uma inibição mais pronunciada de fatores tumorigênicos, tornando a administração combinada de EGCG e IL-1Ra uma abordagem promissora como terapia adjuvante em pacientes com osteossarcoma em outras linhagens de células tumorais a EGCG também se mostraram eficazes na redução da inflamação (Sharma & Kanneganti, 2021; Zhang *et al*., 2021b; Zhang *et al.*, 2023; He *et al.*, 2023).

Entre os mediadores inflamatórios presentes no microambiente tumoral, a IL-1 atua como um fator crucial na carcinogênese associada à inflamação, ela é produzida diretamente por células cancerosas ou por células do microambiente. Em estudos com células e em animais, níveis aumentados de IL-1 foram identificados em vários tipos de tumores humanos, como melanoma, câncer de cabeça e pescoço, cólon, pulmão, ossos e mama. No geral, pacientes que apresentam tumores positivos para IL-1 têm pior prognóstico (Apte *et al.*, 2006; Nike *et al*., 2012; Zhu *et al*., 2016).

Algumas pesquisas avaliaram a combinação do EGCG com outros medicamentos anticâncer, e observaram um desempenho antitumoral sinérgico, podendo ainda reverter a resistência a medicamentos de células tumorais e reduzir a probabilidade de recorrência do tumor após a cirurgia (Meng *et al*., 2017; Byun *et al*., 2018; Liu *et al*., 2019; Almatroodi *et al.*, 2020; Farhan, 2022).

Pesquisas epidemiológicas apresentam ligação entre o consumo de polifenóis naturais e o menor risco de desenvolvimento de câncer. A EGCG é um polifenol muito estudado e apontado com impactos positivos na saúde, porém seu efeito quimiopreventivo depende da bioacessibilidade e interação com os tecidos-alvo. No entanto, sabe-se que o EGCG tem baixa lipofilicidade, que a torna menos possível de passar pelas membranas, especialmente no epitélio intestinal. Como não tem um transporte mediado por receptor, é provável que a permeabilidade de sua membrana dependa da difusão passiva. Na maioria das vezes, uma concentração alta de catequina é necessária para que o EGCG desempenhe seu papel benéfico. Para estudos *in vitro*, uma concentração efetiva de EGCG é geralmente entre 1 e 100 mol/L. No entanto, esse valor é difícil de ser alcançado em condições *in vivo* porque o pico plasmático das catequinas do chá está na faixa sub ou

micromolar baixa (Li *et al*., 2012; Murakami *et al*., 2014; Cai *et al*., 2018; Qu *et al*., 2019).

Muitas pesquisas estão sendo feitas para tornar o EGCG mais biodisponível e melhorar sua baixa absorção pelas células. O uso de nanocarreadores hidrofóbicos têm mostrado alguns resultados promissores. Porém é necessário também estudar outras formas naturais para melhorar sua biodisponibilidade e incentivar o consumo deste polifenol via alimentos, garantido a sustentabilidade e a adoção de uma dieta mais saudável.

Muitos fatores influenciam na bioacessibilidade dos polifenóis, a microbiota intestinal desempenha papéis cruciais na transformação de compostos polifenólicos, influenciando características bioquímicas e contribuindo para variações na resposta a tratamentos de indivíduos. Nesse sentido, atenção especial vem sendo dada à microbiota intestinal.

A composição microbiana intestinal inclui um amplo espectro de espécies de bactérias altamente ativas metabolicamente, apresentando-se como um ecossistema complexo onde trilhões de microrganismos interagem com o sistema hospedeiro. A genética e as condições individuais de vida do hospedeiro influenciam em sua natureza e composição. A microbiota intestinal está envolvida na regulação de nutrientes e em várias vias metabólicas (metabolismo do ácido biliar, metabolismo da colina e metabolismo do triptofano para várias regulações homeostáticas) e na manutenção do sistema imunológico e da saúde geral do hospedeiro (Ashaolu, 2020). Esta microbiota intestinal complexa é composta principalmente por anaeróbios que sustentam a saúde metabólica dos humanos e seu papel de interação com o hospedeiro ainda não está totalmente elucidado (Wang *et al*., 2018; Filosa, Meo, & Crispi, 2018; Alves-Santos, *et al*., 2020; Moorthy *et al*., 2020).

Esses mecanismos são os principais responsáveis pelos benefícios à saúde do hospedeiro, no entanto, também são altamente específicos. O efeito prebiótico dos polifenóis está amplamente associado à modulação de probióticos ou supressão de bactérias patogênicas, resultando na redução de em endotoxinas que induzem resposta imune pró-inflamatória no intestino, além de outros benefícios à saúde, como modulação das fezes, redução do risco de câncer de cólon, doença inflamatória intestinal, obesidade e diabetes tipo 2 (van Duynhoven *et al*., 2013; Cardona *et al*., 2013; Gutierrez-Grijalva *et al*., 2016; Tang *et al*., 2016).

O metabolismo dos flavonóides ocorre principalmente no intestino e fígado. Os microrganismos intestinais usam os polifenóis, não absorvidos, como substratos para suas atividades enzimáticas. Esterases e glicosidases microbianas liberaram as agliconas e oligômeros como um mecanismo primário de quebra de polifenóis. A biotransformação ocorre nos enterócitos do intestino delgado e grosso, e nos hepatócitos, o grupo hidroxila destes compostos sofre glucuronidação, metilação e sulfatação podendo então entrar na circulação sanguínea seguindo para todos os órgãos e depois sendo eliminado pela urina (figura 5) (Tamura *et al*., 2008; Kemperman *et al*., 2010; van Duynhoven *et al*., 2011; Cardona *et al*., 2013; Gutierrez-Grijalva *et al*., 2016; Tang *et al.*, 2016).

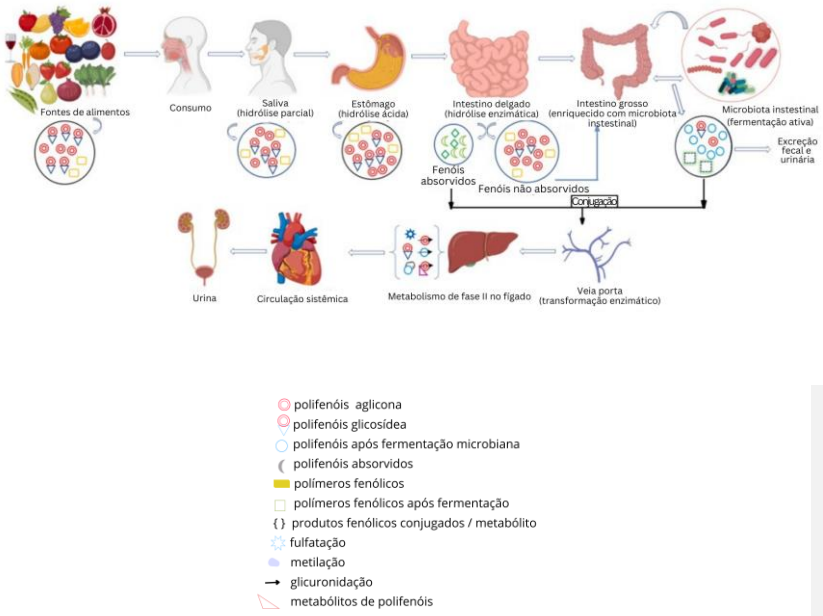

Figura 5: Biotransformação dos polifenóis no hospedeiro;
Fonte: Adaptado de Mithul *at al.*, 2021.

Os compostos polifenólicos encontrados na natureza são moléculas complexas com solubilidade escassa e biodisponibilidade limitada como já mencionado, quando introduzidos no trato gastrointestinal humano por meio da ingestão alimentar ou suplementos, esses compostos sofrem transformações facilitadas por enzimas e

microbiota, resultando em biodisponibilidade aprimorada e propriedades farmacológicas aumentadas.

O processamento de alimentos pode influenciar na disponibilidade dos polifenóis, como por exemplo, na fermentação. Alguns estudos mostraram que os polifenóis são os principais compostos bioativos presentes em alimentos vegetais fermentados, podendo ter sua biodisponibilidade alterada em função da fermentação (Yang *et al*., 2023). Os mecanismos de interações polifenol-microbioma durante a fermentação de alimentos vegetais ricos em polifenóis abordam quatro aspectos: (1) mudanças na atividade funcional de polifenóis em alimentos após a fermentação, (2) enzimas associadas à polifenóis secretadas por microrganismos, (3) vias de biotransformação de diferentes polifenóis e (4) efeitos dos polifenóis em microrganismos que afetam a qualidade destes alimentos (Lorenzo *et al*., 2021).

Nos últimos anos, as pesquisas com alimentos fermentados destacou que os polifenóis nesses alimentos são os principais componentes bioativos que conferem suas diversas atividades benéficas (Shiferaw *et al*., 2020; Di Lorenzo *et al*., 2021).

Comentado [32]: Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., Chen, S., Ye, X., & Wang, L. (2023). Effects of Fermentation on Bioactivity and the Composition of Polyphenols Contained in Polyphenol-Rich Foods: A Review. Foods (Basel, Switzerland), 12(17), 3315. https://doi.org/10.3390/foods12173315

Comentado [33]: Di Lorenzo C., Colombo F., Biella S., Stockley C., Restani P. Polyphenols and Human Health: The Role of Bioavailability. Nutrients. 2021;13:273. doi: 10.3390/nu13010273.

2.2 Alimentos Fermentados

A fermentação é um processo que tem sido usado por milhares de anos, com ação importante na conservação de alimentos e na produção de álcool. Os alimentos fermentados são definidos como alimentos ou bebidas produzidas por meio do crescimento microbiano controlado e da conversão de componentes dos alimentos por meio de ação enzimática.

Konstantinidis (2019) os descreveram como “alimentos que possuem efeitos construtivos em funções-alvo no organismo humano, além dos efeitos nutricionais, visando à promoção da saúde e bem-estar e/ou à redução de doenças crônicas”. Assim, alimentos e bebidas fermentadas são vistos como alimentos que além de oferecer nutrientes, também oferecem outros benefícios à saúde, como prevenção de doenças crônicas não transmissíveis (DCNT) (Konstantinidis *et al*., 2019).

Comentado [34]: Konstantinidi M., Koutelidakis A.E. Functional Foods and Bioactive Compounds: A Review of Its Possible Role on Weight Management and Obesity’s Metabolic Consequences. Medicines. 2019;6:94. doi: 10.3390/medicines6030094.

Comentado [35]: Konstantinidi M., Koutelidakis A.E. Functional Foods and Bioactive Compounds: A Review of Its Possible Role on Weight Management and Obesity’s Metabolic Consequences. Medicines. 2019;6:94. doi: 10.3390/medicines6030094.

A fermentação é principalmente um processo aeróbico que converte açúcares, como glicose, em outros compostos como álcool, enquanto produz energia para o microrganismo ou célula. Bactérias e leveduras são microrganismos com capacidade enzimática para fermentação, gerando ácidos e etanol. Muitos produtos diferentes ao redor do mundo são resultado da fermentação, ocorrendo naturalmente ou por meio da

adição de uma cultura inicial. Diferentes espécies de bactérias e leveduras estão presentes em cada caso, o que contribui para os sabores e texturas únicos presentes em alimentos fermentados. Essas bactérias e leveduras são chamadas de “probióticas” que possuem a seguinte definição da Organização Mundial da Saúde (OMS): “microrganismos vivos que, quando administrados em quantidades adequadas, conferem um benefício à saúde do hospedeiro” (OMS, 2022).

Comentado [36]: Chilton, S. N., Burton, J. P., & Reid, G. (2015). Inclusion of fermented foods in food guides around the world. Nutrients, 7(1), 390–404. https://doi.org/10.3390/nu7010390

Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. [(accessed on 23 September 2014)]. Available online: ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf

Alimentos fermentados são considerados um dos alimentos funcionais registrados desde a civilização humana. Diversos tipos de materiais alimentares, incluindo amidos, vegetais, legumes, raízes, tubérculos, lácteos e carnes são possíveis de serem fermentados. Os métodos de preparação e as matérias-primas utilizadas variam de acordo com a cultura do local. O consumo destes alimentos ganhou atenção dos pesquisadores ultimamente pelos vários benefícios à saúde, os benefícios são tão importantes que pesquisadores sugeriram a inclusão de alimentos fermentados nos guias alimentares em todo o mundo (Chilton *et al*. 2015; Kim *et al*., 2016; Marco *et al*., 2017; Sivamaruthi *et al*., 2018a; Sivamaruthi *et al*., 2018b).

Existem diferentes variáveis no processo de fermentação, incluindo os microrganismos, os ingredientes com seus diferentes nutrientes, tempo e as condições ambientais, dando origem a milhares de variações de alimentos fermentados e até mesmo variações de um mesmo produto fermentado.

Historicamente, a fermentação de alimentos era realizada como método de preservação, pois a geração de metabólitos antimicrobianos (por exemplo, ácidos orgânicos, etanol e bacteriocinas) reduz o risco de contaminação por microrganismos patogênicos. A fermentação também é usada para melhorar as propriedades sensoriais (por exemplo, sabor e textura), com alguns alimentos, como azeitonas, sendo não comestíveis sem fermentação que remove compostos fenólicos amargos. (Villarreal-Soto *et al.*, 2007; Sanlier *et al.*, 2019).

Existem dois métodos principais pelos quais os alimentos são fermentados. Em primeiro lugar, os alimentos podem ser fermentados naturalmente, muitas vezes referidos como "fermentos selvagens" ou "fermentos espontâneos", em que os microrganismos estão presentes naturalmente nos alimentos crus ou no ambiente de processamento, por exemplo, chucrute, kimchi e certos produtos fermentados de soja. Em segundo lugar, os alimentos podem ser fermentados por meio da adição de culturas iniciais, conhecidas como “fermentos dependentes da cultura”, por exemplo kefir, kombucha e natto. Um método de realizar um fermento dependente de cultura é o "retrocesso", no qual uma

pequena quantidade de um lote previamente fermentado é adicionado ao alimento cru, por exemplo, o "*starter* ou arranque" no início da fermentação da kombucha. Os starters usados para iniciar a fermentação podem ser naturais ou starters comerciais selecionados para padronizar as características sensoriais do produto (Dimidi *et al*., 2019) (Sanlier *et al*., 2019) Coton *et al*., 2017; Rezeac *et al*. 2018; Xiang *et al*., 2019).

Comentado [37]: Coton M., Pawtowski A., Taminiau B., Burgaud G., Deniel F., Coulloumme-Labarthe L., Fall A., Daube G., Coton E. Unraveling Microbial Ecology of Industrial-Scale Kombucha Fermentations by Metabarcoding and Culture-Based Methods. FEMS Microbiol. Ecol. 2017;93:fix048. doi: 10.1093/femsec/fix048.

Rezac S., Kok C.R., Heermann M., Hutkins R. Fermented Foods as a Dietary Source of Live Organisms. Front. Microbiol. 2018;9:1785. doi: 10.3389/fmicb.2018.01785

Xiang H., Sun-Waterhouse D., Waterhouse G., Cui C., Ruan Z. Fermentation-Enabled Wellness Foods: A Fresh Perspective. Food Sci. Hum. Wellness. 2019;8:203–243. doi: 10.1016/j.fshw.2019.08.003.

2.2.1 Kombucha

O hábito de beber chá (*Camellia sinensis*) é uma característica da dieta em países Asiáticos, segundo a FAO, é a bebida mais consumida no mundo depois da água. Ultimamente o chá vem sendo consumido indiretamente em muitos países por ser a principal matéria prima da kombucha. Existem diversas classificações em função dos métodos usados para a produção de chá em diferentes regiões. De acordo com o grau de fermentação, o chá geralmente é classificado em três tipos principais, o chá verde (não fermentado), o chá oolong (parcialmente fermentado) e o chá preto (fermentado). A composição "funcional" do chá é identificada em polifenóis, polissacarídeos, *L* -teanina, pigmento, cafeína e saponina, alguns dos quais são metabólitos secundários (Kuo *et al*., 2005).

Kombucha é uma bebida originalmente feita a partir da fermentação de chá (*Camellia sinensis*) por uma cultura simbiótica, seu consumo tem origem na região da Manchúria por volta de 220 a.C e principalmente na dinastia do Imperador Qin Shi Huang que estava sempre a procura de formas de prolongar a sua vida. Posteriormente, um médico chamado Kombu levou a bebida ao Japão para tratar problemas do trato gastrointestinal de um imperador. Em decorrência da expansão das rotas comerciais no início do século 20, a kombucha ficou popular na Rússia e na Europa Oriental (Emiljanowicz & Malinowska-Pańczyk, 2019; Mousavi *et al*., 2020).

Na modernidade, diversas bebidas de kombucha, com infusões variadas e adição de outros ingredientes vegetais, para saborizar, estão disponíveis comercialmente. A composição microbiana e metabólica desses produtos juntamente com os métodos de produção, raramente são relatadas, apesar de modificarem a bebida final, tanto nas características sensoriais, como nos constituintes químicos, impactando nos efeitos à saúde (Emiljanowicz & Malinowska-Pańczyk, 2019; Mousavi *et al*., 2020).

A kombucha tradicional é produzida por fermentação aeróbia de chá verde ou preto e açúcar branco por uma combinação de bactérias e leveduras, conhecida como cultura simbiótica de bactérias e leveduras (*Symbiotic Cultures of Bacteria and Yeasts* -

Scoby). A levedura converte a sacarose em etanol (além de ácidos orgânicos e dióxido de carbono), que as bactérias do ácido acético convertem em acetaldeído e ácido acético. Nesta fase, denominada de F1, o tempo de fermentação fica em torno de 7 a 20 dias, podendo ser observado na literatura até 30 dias. Na maioria das fábricas de produção da bebida a fermentação acontece na temperatura ambiente, assim, quanto menor a temperatura de fermentação, mais tempo é necessário para que esta F1 chegue ao pH ideal de término da primeira fermentação, de 2.8-3.2. A fermentação é caracterizada pela diminuição do pH que ocorre devido à formação de ácidos orgânicos. Além disso, a diminuição do pH é importante para garantir a segurança contra microrganismos patogênicos (pH < 4,2). O pH se torna constante com o avanço da fermentação devido à sua capacidade tampão, assim, é um parâmetro importante para avaliar a fermentação e que justifica a importância de avaliar também a acidez titulável Jayabalan *et al*., 2007; Jayabalan *et al*., 2008; Leonarski *et al*., 2022).

O baixo pH da kombucha, devido principalmente à produção de alta concentração de ácido acético, demonstrou prevenir o crescimento de bactérias patogênicas como *Helicobacter pylori*, *Escherichia coli*, *Salmonella typhimurium e Campylobacter jejuni*. Mesmo em pH neutro e após desnaturação térmica, a kombucha foi capaz de inibir o crescimento de patógenos *in vitro*, sugerindo que outros compostos além do ácido acético exercem efeitos antimicrobianos. (Sreeramulu *et al.*, 2000; Villarreal-Soto *et al.*, 2020).

Após esta etapa, a bebida comercializada passa pelo segundo processo, denominado de F2 (segunda fermentação), onde a bebida é saborizada e envasada preferencialmente com ingredientes *in natura* como frutas, ervas e especiarias. Este processo é caracterizado pela fermentação anaeróbia, onde as características sensoriais da bebida podem se diferenciar principalmente em relação à carbonatação que ocorre de forma natural (Brasil, 2019).

Os microrganismos presentes na fermentação são responsáveis por interações durante um processo simbiótico gerando metabólitos como ácidos (acético, glucônico, glucurônico, cítrico, entre outros), vitaminas hidrossolúveis (B1, B2, B6, B12 e C), etanol, dióxido de carbono e celulose (Jayabalan *et al.*, 2007; Neffe-Skocińska *et al*., 2017; Gaggìa *et al*., 2019; Leonarski *et al*., 2021).

A composição microbiana e metabólica da kombucha varia de acordo com a composição exata do *Scoby*, o tipo e a concentração do chá e do açúcar, a geometria do recipiente de fermentação, concentrações de oxigênio, tempo e temperatura de fermentação, armazenamento e se foi finalizada em F1 ou F2. Outra variação ocorrida na

bebida final em decorrência da sua composição microbiana ocorre nos compostos bioativos. Os microrganismos presentes no *Scoby* produzem enzimas capazes de interagir com os compostos bioativos presentes no chá, degradando moléculas grandes em estruturas menores da mesma classe de compostos químicos (Cotton *et al*., 2017).

As espécies bacterianas e fúngicas que constituem o SCOBY incluem tipicamente bactérias do ácido acético (BAA) (*Acetobacter*, *Gluconobacter*), bactérias do ácido láctico (*Lactobacillus*) e leveduras (*Saccharomyces*, *Zygosaccharomyces*), são os microrganismos dominantes durante a fermentação da Kombucha. *Acetobacter* é um gênero de bactérias do ácido acético caracterizado pela sua habilidade em converter álcool (etanol) em ácido acético na presença de ar. As BAL nem sempre são detectadas na bebida, mas contribuem significativamente para as características sensoriais gerais e principalmente o sabor (Greenwalt *et al*., 2000; De Filippis *et al.*, 2018; Tran *et al*., 2020; Villarreal-Soto *et al*., 2020).

O principal ácido da fermentação da kombucha é o ácido acético, que é produzido por bactérias do ácido acético através da oxidação do etanol ou através da glicólise e do metabolismo do piruvato. Entretanto, a produção de ácido glucônico pela via das pentoses fosfato e até mesmo ácido lático por bactérias do ácido láctico (BAL) através da via Embden Meyerhof já foi documentada (Jayabalan *et al.*, 2014; Coelho *et al*., 2020; Tran *et al*., 2020; Antolak *et al.*, 2021; Laavanya *et al*., 2021; Diez-Ozaeta & Astiazaran, 2022).

O perfil químico da Kombucha é denominado por ácidos orgânicos (principalmente acético, glucônico e glucurônico) e ainda os polifenóis da *Camellia sisnensis*, que podem ser responsáveis pelos múltiplos benefícios à saúde associados ao consumo regular desta bebida. Esses efeitos incluem potencial antitumoral, antidiabético e desintoxicante, melhora da resposta imune e modulação de colesterol plasmático (Yang *et al*., 2009; Jayabalan *et al*.,2011; Jayabalan *et al*., 2014; Cardoso *et al.*, 2020).

A kombucha elaborada com chá verde possui todos os efeitos da EGCG no câncer já descritos nesta revisão em igual ou maior potencial. Em temperaturas ideais de fermentação (25ºC), o conteúdo de polifenóis e consequentemente o seu potencial antioxidante é maior ao final da F1 quando comparado ao chá verde inicial, fato que ocorre em função do processo fermentativo (Cardoso *et al*., 2020; Jayabalan *et al.*, 2008).

O principal grupo de compostos bioativos da kombucha é o de catequinas e mais especificamente a EGCG (Jayabalan *et al.*, 2008; Cardoso *et al*., 2020; Liang *et al.*, 2024). Em kombuchas fermentadas com outras bases de infusões, diferentes compostos caracterizam-se como majoritários. Na composição final da kombucha ainda são

encontrados outros compostos importantes que determinam suas características sensoriais e ações na saúde, como o ácido glucurônico, ácido acético e vitaminas. O galato de (−)-epigalocatequina (EGCG) é a catequina mais abundante no chá verde e consequentemente da kombucha feita a partir dele. Uma xícara de chá verde, normalmente preparada com 2,5 g de folhas de chá, contém 240–320 mg de catequinas, das quais EGCG é responsável por 60–65%. Esse conteúdo pode variar muito de acordo com o percentual de chá usado na fermentação, que pode variar de 2g-10g/L.

Durante a produção do chá preto, as folhas de *Camellia sinensis L.* são submetidas a um processamento que estimula a atividade das polifenol oxidases e a consequente oxidação das catequinas, levando à formação de dímeros e polímeros conhecidos como teaflavinas e tearrubiginas, que são os principais compostos polifenólicos presentes no chá preto. (Aloulou, 2012; Dipti *et al*., 2013; Vina *et al*., 2014; Mousavi *et al*., 2020). Essas diferenças de concentração e tipos de compostos fenólicos podem interferir nas propriedades bioativas da kombucha produzida a partir do chá verde ou preto. Cardoso *et al*., (2020) avaliaram kombuchas elaboradas com chá verde e preto, 127 compostos fenólicos (70,2% de flavonóides) foram identificados nas kombuchas, dos quais 103 foram detectados pela primeira vez e 27 foram encontrados exclusivamente no chá preto, que se destacou por sua maior capacidade antioxidante com maior diversidade e abundância de compostos fenólicos, apesar da kombucha do chá verde ter apresentado potencial antioxidante maior do que o chá verde. No entanto, a kombucha de chá verde exibiu atividade antibacteriana contra um maior número de bactérias e ainda aumentou sua atividade antitumoral, apresentando um valor IG 50 inferior para as linhagens celulares de adenocarcinoma pulmonar e colorretal. Dessa forma, o tipo de chá ou infusão utilizado na produção da kombucha interfere na composição química, perfil e concentração dos compostos bioativo, impactando em suas propriedades na saúde, porém alguns estudos observam que, independentemente do extrato utilizado, os compostos bioativos (principalmente fenólicos) e, também o potencial antioxidante da kombcuha tendem a aumentar durante a fermentação (Jayabalan *et al*., 2007; Cardoso *et al*., 2020).

Outra característica importante a ser avaliada é, o comportamento destes mesmos parâmetros ao final da segunda fermentação, ou seja, quando a bebida está pronta para ser comercializada. É bastante comum que as bebidas comercializadas sejam adicionadas de outros ingredientes, como já descrito anteriormente, dessa forma se faz necessário, além de atentar para as características sensoriais, também observar subprodutos da

indústria alimentícia que possuam propriedades benéficas para a saúde, e possam ser aproveitados a fim de elaborar produtos com melhores perfis nutricionais (Emiljanowicz & Malinowska-Pańczyk, 2019).

Como descrito aqui, vários estudos apontam que o consumo de kombucha promove benefícios à saúde. A maioria dos estudos aborda os efeitos a partir da primeira fermentação somente, que não está disponível para venda e apresenta menor gaseificação. Os produtos disponíveis para venda são oriundos da segunda fermentação, onde é possível fazer a saborização e ocorre a maior formação de gás a partir da fermentação das leveduras. Fato que torna importante o estudo dos efeitos da bebida proveniente da segunda fermentação, que é o produto disponível para o consumidor.

A kombucha, devido às suas características de versatilidade, permite a adição de resíduos agroindustriais com a finalidade de saborização e/ou enriquecimento nutricional, colocando as indústrias da bebida como potenciais consumidores destes resíduos, contribuindo assim para a sustentabilidade.

3. Resíduos agroindustriais

O Brasil é um grande produtor de alimentos, fato que o torna também um grande gerador de resíduos agroindustriais. Apesar do otimismo com o crescimento do agronegócio no Brasil, que representa grande importância econômica, existe preocupação com a quantidade e diversidade dos resíduos agroindustriais e o seu descarte que, apesar de ser biodegradável, precisa de um tempo mínimo para ser mineralizado, se tornando uma fonte de poluentes ambientais. Atualmente vários centros de pesquisa buscam alternativas para a utilização dessa matéria orgânica gerada nos vários estágios de produção de alimentos e que não tem sido descartada corretamente em muitos países. A decomposição desses materiais resulta na produção de chorume, um líquido com grande potencial poluente, uma vez que apresenta baixa biodegradabilidade e possui metais pesados em sua poluição. Este líquido contamina o solo no local em que é formado, penetrando no solo e contaminando os lençóis freáticos. Segundo a Política Nacional de Resíduos Sólidos (PNRS), a reciclagem e reutilização são formas ambientalmente adequadas para a destinação de diversos tipos de resíduos (Huber *et al.*, 2012; Nascimento Filho &Franco, 2015).

A pesquisa moderna aponta esses subprodutos como valiosos, tanto do ponto de vista econômico como nutricional, se tornando de grande importância para as agroindústrias, pois, além de gerar um novo meio de renda, também está contribuindo

Comentado [38]: Emiljanowicz, K. E., & Malinowska-Pańczyk, E. (2019). Kombucha from alternative raw materials – The review. Critical Reviews in Food Science and Nutrition, 60(19), 3185–3194. https://doi.org/10.1080/10408398.2019.1679714

Comentado [39]: HUBER, Karina et al. Caracterização química do resíduo agroindustrial da manga Ubá (Mangifera indica L.): uma perspectiva para a obtenção de antioxidantes naturais. Revista Brasileira de Tecnologia Agroindustrial, v. 6, n. 1, p. 640-654, 2012.

com a preservação do meio ambiente. (Pereira *et al*., 2009; Huber *et al*., 2012; Nascimento Filho; Franco, 2015).

Estima-se que as indústrias de sucos e polpas de frutas gerem aproximadamente 40% de lixo orgânico, que é composto por casca, caroço e bagaço (Nascimento Filho & Franco, 2015). Esforços para usar materiais alternativos de baixo custo, renováveis e ecologicamente corretos, como matérias-primas na elaboração de novos produtos, têm sido feitos por pesquisadores e indústria no mundo todo (Sharmila *et al*., 2020; FAO, 2023).

Os resíduos de frutas têm potencial expressivo para serem utilizados na indústria de alimentos permitindo o enriquecimento nutricional de uma variedade de produtos; isso devido ao conteúdo de nutrientes e compostos bioativos presentes nestas partes das frutas. Um exemplo de fruta que tem seus subprodutos muito estudados é a *Mangifera indica L*., popularmente conhecida como manga.

A *Mangifera indica L*., compreende cerca de 30 espécies de árvores frutíferas da família *Anacardiaceae,* é originária da Malásia e da Índia, sendo cultivada por mais de 4000 anos e produzida em mais de 100 países. Representa uma das culturas de grande relevância no cenário nacional, considerada uma das mais importantes frutas tropicais, sendo o Brasil um dos maiores produtores globais (EMBRAPA; 2023).

Em 2022, a área plantada no Brasil foi de aproximadamente 78 mil hectares. As regiões que concentram a maior produção nacional são Nordeste 76,05% e Sudeste 22,71% do total. Em termos de volumes, em 2022, o Brasil colheu cerca de 1,55 milhões de toneladas. Segundo dados do Observatório da Manga, no ano de 2024 houve um aumento na produção de 45% em relação ao ano anterior, sendo que 80% são para consumo interno (EMBRAPA, 2023). Esses dados apontam para uma grande quantidade de resíduos gerados, sendo agravado pela agroindústria de polpa de frutas que tem sido apontada como uma alternativa de recuperação da economia de algumas regiões produtoras, visando a busca por novos nichos de mercado que não só a comercialização das frutas *in natura* (Oliver-Sinancas *et al*., 2020).

Nos últimos anos, os resíduos da manga têm sido estudados por desempenharem ação importante como um quimiopreventivo promissor no tratamento de diversos tipos de câncer, como de mama e ovário, podendo ser utilizados também como adjuvantes nos tratamentos antineoplásicos convencionais devido aos seus efeitos anti-inflamatórios e antioxidantes. Um dos compostos bioativos presentes nos resíduos da manga é a xantona glicosilada mangiferina (2-C-b-D-glicopiranosil-1,3,6,7-tetrahidroxi-xantona), o

Comentado [40]: PEREIRA, Luiz Gustavo Ribeiro et al. Aproveitamento dos coprodutos da agroindústria processadora de suco e polpa de frutas na alimentação de ruminantes. Petrolina: Embrapa Semi-Árido, 2009.

Comentado [41]: HUBER, Karina et al. Caracterização química do resíduo agroindustrial da manga Ubá (Mangifera indica L.): uma perspectiva para a obtenção de antioxidantes naturais. Revista Brasileira de Tecnologia Agroindustrial, v. 6, n. 1, p. 640-654, 2012.

Comentado [42]: Sharmila G, Muthukumaran C, Manoj N, et al.: Current Developments in Biotechnology and Bioengineering. Chapter 12, Food waste valorization for biopolymer production. 2020;233–249. 10.1016/B978-0-444-64321-6.00012-4

Comentado [43]: FAO: Food Outlook- Biannual Report on Global Food Markets. 2023 . https://www.fao.org/biotech/sectoral-overviews/agro-industry/en/

Comentado [44]: https://www.embrapa.br/observatorio-da-manga

Comentado [45]: Mirza B., Croley C.R., Ahmad M., Pumarol J., Das N., Sethi G., Bishayee A. Mango (Mangifera indica L.): A magnificent plant with cancer preventive and anticancer therapeutic potential. Crit. Rev. Food Sci. Nutr. 2021;61:2125–2151. doi: 10.1080/10408398.2020.1771678.

Lebaka V.R., Wee Y.J., Ye W., Korivi M. Nutritional Composition and Bioactive Compounds in Three Different Parts of Mango Fruit. Int. J. Environ. Res. Public. Health. 2021;18:741. doi: 10.3390/ijerph18020741.

Comentado [46R45]: Shaban N.Z., Hegazy W.A., Abdel-Rahman S.M., Awed O.M., Khalil S.A. Potential effect of Olea europea leaves, Sonchus oleraceus leaves and Mangifera indica peel extracts on aromatase activity in human placental microsomes and CYP19A1 expression in MCF-7 cell line: Comparative study. Cell. Mol. Biol. (Noisy-Le-Grand Fr.) 2016;62:11–19.

polifenol natural encontrado em maior quantidade nas cascas *Mangifera indica L.*, principal responsável pelos efeitos benéficos da manga na saúde (Canuto, 2009; Oliver-Simancas *et al*., 2020; Mirza *et al*., 2021) .

Já foi documentado que o teor de mangiferina varia de acordo com o estágio de maturação da fruta. Azevedo (2006) verificou decaimento do conteúdo de mangiferina, na variedade Tommy Atkins, na medida que o fruto amadurece.

O teor de compostos fenólicos e de mangiferina pode variar de acordo com a cultivar, na Tommy Atkins sua casca apresenta teor de compostos fenólicos totais foi 4444,0 mg/Kg de matéria seca e de mangiferina 1263,2mg/Kg de matéria seca (Berardini *et al*., 2005;).

Estudos utilizando diferentes culturas celulares, com estímulos nocivos variados, confirmaram o potencial antioxidante da mangiferina (Amazzal *et al*., 2007; Lum et al., 2021; Shaban *et al*., 2016; Lebaka *et al*., 2021; Mirza *et al*., 2021).

Além do seu consumo *in natura*, a manga é utilizada para preparar muitos produtos alimentícios, como geleias, licores, sucos, néctares e vinagres. Além disso, é utilizada nas indústrias farmacêutica e cosmética para produzir medicamentos fitoterápicos e cosméticos. Essa vasta gama de utilizações leva a um impacto ambiental importante em relação à geração dos resíduos (Kumar *et al*., 2021).

As evidências sugerem que a casca da manga, prova ser um composto útil e barato no enriquecimento de produtos alimentícios (Huang *et al.*, 2013; Wang *et al*., 2013; Li-li Pan *et al*., 2014; Minniti *et al*., 2023;).

A produção limpa de alimentos, tem promovido a implantação de novos processos integrados, que aliam produtividade e eficiência ambiental, mostrando a grande importância da pesquisa com esses subprodutos (Coelho *et al*., 2014; Le Grand, 2018).

Comentado [47]: Kumar M., Saurabh V., Tomar M., Hasan M., Changan S., Sasi M., Maheshwari C., Prajapati U., Singh S., Prajapat R.K.J.A. Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants. 2021;10:299. doi: 10.3390/antiox10020299.

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4. Desenvolvimentos de produtos alimentícios

Na última década e com mais força, após a pandemia de COVID-19, a conscientização do consumidor sobre a qualidade real dos alimentos, e a importância da dieta na manutenção da boa saúde e prevenção de doenças aumentou. O desejo por parte dos consumidores atualmente é de que os produtos alimentícios não sejam somente convenientes, prontos para consumo, mas que também sejam a fonte de nutrientes essenciais e funcionais. Assim, um aumento contínuo na demanda por alimentos com efeitos desejáveis na saúde vem ocorrendo e afetando o rápido desenvolvimento de um

novo mercado de alimentos. É importante ressaltar a necessidade de evidências científicas que comprovem as alegações de saúde para que o consumidor não adquira alimentos que sejam enganosos (Martirosyan & Singh, 2015; Al-Domi *et al*., 2021; Suaifan *et al*., 2024; FAO, 2024).

Este novo mercado de alimentos saudáveis tem um grande desafio: aliar boa qualidade nutricional, e às vezes ingredientes não tão comuns, às carcterísticas sensoriais satisfatórias (Marco *et al*., 2017; Hazra, Gandhi & Das, 2018; Plaek *et al*., 2019). Para que estes novos produtos sejam aceitos pelos consumidores, além de apresentar essas características sensoriais aceitas, como aparência geral, sabor e textura, é necessário ainda conhecer as intenções do consumidor com o produto em questão. Para isso, é importante aplicar a ciência sensorial ao desenvolvimento de um novo produto.

A ciência sensorial é um campo multidisciplinar que compreende a medição, interpretação e compreensão de respostas humanas às propriedades do produto percebidas pelos sentidos, como visão, olfato, paladar, tato e audição. Embora instrumentos analíticos mostrem-se efetivos na detecção de características físico-químicas importantes na indústria de bebidas, não avaliam a percepção humana. Sendo assim, a análise sensorial mostra-se como ferramenta indispensável para o estudo de bebidas, considerando que, esta é a única forma de avaliar a intensidade de um sabor, avaliar a aceitação e ainda quantificar as características sensoriais do produto (Cardello, 1999).

A qualidade da kombucha foi recentemente estabelecida em 2019 pela Instrução Normativa nº 41 que define o Padrão de Identidade e Qualidade da kombucha. (BRASIL, 2019) Dessa forma a análise sensorial para esta bebida de origem milenar, mas que está entrando no mercado brasileiro recentemente, é importante para atender as necessidades dos consumidores.

Para a introdução de um novo produto no mercado de alimentos e bebidas é necessário, além de atender às necessidades de consumidores e demandas globais de cuidados com a saúde, incorporar processos que contribuam com a preservação do meio ambiente é fundamental.

Em 2015, a ONU aprovou um plano de ações com 17 Objetivos de Desenvolvimento Sustentável (ODS). Esses objetivos devem ser alcançados até 2030 através de uma agenda de ações que visa enfrentar, dentre outras necessidades, a crescente carga de doenças não transmissíveis, incluindo o câncer. Esforços por parte de pesquisadores e governos de muitos países têm sido feitos para estabelecer ações reais para cumprimento desta agenda, na figura 6 estão dispostos todos os 17 ODS (PAHO 2024).

Comentado [50]: Martirosyan, D. M., and J. Singh. 2015. A new definition of functional food by FFC: what makes a new definition unique? Functional Foods in Health and Disease 5 (6):209–223. doi: 10.31989/ffhd.v8i7.531.

Comentado [51]: Hazra, T., K. Gandhi, and A. Das. 2018. Nutritive value and health benefit of fermented milks. Research & Reviews: Journal of Dairy Science and Technology 2 (3):25–28.

Marco, M. L., D. Heeney, S. Binda, C. J. Cifelli, P. D. Cotter, B. Foligné, M. Ganzle, R. Kort, G. Pasin, A. Pihlanto, et al. 2017. Health benefits of fermented foods: microbiota and beyond. Current Opinion in Biotechnology 44:94–102. doi: 10.1016/j.copbio.2016.11.010.

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Neste projeto entendemos que no contexto das DCNT´s, mais especificamente no câncer, a pesquisa na área de alimentos e as ciências médicas devem se aliar a fim de oferecer soluções sustentáveis e saudáveis para cumprir alguns desses objetivos, através da ação feita desde sempre pelos seres vivos, a nutrição. Sendo assim, ao pensar no desenvolvimento de novos produtos alimentícios saudáveis e sustentáveis estamos contribuindo para os objetivos 3,9 e 12.

Figura 6: ODS da OMS, 2015.

Para que os objetivos de desenvolvimento sustentável (ODS) estipulados pela ONU sejam atingidos, se faz urgente a necessidade de interligação, por parte da indústria de alimentos, da ciência de alimentos e das ciências médicas para que produtos diferenciados com reais benefícios à saúde sejam desenvolvidos e ofertados à população (FAO, 2011; Plasek *et al*., 2019; Hardt *et al*., 2022).

Comentado [55]: FAO. Biotechnologies for agricultural development. 2011.: (5) p. 240. https://www.fao.org/4/i2300e/i2300e.pdf

Comentado [56R55]: Plasek, B., Lakner, Z., Kasza, G., & Temesi, Á. (2019). Consumer Evaluation of the Role of Functional Food Products in Disease Prevention and the Characteristics of Target Groups. Nutrients, 12(1), 69. https://doi.org/10.3390/nu12010069

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Capítulo 2

Artigo de Revisão

Kombucha technology: production and legal aspects

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Abstract

Kombucha is a fermented drink with growing interest among the population due to its health benefits. The technological process involved in the production of kombucha is complex and involves the symbiosis between yeast and bacteria. Our study aimed to review the literature and identify factors that influence the fermentation process through a narrative review of the literature. We observed that factors such as time, temperature, quantity and type of tea, geometry of the fermentation vessel and composition of bacteria and yeast influence the quality of the final drink, which can alter the drink's sensory characteristics and health benefits. We conclude that the existence and control of production parameters for the drink is essential, thus ensuring greater standardization and consumer safety.

Keywords: kombucha; scoby; fermented; green tea

INTRODUCTION

Popularly fermented foods are characterized as products obtained through the metabolic interaction of microorganisms and the conversion of components present in fermented bases. As part of this group, we have kombucha, a drink of oriental origin, originally fermented in Cammelia sinensistea with a low final pH, which allows it to be preserved without the use of additives and with the nutritional benefits that tea presents (Jayabalan et al. 2008; Coton et al. 2017; Cardoso et al. 2020; Anantachoke et al. 2023). Kombucha dates back to 220 BC in the Manchuria region in northeastern China. It has now regained prominence and has been widely disseminated in the global market for drinks and products with functional claims. This growth is due not only to the benefits associated with health, but also due to the associated sensory characteristics, such as carbonation and the countless possibilities for diversifying flavors. This ancient way of preparing and preserving drinks provides a product of excellent nutritional quality, even if we consider that the content of polyphenols and flavonoids tends to increase as the days of fermentation and metabolic interactions go by (Duta, Paul, 2019). Thus, it appears that fermentation allows for greater antioxidant activity when compared to unfermented tea, which is directly associated with the content of polyphenols and organic

acids found in the final product (Jayabalan et al. 2008; Jayabalan et al. 2014; Coton et al. 2017).

The objective of this review was to survey the process of obtaining the drink, the characteristics of Scoby, creating theoretical interactions regarding the chemical characteristics and microbiological composition of the drink, the possible benefits and contraindications related to its consumption. Furthermore, we sought to describe some regulations imposed forits production and commercialization and finally, present the control factors that interfere in the fermentation process.

METHODOLOGY

The study is a narrative review of the literature, which in general, aims to elucidate the current state of knowledge on a specific subject through a synthesis, with the aim of identifying unexplored areas of knowledge, facilitating new investigations and laying the foundations for future research on a given topic. The non-systematic approach adopted in the narrative review model is simplified and seeks to provide a timely update on a given topic through broader and more extensive research, without adheringto a specific methodology for carrying out its various stages (Casarin et al. 2020). However, seeking to rely on a standardized line for data collection, even if not directly, using a collection alternative, based on the metadata proposed in the implementation of themethodology Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA)[1]in an adapted way, following the model proposed by Cardoso et al. (2022), which allowed the content covered to be revealed in a critical and concise manner, with the central objective of achieving prospection and elucidation of the topic in a systematic way, even if indirectly, making it possible to map current scientific and technological development, capable of impacting significantly, positively the industry, the economy and society.

[1]The PRISMA methodology is a set of guidelines for systematic reviews and meta-analyses, aiming to improve their quality and transparency. She provides guidance from the preparation of the protocol to the writing of the final report, promoting consistency and methodological rigor. PRISMA assists in the selection of studies, collection and synthesis of data, facilitating critical evaluation and replication of results.

OVERVIEW

Kombucha is a drink resulting from the biometabolic activity of a symbiotic culture of bacteria and yeast, which remains accommodated in a cellulose matrix, popularly known as “Scoby –Symbiotic Culture of Bacteria and Yeast”. In the beverage production process, the biochemical phenomenon of fermentation occurs, where transformations are generated in the tea as a result of the activity of microorganisms, leading to changes in phenolic compounds and the generation of organic acids, which include acetic acid,lactic acid and glucuronic acid (Vitas et al. 2013; Villarreal-Soto et al. 2019; Jakubczyk et al. 2020).

Among the claims attributed to the consumption of kombucha, the antimicrobial, antioxidant, and anticarcinogenic benefits have assumed a prominent role among the therapeutic properties and aroused consumer interest, enhancing its marketing profile (Chakravority et al. 2019; Cardoso et al. 2020; Anantachoke et al. 2023).

The global kombucha production and commercialization market has demonstrated numerous advantages for significant growth and its dissemination on an industrial scale, highlighting it as the fastest growing product in the beverage sector with functional properties (Dutta and Paul, 2019).

With the focus on the development of new drinks and promising products for the food market, accompanied by this trend of demand for more functional products, a marketing race was launched to offer packaged products for immediate consumption based on kombucha, requiring emerging form the implementation of specific legislation that establishes the Identity and Quality Standard (PIQ) of the product, as well as what information should be covered and contained in the means of dissemination and labeling of the product, making it safe for human consumption.

KOMBUCHA: PRODUCTION TECHNOLOGY

In the kombucha manufacturing process, teas derived from Camellia sinensis are the most used as a base, whether combined or isolated (Coelho et al. 2020; Cardoso et al. 2020). To start the fermentation process, in addition to the chosen base, a fraction ofsucrose (5 to 12% (m/v)) is added, which will serve as an energy substrate for the metabolism of the microorganisms involved in the process, which may vary according to the standardization adopted in different processing conditions, acting in

protocooperation (Dutta and Paul, 2019). At the beginning of fermentation, the disaccharide sucrose is hydrolyzed into smaller molecules of glucose and fructose, which will soon be bioconverted to ethanol and CO^2. Ethanol, in the spectrum of fermentation dynamics, is converted to acetic acid, with notable production of gluconic and glucuronic acids throughout the process.The entire mechanism mentioned occurs spontaneously, which according to Coton et al. (2017) can be considered difficult to control, as there are different reactions, varying according to the microbiota involved and the production conditions, which promotesdifferent results in the final drink. The fermentation process can vary from 3 to 60 days. With the union of the base raw material for the beverage production process, fermentation begins, where on average around 7 to 10 days are needed for the product tobegin to acquire specific characteristics. This beginning is called first fermentation and, as it occurs, a new scoby film is formed on the surface of the solution. This cellulose biofilm can reach a thickness of between 8 and 12 mm (Chandrakala, Lobo andDias, 2019). The process takes place at a variable temperature, knowing that between 25 –30 ºC is ideal. The fermentation process occurs even at lower temperatures, but the fermentation speed is slower, taking longer for the drink to acquire the same sensory characteristics (Leal et al. 2018; May et al. 2019).During this period, invertase enzymes, produced by some species of yeast, act to cleavage sucrose molecules, releasing glucose and fructose into the solution, increasing the supply of substrates for metabolism. Glucose molecules are used by bacteria and yeast in energy metabolism, transforming into ethanol and carbon dioxide (May et al. 2019), this carbon dioxide will undergo a reaction with the water in the solution medium, forming carbonic acid (Primiani et al. 2018).

Furthermore, species of microorganism's act in the oxidation of ethanol, producing acetic acid. It appears that in addition to acetic acid, several other organic acids are produced throughout the fermentation process, which contributes to the hydrogen potential (pH) of the drink, which initially hovers around 5, decreasing even further (Amarasingkhe,2018; May et al. 2019). This low pH characteristic of the drink is responsible for conservation without the need for additives, being seen as a very important point of the drink in terms of becoming healthier. Organic acids, D-saccharic acid-1,4-lactone (DSL), and tea polyphenols are the main metabolite components of kombucha (Jayabalan et al. 2014).On an artisanal and industrial scale, scoby is added to

the tea solution with sucrose in a container that can be made of glass or stainless steel. This container must have a wide neck, with an extended exposure area, allowing easy access and surface exposure, sufficient for the exchange of gases with the environment.

Emphasizing that this bottleneck must be covered with a protection that is permeable to these atmospheric gases, but that prevents the entry of insects (Jayabalan et al. 2014).The new fraction of the cellulose film formed on the surface of the container is removed, and the liquid fraction is filtered and the drink is finished for consumption, and can be refrigerated or continued for a second fermentation (Watawana et al. 2015). In this second fermentation, this is where 10 -50% of fruit juice, spices or other ingredients are generally added to the previously fermented kombucha (7-15 days). each trademark. This second fermentation aims to carbonate the kombucha, which must be kept in a closed container (anaerobic fermentation) and stored at room temperature until the desired degree of carbonation that meets legal standards (Santos et al. 2016). The result of this process is a drink that has a slightly carbonated characteristic, inaddition to being very refreshing, composed of several elements beneficial to health (Watawana et al. 2015; Leal et al. 2017; May et al. 2019).

DELINEATION OF CONTROL PARAMETERS DURING FERMENTATION DYNAMICS

Most fermentation processes are aerobiosis and therefore require the supply of oxygen for metabolism to occur. Considering the stoichiometry of respiration, 192 g of oxygen are required for the complete oxidation of 180 g of glucose. However, both components must be in solution before they are available to a microorganism, and oxygen is approximately 6000 times less soluble in water than glucose, so it is not possible to provide a microbial culture with the necessary amount of oxygen to complete the oxidation of glucose or any other carbon source (Villareal-Soto et al. 2019).At the beginning of the process, significant amounts of ethanol and monosaccharides necessary for lactic acid bacteria (AAB) are supplied by Kombucha yeasts. The oxidation of ethanol to acetic acid requires one mole of oxygen (32 g) to completely oxidize 1mole of ethanol (46 g), therefore the activity of AAB as aerobic organisms depends on the transfer of oxygen from the air for fermentation. For this reason, a microbial culture must receive oxygen during growth at a rate sufficient to satisfy the organism's demand (Stanbury et al. 2016).In static

cultures, substrates must be entirely transported by diffusion and oxygen availability can become the limiting factor for cellular metabolism, which can have a negative effect on cellulose production and quality. The kinetic factor that expressesthe relationship between dissolved oxygen and the surface/volume of the medium is the specific interfacial area, which is directly related to other factors, such as the cross section of the reactor and the mass transfer coefficient (Cvetković et al. 2008). This means that the rate of batch fermentation of Kombucha without stirring and without introducing gas depends on the specific interfacial area (which further promotes the idea of the fermentation environment being favorable in relation to the surface area).Cvetković et al. (2008) developed a mathematical model to scale the fermentation of Kombucha tea based on several specific interface areas. Model verification was carried out in large volume reactors (90 L) and very small vessels of 0.33 L.

The model standardizes the optimal conditions as: 70 g/L of initial substrate (sucrose), interfacial area of 0.0231 at 0.0642 cm−1and 14 days of fermentation. They concluded that regardless of the size or volume of the container, if the value of the interfacial area is constant, they can guarantee the production of Kombucha tea with similar properties. In the specific case of batch fermentation of Kombucha tea, several biological factors must be taken into consideration, especially in the absence of agitation, where microbial disintegration can occur between the aerobic acetic bacteria that will tend to occupy the surface layer and the yeast that can precipitate at the bottom of the container (Lončar et al. 2006), which can lead to negative effects on the fermentation process.Microbial cellulose has already been well studied by some authors over the last few decades (Czaja et al. 2006; Campano et al. 2016), and the available information that defines the optimal reactor conditions for its development, such as surface/volume or surface/height are limited. To investigate the influence of volume on processing, Lončar et al. (2006) worked with different conditions and found that the best geometric conditions for intensifying fermentation were obtained with a reactor with a volume of4L and a diameter of 17 cm. Goh et al. (2012) investigated the relationship between the yield, the properties of the biofilm produced from Kombucha fermentation and the surface area and found that biofilm production increased with an intensification of the surface area and decreased with a wider depth. This can be explained because the metabolic process is completely aerobic and is constantly generating carbon dioxide, which can be trapped in the film and accumulate in

greater quantities, especially in deeper environments.However Caicedo, Da França & Lopez (2001) found that although the surface area is decisive, the height is still important, as a minimum height is necessary for the development of the film, taking into account the production of several layers of cellulose throughout fermentation, which will occupy part of the initial volume. Permeating the fermentation pattern, it is noted that there are many variables that interfere in the fermentation process and, consequently, in the sensorial characteristics of the drink. In this way, different final products are offered to the consumer and the responses in relation to the health benefits and also the acceptance of the drink, making it necessary for standards and regulations to be established to guarantee the quality of the product that reaches the consumer (Dutta and Paul, 2019).Throughout this process, there are several factors involved in the excellence of the fermentation, as well as the quality of the product obtained at the end, which permeate the quality of the extract used as a liquid base for the culture, as well as the microorganisms, which make up the structure of the scoby. The type of substrate selected, the time and temperature released and the pH of the medium, are relevant factors that directly imply how this mechanism presented so far will develop (Villareal-Soto et al. 2018). Frame 1 presents different time and temperature conditions adopted in the fermentation of kombucha in several studies cited in the literature, which used different base and control parameters.

Frame 1-Time and temperature conditions adopted in the fermentation of kombucha in several studies in the scientific literature.

Fermentation time	Temperature used (°C)	Reference
21 days	24 °C	Jayabalan et al. (2010)
10 days	28 °C	Malbaša et al. (2011)
8 days	30 °C	Goh et al. (2012)
10 days	23 °C	Marsh et al. (2014)
10 days	30 °C	Lončar et al. (2014)
8 days	Room temperature	Nguyen et al. (2015)
21 days	28 °C	Chakravortya et al. (2016)
12 days	30 °C	Ayed; Abid; Hamdi, (2017)
21 days	20°C (CHV) e 30° (CHP)	Filippis *et al.* (2018)

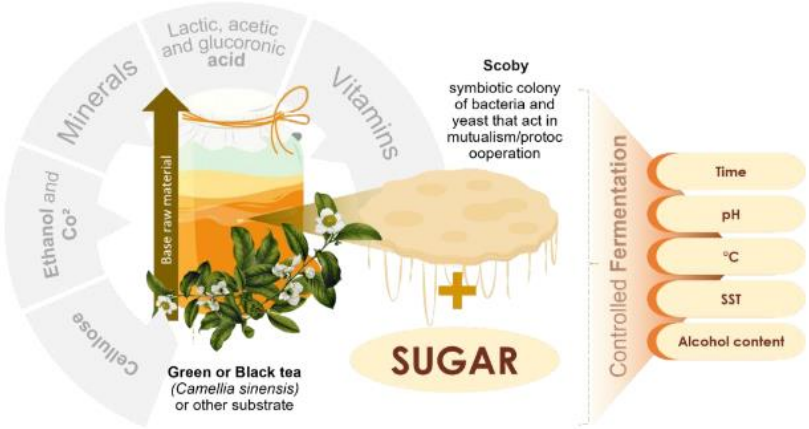
Caption: CHV = green tea; CHP = black tea.Source: Authors, 2024.

May et al. (2019) states that, when carbon levels are very low, new substrates in addition to sucrose can begin to be used by bacteria and yeast, envisioning alternative routes

for obtaining carbon molecules, such as ethanol. However, it should be noted that such alternative mechanisms also impact the metabolites produced throughout fermentation. It is known that sucrose is the carbon source most used in this process, however, an important fraction of this substrate is not used during fermentative metabolism (Jayabalan et al. 2014) and when adding other types of sugar, such as lactose or fructose, can have impacts on fermentation and the final product.In the study by Malbaša, Lončar and Djurić (2008), in which the elaboration of variations of kombuchas fermented in solutions prepared by the infusion of black tea and sucrose and black tea and sugarcane molasses, it was observed that in the drink prepared with the addition of sucrose as the main carbon source, a greater production of acetic acid was identified, which was proportionally associated with a reduction in the pH values of the medium. In the sample that used sugarcane molasses as an energy substrate to obtain carbon, a higher concentration of lactic acid was noted. Traditionally, pH tends to decrease during the fermentation process, this parameter being related to safety against the growth of pathogenic species of microorganisms, with the inhibition range plotted, normally at pHs close to 4.2 (Hur et al. 2014; May et al. 2019). Jayabalan et al. (2014) point out that the pH range of kombucha tends to decrease mainly in the initial days of fermentation, tending to stabilize due to the buffering effect exerted by the acids in the solution. Malbasa et al. (2011) explain that this phenomenon of "plateau" of equilibrium at a given moment of fermentation, due to buffering, is due to the fact that the synthesis of weak organic acids interacts with mineral components present in the solution, mainly those derived from tea.In the broad commercial spectrum, the final beverage is composed of sugars (including sucrose, glucose and fructose), organic acids including acetic acid, gluconic acid, glucuronic acid, lactic acid, DSL, citric acid, oxalic acid and pyruvic acid (Jayabalan et al. 2007) B vitamins and vitamin C (Bauer-Petrovska and Petrushevska-Tozi, 2000), theophyllines, tea polyphenols, flavonoids, various amino acids and proteins, ethanol, biogenic amines, purine bases, hydrolytic enzymes, minerals (mainly copper (Cu), Iron (Fe), Zinc (Zn), Nickel (Ni) and Manganese (Mn)) and metabolites secreted by yeast and bacteria (Malbaša et al. 2011).Tea itself contains the main polyphenolic catechins described in the literature, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Black tea is considered a fully fermented form of tea as the production process creates a small particle size in the tea leaves and a larger surface area for enzymatic oxidation. During the fermentation of black tea,

quinones react with catechins and produce new compounds, such as theaflavins and thearubigins. Furthermore, the catechins present in green tea are also partially converted into theaflavins (Haruthairat Kitwetcharoen et al. 2023). The phenolic compounds in kombucha are bioconverted into smaller biological molecules, through the fermentation process that occurs in an acidic environment or through enzymatic activity, guided by the metabolism of bacteria and yeast. For example, the observed increase in overall catechin content in green and black tea kombucha subsequent to fermentation can be attributed to the biotransformation process in which epigallocatechin-3-gallate (EGCG) undergoes conversion to epigallocatechin gallate(ECG) andepicatechin(EC), by enzymes released by microbial communities in an acidic environment. The mechanism involved occurs by the hydrolysis of EGCG into smaller molecules and converted into epigallocatechin(EGC), EGCG and EC (Jayabalan et al. 2008). Other molecules such as theaflavins and thearubigin, which are derived from the polyphenol complex found in black tea and are associated with color changes in tea, are also found (Martínez-Leal et al. 2018). It is found that the lighter color of a fully fermentedkombucha can be attributed to the conversion of theaflavins into theobromine (Jayabalan et al. 2007; Torre et al. 2021). In the scientific field, it is possible to verify the use of other means of solution, used as alternative bases for the production of the drink, for example, wheatgrass juice (Sun, Li, Chen, 2015); soy whey (Tu et al. 2019), matte tea (Santos et al. 2009), coffee (Watawana et al. 2015) and coconut water (Villareal-Soto et al. 2018).

Figure 1 briefly presents the main actors involved in the obtaining process and the main components found in kombucha.



pH: hydrogen potential; °C: Degrees Celsius; SST: Total soluble solids (°Brix); CO^2: Carbon Dioxide.
Source: Authors, 2024.

DYNAMICS OF PROTOCOOPERATION OF THE MICROBIOLOGICAL ECOSYSTEM AND OBTAINING THE DRINK

The complexity of kombucha fermentation kinetics occurs mainly due to the number of microorganisms involved and their interactions (Kitwetcharoen et al. 2023). Most species excrete metabolic products that can stimulate or inhibit the specific growth rate of other species, establishing commensalistic or amensalistic interactions that require a clear understanding of this coexistence phenomenon. Some groups of bacteria such as lactic acid and acetic acid bacteria (LAB and AAB), as well as yeast species such as Saccharomyces cerevisiae, have well-established roles in this dynamic.The complexity of the system encompasses countless species that are still unknown, both in relation to their genetics and bioactivity, as well as their interactions, and some obstacles prevent the complete understanding of these ecosystems, with this diversity and complexity of microbial communities being the first of them, mainly in the delimitation of such as, some microorganisms can act in parallel, while others act sequentially with dominant evolution during the fermentation process (Chakravorty etal. 2016). Specifically, when dealing with kombucha, the supernatant biofilm of microorganisms (scoby)is used as a key and essential element for the occurrence

and maintenance of this process, having a unique and very complex microbiota, which can be divided into two fractions, namely, one recognized in biological material and the other, found in the drink itself (Chakravority et al. 2016). The main bacteria identified in scoby are the following strains: *Acetobacter xylinodes, komagataeibacter xylinus, Gluconacetobacter xylinus, Acetobacter pasteurianus and Acetobacter aceti* (Dutta and Paul, 2019). Jayabalan et al. 2007 point out that a range of yeasts have also been identified as elements present in the composition of scoby, including *Saccharomyces, Saccharomicodes, Schizosaccharomyces, Brettanomyces/Dekkera, Candida, Torulospora, Koleckera, Pichia, Mycotorula and Mycoderma*,promoting the idea that in addition to bacteria acetic yeasts normally cited as elements present in the composition, there are many species of yeast, with traditionally recognized strains. What was encouraged by Chakravorty et al. (2016), who evaluated the microbiological and chemical profile of kombucha, using modern genetic sequencing tools and observed that the genus *Candida* was the predominant yeast in the two portions evaluated (biofilm and liquid) of the kombucha used for the study. In disagreement, Marsh et al. 2014, when analyzing a sample of kombucha from the perspective of microbiology, showed the dominance of *Saccharomyces* and *Zygosaccharomyces* in the analyzed samples, with the first yeast being found in very low proportions and the second not found in other studies, such as that of Chakravorty et al. (2016). In a comprehensive way, Table 1 presents some of the microorganisms found mapped in the drink and in the scooby according to the scientific literature mapped.

BRAZILIAN LEGISLATION: ADOPTION OF IDENTITY AND QUALITY STANDARDS (PIQ) AS AN ELEMENT OF SECURITY FOR THE INDUSTRY AND THE CONSUMER

The production of metabolites such as ethanol and acetic acid in kombucha acts preventively on the growth of microorganisms with pathogenic potential, inhibiting their growth by modifying the culture medium. However, during the beverage production process, having faithful control over the physical-chemical parameters of the product is essential. For the food industry to guarantee the production of a drink or food with quality and safety, it must meet the standards established by regulatory agencies in the national territory, such as the Ministry of Agriculture, Livestock and Supply (MAPA) and the National Health Surveillance Agency (ANVISA). Following this trend in the population's consumption pattern, added to the great interest in expanding fermentation processes at an industrial level, MAPA, in June 2018, published a public consultation,

over a period of 75 days, exposing the scope of Ordinance No. 64, of May 14, 2018, in which it provides for the project to implement the Normative Instruction (IN), which aims to define the Identity and Quality Standard (PIQ) specific to kombucha throughout the national territory.The result of this public call was Normative Instruction nº41/2019 (Brasil, 2019), published in September 2019, in the Official Gazette of the Union. The publication of this normative instruction placed Brazil in the position of the first country in the world to have a specific standard regarding the characteristics of kombucha, establishing and demanding standards from manufacturers.IN nº41/19 defines kombucha as "The fermented drink obtained through aerobic respiration and anaerobic fermentation of the must obtained by the infusion or extract of *Camellia sinensis* and sugars by symbiotic culture of microbiologically active bacteria and yeasts (scoby)(Brazil, 2019). The entire scope of standards established in this IN apply only to kombuchas obtained industrially, where technologically adequate processing is expected, meeting the established parameters. Kombucha must be composed of drinking water, infusion or aqueous extract of *Camellia sinensis*, sugars, according to specific ANVISA legislation (Brazil, 2005), in addition to the symbiotic culture of bacteria and yeast (Scoby)suitable for alcoholic and acetic fermentation, as long as its safety to human health is guaranteed. The analytical parameters of kombucha, required by Brazilian legislation, are shown in Frame 2.

Table 1 - Main microorganisms found in kombucha and scoby according to scientific literature.

YEAST		BACTER
Arxula adeninivorans	*Meyerozyma caribbic*	*Acetobacter aceti*
Brettanomyces lambicus	*Meyerozyma guilliermondii*	*Acetobacter nitrogenifigens*
Brettanomyces claussenii	*Mycoderma sp.*	*Acetobacter okinawensis*
Brettanomyces custersii	*Mycotorula sp.*	*Acetobacter pasteurianus*
Brettanomyces/dekkera anômala	*Saccharomyces cerevisiae*	*Acetobacter peroxydans*
Brettanomyces/dekkera bruxellensis	*Saccharomyces ludwig Naumovozyma*	*Acetobacter sp.*
Cândida índia	*Pichia fermentans*	*Acetobacter syzygii*
Cândida sp.	*Pichia membranifaciens*	*Acetobacter tropicalis*
Candida kefyr	*Pichia mexicana*	*Bacterium gluconicum*
Candida krusei	*Pichia sp.*	*Bifidobacterium*
Candida stellata	*Saccharomyces uvarum*	*Collinsella*
Candida stellimalicola	*Saccharomycodes ludwigii*	*Enterobacter cancerogenus*
Candida tropicalis	*Saccharomycopsis fibuligera*	*Enterobacter cloacae*
Candida parapsilosis	*Schizosaccharomyces pombe*	*Enterobacter ludwigii.*
Debaryomyces hansenii	*Sporopachydermialactativor*	*Gluconacetobacter europaeus*
Dekkera anomala	*Starmeraamethionina*	*Gluconacetobacter intermedius*
Dekkera bruxellensis	*Starmeracaribae*	*Gluconacetobacter kombuchae*
Eremothecium ashbyii	*Torulaspora delbrueckii*	*Gluconacetobacter rhaeticus*
Eremothecium cymbalariae	*Torulopsis sp.*	*Gluconacetobacter sp.*
Halomonas sp.	*Zygosaccharomyces bailii*	*Gluconacetobacter xylinus*
Hanseniaspora uvarum	*Zygotorulaspora florentina*	*Gluconobacter entanii*
Hanseniaspora meyeri	*Zygowilliopsis californica*	*Gluconobacter oxydans*
Hanseniaspora valbyensis		*Gluconobacter saccharivorans*
Hanseniaspora vineae		*Gluconobacter sp.*
Herbaspirillum sp.		*Komagataeibacter hansenii*
Kazachstania telluris		*Komagataeibacter rhaeticuse*
Kazachstania exigua		*Komagataeibacter xylinus*
Kloeckera apiculata		*Lactobacillus fermentum*
Kluyveromyces marxianus		*Lactobacillus nagelii*
Lachancea thermotolerans		
Lachancea fermentati		
Lachancea kluyveri		

Leucosporidiella *Merimblaingelheimense*		*Lactobacillus satsumensis* *Lactococcus* *Oenococcus oeni* *Propoonibacterium* *Ruminococcaceae incertae sedis* *Weissella*

Source:Marsh et al. 2014; Reva et al. 2015; Chakravorty et al. 2016; Coton et al. 2017; Gaggia et al. 2018, 2019; Sinir, Tamer and Suna, 2019; Dutta & Paul, 2019.

Frame 2 -Controlparameters included in IN nº 41 of September 17, 2019, which regulates the PIQ of kombucha.

Parameter	**Minimum**	**Maximum**
Hydrogenion potential (pH)	2,5	4,2
Alcohol content (% v/v) alcohol-free kombucha	-	0,5
Alcohol content (% v/v) kombucha with alcohol	0,6	8,0
Volatile acidity (mEq/L)	30	130
Pressure (atm at 20ºC) in kombucha added with CO2	1,1	3,9

In addition to the required parameters, IN nº 41/19 contains other inspection and care rules, which aim to guarantee the safety of the product for human health. In finished kombucha, the presence of viable microorganisms may occur, due to the detachment ofscoby fractions, and their addition to the packaged product after the fermentation process is strictly prohibited (Brasil, 2019). In order to overcome this impasse, the legislation authorizes the use of technological processes, such as pasteurization, filtration, centrifugation, among others.Pasteurization occurs by raising and reducing the temperature of the product, leading to a reduction in the microbiological load after fermentation, with the aim of maintaining a degree of stability to the growth of microorganisms naturally present in the drink, which present a potential for toxicity to the individual. Furthermore, even if the presence of viable microorganisms is verified, the product cannot exploit this characteristic, pointing out supposed functional properties or health benefits, in labeling and/or advertising, resulting in legal administrative measures provided for within the scope of legislation (Brazil, 2019).In the analysis guided by Alencar et al. (2020) in which they sought to evaluate the

suitability of kombucha labels sold in Brazilian territory, it was observed that of the different brands analyzed, the vast majority had some characteristic that did not comply with specific legislation (IN nº 41/2019). Of the products analyzed, the following names were identified in the label structure: “fermented drink”, “probiotic drink”, “live drink”, “Kombucha tea”, “Kombucha culture”, “symbiotic colony” and “live organisms”. The authors highlight that this diversity in terms of drink names can be explained by the lack of information held by manufacturers, resulting from gaps in current legislation for characterizing this drink, which leads to highlighting the importance of legislation on food labeling (Alencar et al. 2020).

In addition to bringing a new name to the product, the regulations also align topics highlighting what can be optionally used as an additional ingredient in the beverage manufacturing process, as long as they are included on the label, such as: the infusion of plant species in water or its extracts, fruits, vegetables, spices, honey, molasses and other sugars of vegetable origin, in addition to other components provided for in ANVISA legislation (RDC nº 54 of November 12, 2012), such as fibers, salts minerals and carbon dioxide (CO^2) used in industrial carbonation (Brazil, 2012; 2019). Adding any ingredient that is not permitted, according to current specific ANVISA legislation, may be considered an adulterant product. The body approved the use of new ingredients that can be used in the beverage manufacturing process, including natural colorings and flavoring compounds, in accordance with RDC nº. 2, of January 15, 2007 and nº. 5, of January 15, 2007 for non-alcoholic kombucha. Adding volatile acids, synthetic or from exogenous sources, which do not come exclusively from the fermentative process of inputs and bacterial metabolism, constitutes a breach in the scope of the legislation and is characterized as a crime of industrial responsibility, with MAPA being responsible for registering and monitoring the establishments and products, ensuring that they comply with current legislation (Brazil, 2007).

CONCLUSION

Kombucha presents itself as a fermented drink with growing popularity, driven by its sensorial characteristics and potential health benefits. The production process, although traditional, involves a complex interaction of bacteria and yeast, resulting in aunique microbiological ecosystem that requires rigorous control to guarantee the safety and quality of the final product.Research into kombucha, encompassing several areas of knowledge, is fundamental to elucidate the fermentation mechanisms, optimize

the production process, characterize the composition and bioactivity of the drink, and explore new bases and ingredients. Furthermore, studies on health benefits, including antioxidant, antimicrobial and anticarcinogenic properties, are crucial to substantiate functional claims and ensure safe consumption. The development of the kombucha industry in Brazil depends on the synergy between research, technological innovation and regulatory rigor, driving the production of a safe, high-quality drink with the potential to add value to the production chain and promote the health of the population.

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Capítulo 3

Resultados: Artigo Original 1

Manuscrito submetido no periódico ***Int. J. Food Sci. Technology*** em 14 de junho de 2024.. **Anexo 2** – Comprovante de submissão.

Physicochemical, microbiology and sensory characteristics of kombucha prepared with tommy mango peel flour.

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ABSTRACT

Food choices throughout life are related to greater or lesser risk factors for diseases, therefore it is necessary for the food industry to offer products with nutritional quality and sensorial acceptance. Fermented foods are a good option due to the beneficial compounds generated in the fermentation process and low pH that allows conservation without additives. Thus, the objective of the study was to produce and include tommy mango peel flour in the production of kombucha and evaluate its effects on physicochemical and sensory properties, antioxidant capacity and microbiological profile. Kombucha was developed with green tea and the addition of tommy mango peel flour (10 and 20%). The kombuchas were evaluated in the first fermentation (aerobic) and at the end of the second fermentation (anaerobic), the granulometry and colorimetry of the flour and the antioxidant profile were evaluated. The physical-chemical profile of the drink was evaluated by pH, soluble solids and acidity, phenolics by folin-cioconteu and antioxidants by DPPH, ABTS, ORAC. Microbiome analysis was performed by 16s DNA extraction. For sensory analysis, an affective test was carried out for global assessment, flavor, texture and oral perception. We observed that the phenolic content antioxidant capacity are higher in the second fermentation and the addition of FCMT increases this profile. We noticed that the antioxidant capacity of second fermentation kombucha is higher than that of green tea and that the greater the addition of mango peel flour, the greater the antioxidant capacity. The most abundant bacterial genera were Liquorilactobacillus nagelii (72%), *Acetobacter* (13%) and *Komagataeibacter* (12%) and for fungi (90%) *Brettanomyces/Dekkera bruxellensis.* The drink achieved different levels of acceptance among consumers and non-consumers only in terms of flavor, proving to be a good alternative for the food industry which, by using a mango by-product, adds nutritional value to the drink.

Keyword: kombucha; antioxidant; green tea; fermented food

Introduction

Food intake is one of the main routes of human exposure to different compounds, providing the supply of a complex mixture of chemical substances, which can have positive or negative effects on the consumer's health. Food choices throughout life can lead to a gradual loss of physiological integrity, which constitutes the main risk factor for important diseases such as chronic non-communicable diseases (NCDs) (Vaiserman et al., 2016; Isaksen & Dankel., 2023). In this sense, scientific evidence points to the importance of food choices for preserving health, especially today, where the food industry offers practical and low-cost products, but with low nutritional value and also with chemical additives known to be harmful to the human body and to soil and water (Vaiserman et al., 2016; Kim & Adhikari, 2020).

Given these factors, a growing portion of the population has shown greater concern when choosing foods, making it necessary to develop products that have satisfactory nutritional quality and sensory characteristics. Fermented foods become a healthy food option, they are defined as foods or beverages produced through controlled microbial growth and the conversion of the components of these foods through enzymatic action (Kim & Adhikari, 2020).

Kombucha is a type of drink of ancient oriental origin, fermented in *Camellia sinensis* tea with a low final pH, which allows it to be preserved without the use of additives and with the nutritional benefits that this leaf presents, in addition to additional compounds produced by the fermentation process, such as organic acids. Lately, consumption of the drink has been growing in Europe and the Americas, not only because of the health benefits associated with fermentation, but also because of the sensorial characteristics of the drink itself, such as carbonation and the different flavoring possibilities. Therefore, this ancient way of preparing and preserving drinks provides a product of excellent nutritional quality, also considering that the content of polyphenols and flavonoids, as well as the antioxidant activity of tea, increase with fermentation (Jayabalan et al., 2007; Coto et al., 2017; Cardoso et al., 2020).

The inclusion of other ingredients rich in bioactive compounds can enrich kombucha and increase its beneficial potential. Several studies indicate that antioxidant compounds in the diet and the combination of protective substances from plants have

helped to boost the development of processes for obtaining functional foods. As an example, we have mangiferin, a phenolic compound present in mangoes, mainly in the peel, which has important antioxidant and anti-inflammatory activity both in vitro and in vivo (Amazzal et al., 2007; Lum et al., 2021). Among the many varieties, *Tommy Atkins* is the most produced and has the largest share in the volume sold of mango in the world, mainly due to its intense color, high production and resistance to transportation over long distances (FAO, 2024).

The global fruit market is forecast to grow by 5% per year until 2029, part of this production is used by the cellulose manufacturing industries and in the fruit pulp industry, where the peel is discarded. (Marçal & Pintado, 2021). The full use of food by industries is necessary, considering that the United Nations (UN) established 17 Sustainable Development Goals (SDGs) to overcome the biggest challenges of our time, take care of the planet and improve everyone's lives through sustainable and transformative measures to be achieved by 2030. Thus, the development of truly healthy, sustainable and additive-free foods and beverages meets some of these objectives, such as SDG 3, which deals with ensuring a healthy life for all, SDG 9 which addresses sustainable and innovative industrialization and SDG 12 which addresses sustainable production and consumption patterns. Knowing the use of this part of the fruit and its potential health benefits, mango peel flour becomes a potential ingredient to be used to enrich fermented drinks (Xie et al., 2023; Kucuk et al., 2024).

In this context, production methods and the effectiveness of the final product must be studied to increase the scientific basis for sustainable industrialization and the introduction of innovative products that allow the insertion of bioactive compounds with the aim of promoting quality of life and prevention. of chronic non-communicable diseases. The objective of this study was to produce and include tommy mango peel flour, as an ingredient, in the production of a kombucha model and evaluate its effects on the physicochemical and sensorial properties, antioxidant capacity and microbiological profile.

Materials and Methods

Acquisition and processing of tommy mango peel

Processed and packaged Tommy Atkins Mango (*Mangifera Indica L.*) peels were purchased from a supermarket that produces fruit salads, located in the city of Petrópolis, Rio de Janeiro, Brazil. Next, the material was separated and subjected to conventional drying in a forced air circulation oven (Marconi(R), MA035/5) at 60°C for 26 hours. After drying, the peels were crushed in a porcelain grail, until they formed a type of finely pulverized powder, called Tommy Mango Peel Flour (FCMT). The FCMT was produced in a single batch and stored at -22°C in a freezer until use and subsequent analysis.

Physicochemical characterization of tommy mango peel flour

The physicochemical characterization of FCMT was carried out in triplicate, and followed the following analyses: moisture, ash, total lipids, crude protein, carbohydrates (by analytical difference) and crude fiber, following the methodology described by the Adolf Lutz Institute (IAL, 2008). The results were expressed as the average values per 100g of the product. The moisture content was determined according to the weight loss of the product using the oven method, where the sample was subjected to heating at 105°C, until constant weight. The fixed mineral content (RMF) was determined by the residue method by incineration in a muffle furnace at 550°C. The determination of crude protein was carried out using the Kjeldahl Method, which is based on the determination of total nitrogen, using the global correction factor of 6.25. Total lipids were determined using the direct Soxhlet extraction method, using the petroleum ether solvent. Carbohydrates were estimated indirectly by analytical difference: 100 (%) – (proteins + lipids + RMF).

The colorimetric coordinates were determined through the reading system of the parameters L*, a* and b* using a portable colorimeter (Delta Vista Color®, model 450 G). The particle size of the FCMT was determined using a set of sieves positioned under a vibrating platform with varying spacings in increasing order of mesh opening (850, 600, 250 and 150 µm). The samples (100 g) were placed on the largest opening sieve and subjected to constant agitation for 10 minutes on an electromagnetic stirrer (BERTEL®, 220 V, 360W). Afterwards, the fractions retained on each sieve were weighed and the percentage of material retained on each sieve (%R) and the fineness modulus (MF) were

calculated. The FCMT also followed the flow of characterizing the antioxidant capacity using the methodologies to be described below.

Kombucha production

For the experiment, a large batch of kombucha was produced, with the aim of ensuring that there would be no difference in the composition of the final drink, supporting the maintenance of the composition of the Scooby used in fermentation metabolism. Initially, an infusion of *Camellia Sinensis* tea (green tea) was made, acquired from a producer located in the city of São Paulo/Brazil. The drink was prepared at a concentration of 8 g/L of powder and infused with water at 75 °C for 2 minutes. After infusion, the supernatant leaves were removed using a stainless steel sieve and 50 g/L of sugar (sucrose) were added and the teas were kept in an ice bath until they reached a temperature of 25 °C. Then, 10% (m/v) of SCOBY (Enutra® foods and beverages) and 100 mL/L of a batch of previously fermented and ready-to-consume (starter) kombucha were added to inhibit the growth of undesirable microorganisms (F1). Kombucha fermentation was carried out at 20 °C for 10 days to obtain a good quality drink (Neffe-Skocińska, Sionek, Ścibisz, & Kołożyn-Krajewska, 2017). After 10 days of the initial fermentation, the second fermentation was carried out in anaerobic mode (F2) at 20ºC. F1 was filtered through a sterilized cotton fabric filter, added with 10% or 20% FCMT and placed in amber glass bottles with a capacity of 355 mL and stoppered with a steel lid suitable for these bottles, ensuring an anaerobic environment, foreseen in the study design in F2. The bottles had an Olec® manometer attached to the neck to measure carbonation over the 10 days of fermentation in the second period (F2). At the end of 10 days of the second fermentation, the carbonation pressure was evaluated and presented in pound force per square inch (PSI). These specifications followed the standardization of a local kombucha manufacturer, which already offers the product on the consumer market, meeting the product's identity and quality standards, in accordance with the legislation, which establishes the following parameters: pH (2.5- 4.2); Volatile acidity (30-130mEq/L); pressure (atm at 20°C) of kombucha added with CO_2 (Brazil 2019).

After the fermentation time, the drink was filtered (Whatman #1 qualitative filter paper) and aliquots were divided into eppendorf microtubes, centrifuged at 10.000 rpm for 10 minutes and stored at -18 °C until further analysis. drinks are presented in Figure 1.

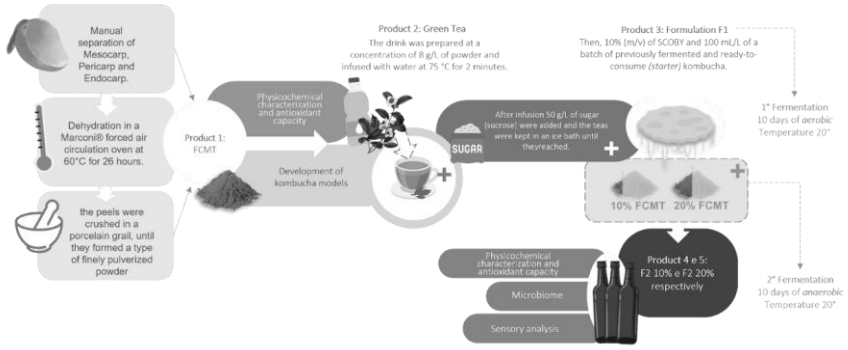

Figure 1. Kombucha production process.

Analysis of the physical-chemical composition of kombucha

All physicochemical analyses were performed in triplicate according to the AOAC methodology (Association of Official Analytical Chemists, 1996). The hydrogen potential (pH) of the drinks was determined using a pH meter (Tecnopon, Piracicaba, Brazil) and expressed as the negative logarithm of the concentration of hydrogen ions in a solution. Total soluble solids were determined by refractive index with a portable refractometer at 20 °C and the results were expressed in °Brix. The titratable acidity was determined by titration with 0.1 N NaOH, the results were expressed as $g.100^{g-1}$ of citric acid per 100 mL of sample.

Phenolic compounds

The content of total phenolic compounds (TPC) of drinks was determined according to the Folin-Ciocalteau (FC) procedure following the methodology adapted from Abreu et al. (2019). The analyzes followed reading at a wavelength (λ) of 750 nm, in a spectrophotometer (SpectraMax i3x multimode microplate reader, California, United States States). A gallic acid standard curve was used as a calibration standard and results were expressed as mcg of gallic acid equivalents (GAE) per mL of sample on a dry basis.

Antioxidant capacity

The antioxidant capacity was analyzed by the methods: DPPH, ABTS and ORAC. The experiments were performed in triplicate. All were performed using the adapted methodologies described by Abreu et al. (2019).

Microbiome analysis

After centrifuging the samples the supernatant was discarded and the pallet of microorganisms from the liquid sample were collected by centrifugation, mixed with biofilm fragments, and used for total DNA extraction. From this DNA, the 16S (for bacterial analysis) and ITS (for fungal analysis) genes were amplified. The amplified fragments were then sequenced on the Illumina NextSeq® platform, and the sequences were analyzed with Qiime software to identify the microorganisms present in the samples and their respective percentages therein. These results were then compared with the literature to determine the phenotypic characteristics of the microorganisms found.

Sensory analysis.

Those who met the inclusion criteria (voluntary participation, being 18 years of age or older and spontaneously accepting to contribute by reading and agreeing to the Informed Consent Form) presented by the collection team participated in the research. This research was approved by the Research Ethics Committee (CEP) under CAAE number: 59033722.3.0000.5245 guaranteeing the secrecy and privacy of those involved. An acceptance test was carried out with kombucha added with 20% FCMT. 162 tasters were subjected to the affective test, where they fell into the spectrum of kombucha consumers and non-kombucha consumers. The attributes evaluated were color, flavor, general appearance and oral perception (related to carbonation). A nine-point hedonic scale was used, where 1 implied the term “extremely disliked” and 9 “extremely liked”, in addition to the purchase intention test also carried out with a five-point hedonic scale (Worch, 2012).

All tasters were informed that it was not mandatory to ingest the entire product and between each portion they were offered a glass containing mineral water at room temperature, so that the previous sensations were neutralized and the procedure could be repeated for all samples. To characterize the tasters, education, whether or not they consume kombucha, frequency and reason for consumption were included in the questionnaire.

Statistical analysis

The results of proximate composition, colorimetric analysis, and texture were submitted to analysis of variance (one-way ANOVA) with Tukey's post-test, with a significant difference at $p \leq 0.05$. The analyzes were carried out using GraphPad Prism software version 5.0.

Results and Discussion

Mango peel flour analysis

Data on the proximate composition and granulometric profile of Tommy da mango peel flour are presented in Table 1. It was observed that FCMT has a low content of total proteins and lipids (0.82 and 2.05 g% respectively) however, it has an excellent total fiber content (38.80g%) in the portion. Chen *et al.* (2019) reported the proximate composition of mango peel powder, a behavior similar to that of the present study was observed, where a low concentration of proteins and lipids was observed (6.8 and 0.9 g% respectively) and a high concentration of fiber (44.4 g%) in the portion.

The total fixed mineral residue content of FCMT was similar to that reported by Mayo-Mayo et al. (2020) for mango peel (2.31 and 2.44 g% respectively), with values plotted at 2.39 g%. The moisture value (%) verified in the FCMT (5.90 g%) is below the value plotted by other authors for the same food matrix. Rybka et al. (2018) described mango peel flours from different cultivars, and found a moisture content of around 8.17g% for Tommy mango in the same drying condition presented by the present study. Chen et al. (2019) and Roidoung, Ponta and Intisan (2020) found more approximate values, plotted at 7.1 and 7.23 g% respectively. The use of FCMT in the formulation of new foods can significantly contribute to the full use and addition of value to the raw material and product, helping and directly impacting the reduction of operational production costs, when compared to the use of products that use noblest parts of the fruit, such as the Pulp (Jahurul et al., 2015).

The evaluating the granulometric profile of this flour becomes essential, since the size of the particles can influence the digestibility and bioavailability of the nutrients present, and it can be considered that the smaller the particle size, the better the use of nutrients. (Almeida et al., 2020). The FCMT presented particle size distribution classified

as powder with a low fineness modulus (FM%: 0.0850), considering the retention accumulated on the normal series sieves (100 MESH / 150 µm).

Still in relation to the physical-chemical aspects of flour, colorimetric analysis constitutes an important element that defines, in theory, the quality in the standardization of food products (Menezes Filho et al., 2019). There was high brightness intensity (L^* = 57.26) in the FCMT, with chroma b^* highlighted in the colorimetric spectrum trend (b^* = 34.0) more red/yellowish. Lario *et al*. (2004) highlights that drying processes determine color changes and induce darkening of food matrices, tending towards the brown spectrum, which is defined as a yellow color with low luminosity. The colorimetric pattern found in FCMT in the present study is in line with the results found by Aziz et al. (2012), who found chroma b^* varying from 34.46 to 39.22 in samples of ripe mango flour.

Table 1. Proximate composition, granulometric profile and colorimetry of tommy mango peel flour.

Centesimal composition		***Colorimetry***		**Granulometric profile**		
Parameters	g (%)	**Parameters**		Slaves (MESH/µm)	Retained weigth (g)	Accumulated retention (%)
Moistrure	5,90	*L**	57,16	20 / **850**	0,17	0,17%
Ash	2,39	*a**	8,81	28 / **600**	3,29	3,48%
Fiber	38,80	*b**	34,00	60 / **250**	11,13	14,66%
Proteins	0,82	*ΔE*	0,40	100 / **150**	8,46	23,17%
Lipids	2,05			**Tray**	76,44	-
Carbohydrates	50,50			**Fineness Module**		0,0850
Caloric value (kcal)	222,0					

L*: brightness; a*: Chroma a; b*: Chroma b; *ΔE*: Delta E; g: grasskcal: kilocalorie.

Sourse: Autors, 2024.

Physicochemical parameters of kombucha

The physicochemical characterization of the formulated drinks are presented in Table 2. The kombucha was evaluated 10 days after the start of the first fermentation (T0) (20ºC ± 3ºC) and 10 days after the second fermentation (T1) (20ºC ± 3ºC). The values obtained for acidity, pH, °Brix and reducing sugars of tea and Kombucha throughout the fermentation are described in Table 2. It can be observed that the volatile acidity increased, while the pH and sugar content decreased throughout the fermentation. It can

be observed that the pH of the tea was 4.8 ± 0.1 and kombucha, from time zero pH of 3.2 ± 0.1 due to the addition of the starter, which already has an acidic pH (2.4). After 10 days of fermentation, there was a reduction in pH to 3.1± 0.2 (F2 20%), $p > 0.05$. These results are similar to those found in the study by Perioto et al. (2022) who obtained a pH of approximately 4.39 at the beginning of fermentation and 3.5 on the seventh day, while Cardoso et al. (2020) and Yang et al. (2022) found a pH of 3.2. Fermentation of kombucha decreases pH due to production of organic acids. The high acidity is due to the production of several organic acids during the tea fermentation process, the main ones being acetic acid and gluconic acid (Jayabalan et al., 2007; Bhattacharya et al., 2016).

The average acidity in acetic acid went from 1.04 g/L at time zero (beginning of fermentation) to 1.06 g/L (F2 10%) and 1.07 g/L. /L (F2 20%) at the end of the second fermentation, while it was observed that in non-inoculated tea the percentage was 0.25 g/L, corroborating the pH value that decreased during the fermentation process. According to Simir (2019) the drink will be more sensorially accepted and pleasant, with a less acidic flavor, when fermentation is completed with total acidity between 4 and 5 g/L. Jayabalan et al. (2007) did not detect acetic acid value on the first day of fermentation and in 7 days of fermentation they found a value close to that found in this work, being 1.64 g/L, using the same green tea substrate for the production of the drink. Cardoso et al. (2020) described a kombucha green tea that had a total acidity of 0.36% (w/v acetic acid) after 10 days of fermentation. Our results demonstrated an average of 0.19% was obtained in 10 days of fermentation of kombucha with green tea.

In kombucha, organic acids are generally obtained through the fermentative process, carried out by the symbiosis of bacteria and yeast. However, the composition of the microbial community, fermentation temperature, concentration of reagents and other parameters may vary during the process, which may reflect a variation in total acidity values. The concentration and composition of different organic acids, especially the proportion of acetic acid in relation to gluconic acid, determine the flavor and aroma of products. The variation in total acidity values between the triplicates of the analyzed samples suggests a difficulty in standardizing the manufacturing process.

In table 2, it can be seen that soluble solids decreased during fermentation. The total soluble solids content is explained by the concentration of sugars added to the tea used as a substrate in the fermentation process (Dada et al., 2021; Zhang., 2021) and by

the addition of tea. In our work, a decline in solids content was found probably due to sugar consumption. The reducing sugar content indicates that during the fermentation process, yeast and acetic bacteria hydrolyze the non-reducing sugars (sucrose) in kombucha, converting them into glucose and fructose (Santos et al., 2018). It was observed a change in the reducing sugar content in formulation F2 10% (10.01 ± 0.02 g/L to 10.02 ± 0.01 g/L -F2 10%) and 9.71 ± 0.02 g/L (F2 20%) from time zero to the tenth day. of fermentation. However, according to Jaybalan et al. (2014), sugar, an important source of carbon, where much of the sucrose is not completely consumed in the first 10 days of fermentation, is also a major constituent of kombucha, as are the products of its hydrolysis by yeast: glucose and fructose. The author states that the remaining sucrose can be used by acetic bacteria as a carbon source to produce a cellulose network as a secondary metabolite of fermentation, giving rise to a new SCOBY, mainly the bacteria *Acetobacter xylinum*.

Perioto et al. (2022) obtained reducing sugar values of 45 g/L at zero fermentation time and approximately 120 g/L at the end of 7 days, values greater than twice the initial concentration. This initial hydrolysis of sucrose is attributed to the action of yeast, as fermentation progresses, yeasts use sugar anaerobically to produce ethanol, while acetic bacteria use sugar and ethanol to produce gluconic acid and acetic acid, respectively. Thus, there is variability in the reducing sugar content in different kombuchas and sugar consumption depends mainly on the fermentation time. In this work we chose to carry out the first fermentation in 10 days.

Table 2. Physicochemical composition of green tea and kombucha produced with aerobic fermentation followed by anaerobic fermentation.

	Green Tea	**F1**	**F2 10%**	**F2 20%**
pH	4,8 ± 0,1[a]	3,2 ± 0,1[b]	3,2 ± 0,1[b]	3,1 ± 0,2[b]
% Total molar acidity (v/m)	0,04 ± 0,05[a]	0,55± 0,02[b]	0,58 ± 0,04[b]	0,61 ± 0,07[c]
Acetic acid (g/L)	0,25 ±0,02[a]	1,04±0,04[b]	1,06±0,01[b]	1,07±0,02[b]
Reducing sugars (g/L)	1,71 ± 0,02[a]	10,01 ± 0,02[b]	10,02 ± 0,01[b]	9,71 ± 0,20[b]
Total Soluble Solids (ºBrix)	5,60 ± 0,10[a]	7,30 ± 0,10[b]	3,50 ±0,10[b]	3,40 ± 0,01[b]

Equal letters on the same line indicate that the values are not statistically significant ($p \leq 0.05$) according to the Tukey test. F1 = kombucha with green tea, starter, scooby and sugar. F2 10% = kombucha (F1 10 days) added with 10% mango peel flour after 10 days of second fermentation. F2 20% = kombucha (F1 10 days) added with 20% mango peel flour after 10 days of fermentation.

Total phenolic compounds content

Total concentrations of phenolic substances for the evaluated samples (Fig. 2). shows that this process increases significantly during the fermentation process ($p < 0.05$) compared to the initial (F1) and green state. Cardoso et al. (2020) also found higher values of total phenolics in kombucha after 10 days of fermentation, however in this study only the first fermentation was analyzed and in our study we evaluated the complete fermentation of the product that is sold. It is worth mentioning that there are many concentrations of tea studied, which can significantly impact the total phenolic concentration. In our study, 8g/L of *Camellia Sinensis* herb (green tea) was used. It can be highlighted that the fermentation process increases the total phenolic content in kombucha made with green tea and that the second fermentation under anaerobic conditions further favors the increase in phenolic content and that the higher concentration of mango peel flour in this second fermentation it does not interfere with the total content, showing no statistical difference between them ($p < 0.05$). The chemical composition of kombucha varies considerably according to the type of tea used and the parameters established during fermentation (Chu and Chen, 2006, Ivanišová et al., 2020, Jayabalan et al., 2008, Villarreal-Soto et al., 2018, Villarreal-Soto et al., 2019). Green tea is obtained from the fresh leaves of *Camellia sinensis L*. and catechins are the main polyphenols found in the drink (Senanayake, 2013).

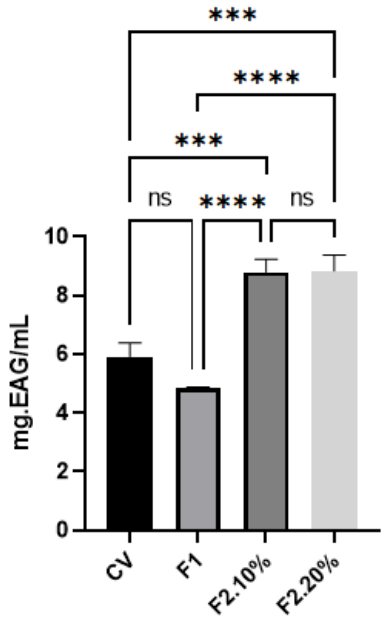

Figure 2. Content of total phenolic compounds obtained in green tea and kombuchas obtained from green tea in first and second fermentation. Presence of (*) indicates statistical difference ($p < 0.05$); *There is no presence of "ns" statistical difference.*

Antioxidant capacity

The chemical diversity of antioxidants and the reaction behavior to different types of radicals and oxidants make it difficult to consolidate a universal method for determining antioxidant activity, therefore requiring evaluation using different methods (Becker et al., 2019). Due to this issue, the ABTS, DPPH and ORAC methods were chosen for this study.

In our study, considering the DPPH method, we observed that the antioxidant capacity of second fermentation kombucha is higher than that of green tea and that the greater the addition of manga peel flour, the greater the antioxidant capacity. Using the ORAC method, there was no increase in antioxidant potential as a result of the fermentation process, but the fermented beverage maintained a high antioxidant potential, just like green tea. Regarding the ABTS method, we observed that in the first fermentation there was a reduction in antioxidant potential, which was recovered in the second

fermentation. The antioxidant capacity of green tea kombuchas can be explained by the high concentration of total phenolics and also by the diversity and abundance of phenolic classes (Cardoso et al., 2020; Yang et al., 2022). Malbaša et al. (2011) observed that green tea kombuchas can have great antioxidant capacity depending on the type of starter culture used in the production of kombucha at 28 °C for 10 days. In addition to the concentration and composition of phenolics in kombucha, other metabolites produced during fermentation, such as ascorbic acid and other organic acids, can also modify the antioxidant capacity of kombucha (Jayabalan et al., 2007; Malbaša et al., 2011). In the study by Yang (2022), several commercially available brands of kombucha were analyzed and there was great variation in the phenolic content. The antioxidant capacity of kombucha is also affected by temperature (Jayabalan et al., 2008; Qu et al., 2020) and fermentation time (Chu & Chen, 2006), in our study the fermentation temperature was around 20ºC , this temperature was lower than most studies, which may have influenced the antioxidant potential of the beverage studied (Villarreal-Soto et al., 2019; Tran et al., 2020). Our results differ from some authors who found an increase in antioxidant capacity by different methods in first fermentation kombucha compared to green tea. (Jayabalan et al., 2008; Malbasa et al., 2011; Cardoso et al., 2020;) Our results indicate that although the low temperature may have negatively influenced the antioxidant potential of the drink, the addition of FCMT represented a positive effect, recovering this antioxidant capacity compared to green tea. Neffe-Skocinska et al. (2017) tested three different temperatures (20 °C, 25 °C and 28 °C) in 10 days of fermentation, evaluating factors such as pH and organic acids, and defined the best temperature as 25 °C during this period. We chose to use the lowest temperature (ambient 20ºC), to portray the conditions used by a kombucha factory located in a mountain town, and present a solution to improve this antioxidant capacity, such as the addition of FCMT. In fact, the addition of FCMT increased the antioxidant potential of kombucha, proving to be an effective solution for factories that are located in places with a milder climate and choose not to use air conditioning, which increases the cost of production. Thus, the use of FCMT, which is the by-product of manda, most of the time discarded incorrectly, proves to be an important ingredient in the production of drinks, such as kombucha, meeting one of the UN SDGs.

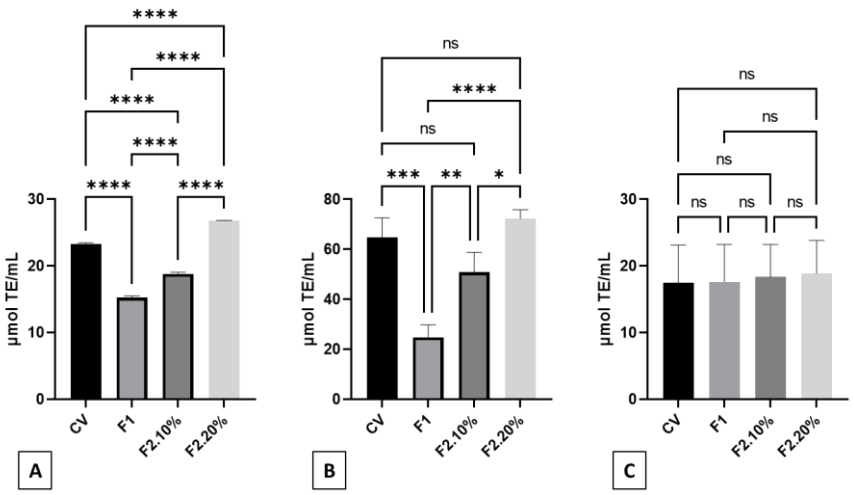

Figure 3. Antioxidant capacity of green tea, first fermentation kombucha (F1), second fermentation kombucha added with 10% mango peel flour (F2 10%) and second addition kombucha added with 20% mango peel flour (F2 20%) by different methods.
A. DPPH test; B. ABTS test; C. ORAC test.. *Presence of * indicate statistical difference ($p < 0.05$); Presence of "ns" there is no statistical difference.*

Microbiome Analysis

Within the SCOBY consortium, a series of symbiotic interactions occur and, due to their complex nature, the interactions between microorganisms and the fermentation environment are the subject of several researches, aiming to understand the genetic sequencing of different microorganisms.

As in the studies carried out by Gaggìa et al. (2019) the present work presents similarities in the bacterial composition, based on the sequencing of the 16S gene. Simultaneously, *Acetobacteraceae* was found as a large family of bacteria, with *Komagataeibacter* spp. as one of the dominant genera, but *Lactobacillaceae, Paenibacillaceae*, *Staphylococcaceae*, *Streptococcaceae*, *Lachnospiraceae* were also detected. Through the taxonomic classification of these communities, the presence of 2 recognized phyla, 4 classes, 8 orders, 9 families, 13 genera and 10 species was observed (supplementary material). For F1, (fig. 4-A) the most abundant bacterial genus is *Liquorilactobacillus nagelii* (72%), followed by the genera *Acetobacter* (13%) and *Komagataeibacter* (12%). Figure 4 shows the proportion of most abundant bacteria in

sample F1, sample F2 did not present a sufficient number of bacteria for detection, this can be justified by the fermentation temperature (20ºC) which is the ideal temperature for the growth of yeasts and not bacteria. Therefore, the increase in fungus would have limited bacterial growth in the second fermentation, making its quantification impossible.

Acetobacter and *Lactobacillus* were the main bacterial genera in the commercial Kombucha starter investigated by Tu et al. (2019). As it is a fermented drink, the abundance of the taxon *Liquorilactobacillus nagelii* is justified in this work, as it is characterized as an obligatory homofermentative *lactobacillus* that can ferment hexose sugars, such as galactose, glucose, fructose, sucrose, mannose, N-acetyl glucosamine, maltose and trehalose into lactate through glycolysis, essential for kombucha to have its specific nutritional and sensory characteristics (Yetiman and Ortakci, 2023). The *genus Komagataeibacter* is the most efficient taxon in the synthesis of the nanofibrillar form of cellulose, using UDP-glucose as a substrate in the glycolytic pathway (Cannazza et al., 2021), being essential for the formation of the cellulose biofilm (scoby) responsible for the process of obtaining of the drink, comprising one of the viable encodings, most representative in the analyzed sample.

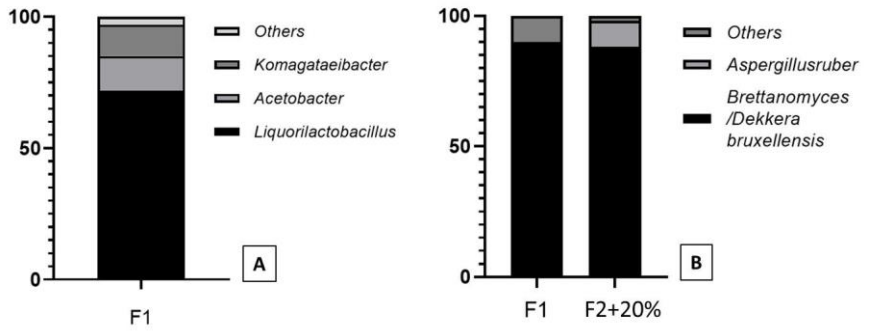

Figure 4. Proportion of most abundant bacteria and fungi in kombucha. A. bacteria in first-fermentation kombucha (F1); B. bacteria and fungi in first fermentation kombucha (F1), second addition kombucha added with 20% mango peel flour (F2 20%).

Fungal community

It is known that yeasts are normally found inside the cellulose biofilm used to obtain kombucha, where oxygen concentrations are low, allowing the creation of an environment that guarantees the occurrence of anaerobic fermentation (Coelho et al.,

2020). Their diversity varies depending on their origin, substrate used for metabolic pathways and conditions for biofilm production and they play a crucial role in the principle of co-culture, through the production of invertase, which breaks down sucrose in the medium into reducing sugars, which are more accessible to any microbial member of the Kombucha consortium (Devanthi et al., 2019; 2022).

Sequencing of the ITS region resulted in 68,121 and 96,959 sequences for F1 and F2+20% respectively. The taxonomic classification of the community observed the presence of 4 recognized phyla, 13 classes, 34 orders, 54 families, 63 genera and 70 species, highlighting that the diversity of fungi evaluated was greater than the bacterial diversity analyzed by sequencing the 16S gene. Analyzing only the identified fungi, both samples showed the majority (90%) of the genus *Brettanomyces/Dekkera bruxellensis*. Added to this, sample F2 20% also presented 10% of *Aspergillus ruber* while sample F1 presented several other fungi in a low proportion. *A. ruber* is a popular fungus because it has a substantial amount of secondary metabolites belonging to different classes in which some of them present promising biological activities. Some of these metabolites showed excellent biological activities, particularly anticancer and antimicrobial. (Rateb & Ebel, 2011). The Figure 4 - B shows the proportion of the most abundant fungi in the drink samples.

Numerous studies are being conducted to describe the diversity of yeasts found in kombucha. When analyzing the potential of symbiotic cultures of kombucha (formed by bacteria and yeast) on cellulose yield in molasses medium, Devanthi et al. (2021) observed that the most dominant isolated yeast, identified by genome sequencing, through the 26s rRNA gene, was similar to the sequence of *Brettanomyces/Dekkera bruxellensis* (around 99.83% similarity) and as in kombucha , its presence in abundance is also marked in other fermented products, such as wines and beers for example (Serra , Funch & Forster, 2019), which could also be verified in the present study. Gülşah et al. (2019) found in their study that the predominant genus in kombucha samples was *Zygosaccharomyces* (84%) and that the genus *Dekkera* appeared in a smaller proportion (6%).

Most microbial species excrete metabolic products that can stimulate or inhibit the growth of other species, establishing interactions that need to be further explored (Gomes et al., 2019; Villarreal-Soto et al., 2019).

Sensory evaluation

The sensory test was carried out by untrained individuals (n=167) and separated by whether they were consumers (n=65) or not of kombucha (n=112). Among consumers, 60% reported consuming kombucha as bringing health benefits, 26% for health reasons and 9% because it is a tasty drink and 5% had no specific reason. The majority (62%) of consumers consumed the drink at least once a week and 70% had a higher level of education.

Table 3 presents the results of the acceptance test carried out with a hedonic scale for Kombucha with 20% FCMT added by Kombucha consumers and non-consumers. Overall, FCMT kombucha received high scores on all attributes evaluated by both kombucha drinkers and non-kombucha drinkers. For the attributes color, oral perception (carbonation) and overall impression there was no significant difference between kombucha consumers and non-kombucha consumers. The attribute with the highest average was the general impression in both groups of tasters and the one with the lowest average was flavor, an attribute with the highest average in the group of kombucha consumers. This result can be explained by the fact that kombucha is a drink with very particular sensory characteristics, such as acidity and bitter flavor that occur due to the formation of organic acids during the fermentation process (Jayabalan et al., 2014). Vázquez-Cabral et al. (2014) analyzed the global acceptance of a kombucha with green tea and using the same hedonic scale obtained similar results. These characteristics can be attenuated by the inclusion of fruit juice and/or seasonings, which did not occur in this study, therefore the flavor of our product is very characteristic of natural kombucha, as the addition of FCMT does not significantly alter the flavor of the drink.

Even with the lowest flavor score among non-consumers, the intention to purchase the product showed no difference when treated statistically in the evaluation of the two groups, being 4.1 for consumers and 3.9 for non-consumers on a 5-point scale. Barbosa, Costa and Araújo (2020), evaluated the intention to purchase kombucha with 100 participants, of which 67% gave grades 4 and 5 (they would buy and would certainly buy). According to the description of the tasters' characteristics, we can observe that 70% of consumers have a higher level of education, and more than 80% of them consume

kombucha for reasons related to its potential health benefits. This information directs producers to the most appropriate flavor choices and the use of the information in product labeling and advertising. Our kombucha showed greater antioxidant potential with the addition of 20% FCMT, making it a product with potentially beneficial health benefits, in line with consumer preferences.

Table 3 - Results of the general acceptability test of kombucha added with Tommy mango peel flour.

Atributes	Consumers	Non-consumers
Color	7.39 ±1.23[a]	6.70±1.13[a]
Flavor	7.04 ±1.62[a]	5.84±0.90[b]
Mouth perception	7.05±1.04[a]	6.11±1.10[a]
Global impression	7.40±1.06[a]	6.38±1.08 [a]
Buy intention	4.10 ±0.50[a]	3.90±0.40[a]

Equal letters on the same line indicate that the values are not statistically significant ($p \leq 0.05$) according to the Tukey test.

Conclusion

The results obtained demonstrate that fermenting kombucha at lower temperatures can reduce its antioxidant capacity, but that the addition of mango peel flour recovers this antioxidant potential. The ready-to-consume drink presented a microbiological profile only for fungi and was accepted by kombucha consumers, showing that the addition of peels can increase the nutritional value of industrialized products and also contribute to reducing the incorrect disposal of these wastes.

Despite the amount of research already carried out, more investigations are needed into the sensorial, physical-chemical characteristics and antioxidant capacity of kombucha, especially in the second fermentation, and in the different storage times, which is the product received by the consumer. This research could lead to a better definition of the quality of kombucha and better control of its production process to obtain a drink with greater health benefits and more accepted sensory characteristics.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Capítulo 4

Resultados: Artigo Original 2

Effects of kombucha with added tommy mango peel flour on the cytotoxicity and in vitro antitumor activity of colorectal adenocarcinoma (Caco-2) and human osteosarcoma (MG-63) cell lines

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ABSTRACT

In the epidemiological scenario, neoplasms continue to be a major contributor to the global mortality rate, demonstrating their importance in the health sectors. Food choices are an important risk factor for the development of cancer. In this sense, it is necessary to offer more natural alternatives on the food and beverage market that still have some protective effect against cancer. Fermented foods, such as kombucha, have this potential. The aim of the study was to develop and evaluate the cytotoxicity and in vitro antitumor potential of a kombucha model with added tommy mango peel flour in human osteosarcoma (MG-63) and colorectal adenocarcinoma (Caco-2) cell lines. The kombucha formulas for the first (aerobic fermentation) and second fermentation (anaerobic fermentation) were formulated with 20% Tommy Atkins mango peel meal.

We analysed cytotoxicity, cell viability and cell death by MTT, clonogenic assay and beta-galactosidase assay, and apoptotic profile by cytometry. The results show that both osteosarcoma cells (MG-63) and colorectal adenocarcinoma cells (Caco-2) were sensitive to treatment with first and second fermentation kombucha. The osteosarcoma cells were more sensitive to the kombucha treatment.

Key words: kombucha; fermented drink; Caco-2; MG-63; cytotoxicity

Introduction

In the epidemiology scenario, neoplasms continue to be a major contributor to global mortality rates, demonstrating their importance in the health fields(1). The wide ranging epidemiologic and social impact of these diseases encourages continued efforts to develop various therapeutic strategies aimed at their control that may improve prognosis. Among cancer subclasses, colorectal adenocarcinoma and osteosarcoma are models that differ markedly in their pathophysiology and features. Although their origins are different, both may involve common molecular mechanisms, such as mutations in cell growth-regulating genes and apoptosis, and can also be studied to better understand the processes of metastasis and resistance to therapy, which are common challenges in the treatment of different types of cancer, from the initial molecular changes to disease progression (2,3).

In general, adenocarcinoma of the colorectal is a very common type of cancer originating from the glandular cells of the colorectal and is often associated with risk factors such as diet, family history and inflammatory bowel disease (4,5). Osteosarcoma, on the other hand, is a primary bone cancer that originates from bone-forming cells, occurs predominantly in adolescents and young adults, has a poor prognosis and is often associated with genetic factors and certain pre-existing conditions, including those with a genetic background (6,7).

The dynamic interaction between disease burden and treatment innovation highlights the importance of continued progress in the field of oncology to overcome the multifaceted challenges posed by the disease. In this context, it is important to emphasize that dietary choices throughout life are an important factor in prevention and clinical management (8). According to the International Agency for Research on Cancer (IARC), dietary habits observed from childhood to adulthood can directly influence cancer

development at different stages. Dietary patterns that emphasize the consumption of more natural foods over processed foods such as refined grains, sugary drinks and sweets are associated with a lower risk of developing an irregular and potentially neoplastic cell cycle (3).

Awareness of the introduction of a high nutritional value dietary model is gaining attention, particularly promoting the group of fermented foods and beverages (9). Fermentation began as a method of preserving food, but today its technological potential is well known, as it acts on the physico chemical transformation of the product during the process, leading to the production of bioactive molecules that provide a range of health benefits (9) and even have effects on the progression of cancer, as is the case with kombucha (10,11).

Kombucha is a type of drink of oriental origin, originally fermented in *Cammelia sinensis* tea, with a low final pH, which allows it to be preserved without additives and with the nutritional benefits of the plant. Consumption of the beverage has increased in Brazil and other European countries, Canada and the United States, not only because of the health benefits associated with fermentation, but also because of the sensory properties of the beverage, such as carbonation and the possibilities for diversification of flavors and applications (10,11). The growing consumer interest is related to its multiple functional properties, such as its anti-inflammatory potential and antioxidant effects (12). The global kombucha market will reach a value of 11.8 billion US dollars by 2030, with annual growth of 16.8 % between 2022 and 2030 (13).

Considering the urgent need to provide the population with healthier and more natural foods that can contribute to the prevention of chronic non-communicable diseases (NCDs), such as cancer, the present work aimed to develop and evaluate the cytotoxicity and in vitro antitumor potential of a kombucha model to which mango peel flour was added in colorectal adenocarcinoma (Caco-2) and human osteosarcoma (MG-63) cell lines.

Materials and methods

Samples

Acquisition and obtaining of tommy mango peel flour (TMPF)

The processed and packaged Tommy Atkins Mango (Mangifera Indica L.) peels were purchased from local retailers, specialized in the processing of horticultural matrices for consumption, in the city of Petrópolis (22° 30′ 17″ South, 43° 10′ 56 ″West), Rio de Janeiro, Brazil. The material was taken to the Food Technology Laboratory of the Arthur Sá Earp Neto University Center (UNIFASE) in Petrópolis, washed in running water and sanitized in a 200 ppm sodium hypochlorite solution, remaining immersed in the solution for 20 minutes. Next, it was sliced into a "Julienne" cut (1 finger thick) and taken for conventional drying in a forced air circulation oven (Marconi®, MA035/5) at 60 °C for 26 hours. After drying, the material was crushed in a porcelain grail, until it formed a type of finely pulverized powder, called Tommy Mango Peel Flour (TMPF). The TMPF was produced in a single batch and stored at -80 °C in an ultrafreezer until use and subsequent analysis.

Out of concern for the quality and health safety of the extracted shells used for the production of TMPF, it is noted that the place where this raw material is extracted has a certification of sanitary production issued by the bodies responsible for municipal supervision.

Preparation of kombucha samples

For the experiments, a large batch of kombucha was produced, ensuring homogeneity between treatments until the end of the experiments. The development of the product began with the infusion of green tea, obtained from the *Camellia sinensis* herb, acquired from a producer located in the city of São Paulo/Brazil (23° 32′ 56″ South, 46° 38′ 20″ West). The drink was prepared at a concentration of 8 g/L of powder and infused with filtered water at 75 °C for 2 minutes. After infusion, the supernatant leaves were removed using a stainless steel sieve and 50 g/L of refined sugar (sucrose) were added. After homogenization, the solution was kept in an ice bath until it reached 25 °C, where an aliquot, called "GT", was removed. At 25 °C, 10% (m/v) of Symbiotic Culture of Bacteria and Yeast (SCOBY) donated by a commercial kombucha producer, located in the city of Petrópolis, RJ, Brazil (Enutra®) were added and 100 mL/L of a batch of previously fermented kombucha (Startup), creating an environment of proto-cooperation between microorganisms, preventing the growth of undesirable microorganisms.

The initial fermentation of Kombucha was carried out at 20 °C for 10 days, and the product obtained from this period was called "F1". After 10 days of initial

fermentation, a second anaerobic fermentation was carried out. F1 was filtered through a cotton fabric filter, sterilized and added with 20% TMPF, and then placed in amber glass bottles (355 mL) and stoppered with a suitable steel lid, ensuring an anaerobic environment. The bottles had a manometer (Olec®) attached to the neck, to monitor carbonation over a period of 10 days. At the end of 10 days of the second fermentation, the carbonation pressure was evaluated and presented in pound force per square inch (PSI). These specifications followed the standardization of a local kombucha manufacturer, which already offers the product on the consumer market, meeting product identity and quality standards, in accordance with national legislation, which establishes the following parameters: pH (2.5 - 4.2); Volatile acidity (30 - 130 mEq/L); pressure (atm at 20°C) of kombucha added with CO2 (Brazil 2019). At the end of this period, the product obtained was called "$F2^{20\%}$". After the fermentation time, the drink was filtered (Whatman #1 qualitative filter paper) and aliquots were divided into Eppendorf microtubes, centrifuged at 10,000 rpm for 10 minutes and stored at -80 °C until further analysis.

Cellular assays

Reagents and solutions

Ciprofloxacin 2 mg/mL (200x diluted) (EUROFARMA, 2299181); Dimethyl sulfoxide (DMSO) (Auros Química, 1821); Dulbecco's Modified Eagle High Glucose (DMEM-HIGH glucose) (SIGMA-ALDRICH, D6426-500ML); Dulbecco's Modified Eagle Medium Low Glucose (DMEM-LOW glucose) (SIGMA-ALDRICH, D6046-500mL), Glycerol for molecular biology ≥99.0%. (Sigma-Aldrich N 200-289-5, G2025-1L), Senescent Cell Histochemical Staining Kit (SIGMA-ALDRICH, CS0030-1KT), Apoptosis Detection Kit: FITC Annexin V/Dead Cell Kit (BD, 556547), MTT (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium (SIGMA-ALDRICH, M5655-1G), Phosphate Buffer Saline (PBS) (GIBCO, 20012-027), Triton ™ X-100 (T9284-500ML, Sigma); Fetal bovine serum (FBS) (GIBCO, 12657-029). All chemicals were analytical grade.

Cell culture and treatment protocol

The certified cell lines of human colorectal adenocarcinoma (Caco-2) and human osteosarcoma (MG-63) were provided by the Laboratory of Biology of Eukaryotic Cells, INMETRO, Rio de Janeiro, Brazil. To control and guarantee the quality of the

experiments, cellular authenticity tests were carried out through the analysis of the Short Tandem Repeats (STR) marker, serving as a basis for screening possible cross-contamination with other strains, in addition to specific microbiological tests. To check for the presence of bacteria and fungi, the automated equipment BACTEC™ FX BD (Becton-Dickinson) was used, with culture flasks for the growth of anaerobic (Anaerobic Plus, 3221270) and fungal (Myco Lytic, 3249457) microorganisms. For the presence of mycoplasma, the bioluminescence method was used, Lucetta™ 2 system (Lonza, MycoAlert® PLUS Mycoplasma Detection Kit, LT07-710).

The cells were cultivated in cell culture flasks, regularly under an atmosphere of 5% CO2, in DMEM-LOW glucose, supplemented with L-glutamine and 20% fetal bovine serum (FBS) and in DMEM-HIGH glucose, supplemented with L -glutamine and 10% SFB, for Caco-2 and MG-63, respectively. In time, all treatments received the addition of 0.5% of the antibiotic "Ciprofloxacin". Stock cultures in flasks were grown to 80% confluency and routinely subcultured. Cell morphology and confluence were observed using a microscope (EVOS™ Thermo Scientific AMF 5000®).

Cell viability assay

Cell viability was determined using the MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide) method (14). Caco-2 and MG-63 cells were plated in 96-well microplates at a density of 1.0 × 104 cells/well and incubated overnight (5% CO2 - 37 °C). After adhesion, the cells were exposed to treatments at different concentrations (8 different concentrations, in 1:2 dilution series from 1:2 to 1:256 for green tea (GT) and kombuchas samples at different fermentation times and preparation conditions (F1 and $F2^{20\%}$) for 72 hours. One of the microplate columns was used as a positive control, being exposed to 1% Triton™ X-100 detergent and incubated for 20 minutes to completely kill the cells. intended for negative control, not receiving any type of treatment, maintaining ideal cultivation conditions. After the period, the culture medium was removed and 100 µL of MTT (1 mg/ mL) were added. The MTT was removed and 100 µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan, formed by the action of mitochondrial reductase. The results were read in a spectrophotometer (Thermo Multiskan GO cuvette NS: 1510-01941-C) by absorbance, using a wavelength of 570 nm. Cell viability was calculated in comparison with the control (100%).

Colony formation/ clonogenic assay

The strains were seeded in 35mm plates (in a 6-well system) at a density of 10 and 100 cells/cm² and incubated overnight (5% CO^2 - 37 °C) - time zero (t0). On the following day, the cells were treated in triplicate with concentrations of 1:4; 1:8; 1:16; 1:32 and 1:64 and incubated for 10 days (t10). After this time, the culture medium was removed and the cells were washed with PBS saline. To count colonies, 4% buffered formaldehyde at pH 7.2-7.4 was used for fixation (700 µL/well) and an optical microscope with phase contrast inversion was used. Colonies with ≥ 50 and < 50 cells were considered complete colonies, following Franken et al. 2006. The data shown represent the mean ± standard deviation.

β-Galactosidase enzyme activity assay

The cells were grown in 35 mm culture dishes, inserted into a six-well system, cultivated to form colonies, and were analyzed using the biomarker for the enzyme β-galactosidase, following the guidelines described in the Histochemical Cell Staining Kit Senescent (Sigma-Aldrich CS0030®). Cells stained blue were considered positive for SA-βgal. The results were expressed as the percentage of colonies containing more than 40% of cells with blue staining in the cytoplasm, in relation to the total population of colonies quantified, using an optical microscope (EVOS™ Thermo Scientific). The procedure continued until blue coloration was noticed in the cell cytoplasm, reaching maximum coloration after 16 hours. Quantification was carried out using an optical microscope (EVOS™ Thermo Scientific AMF 5000) in a systematic way.

Cell viability and apoptosis assay

Samples of 5 x 10^5 cells in suspension were analyzed using the FITC Annexin V Apoptosis Detection kit (V13242 - INVITROGEN®). Each cell type was divided into two groups: negative control (optimal culture medium) and positive control (optimal culture medium + treatments, at concentrations of 1:4 or 1:8 mL/mL). The cells were incubated for 72 hours at 37 °C in an oven with CO_2. The cells were trypsinized, counted in a Neubauer chamber and centrifuged in PBS solution. The supernatant was discarded and the cells were resuspended in Binding Buffer (2 x 10^5 cells/100 µL). Cell viability and apoptosis were measured using flow cytometry, conducted at the Cell Biology Laboratory (INMETRO), Rio de Janeiro, Brazil, using a flow cytometer (FACSAria III, BD Biosciences). Labeling was analyzed to determine the apoptotic rate. The percentage of total apoptotic cells was calculated by adding the percentages of early apoptotic cells

(annexin V+/PI−) and late apoptotic cells (annexin V+/PI+) and the data analyzed using FlowJo software (FlowJo v. 1.2).

Tabulation, treatment and statistical analysis of data

The results presented are the mean and corresponding standard deviation of triplicates. Data were analyzed using GraphPad Prism 9.0.0 statistical software (GraphPad Software Inc., San Diego, CA, USA). Analysis of variance (ANOVA) was used and Tukey's post-test was used with a 95% confidence level.

Results and discussion

Cell Viability

It was found that treatment with kombucha decreased the viability of MG-63 and Cacao-2 cells after 48 hours of treatment at different concentrations (Figure 1). The MG-63 cell line showed greater sensitivity to samples F1 and F220%, with viability decreasing below 50% (IC50) compared to the negative control group (CTRL-; live cells without treatment) when treated under the proposed conditions of a 1:2 to 1:8 dilution series. In the Caco-2 cell line, lower viability was observed in cells treated with F220% under the same conditions, with a dilution series similar to MG63, except at 1:8, where viability remained above 50% compared to CTRL. In parallel, in MG63 cells, it was observed that the non-fermented green tea infusion (CTRL product) had a higher potential to reduce viability under the highlighted treatment conditions, with greater effects at concentrations of 1:2 and 1:4. In contrast, in Caco-2 cells, F1 and F220% showed a cytotoxic effect (more than 50% cell death compared to CTRL) in the 1:4 treatment, but in green tea this concentration maintained viability at 50%, which does not represent cytotoxicity for this assay. The viability indices obtained for the 1:2 treatments with F1 and F220% were higher than the corresponding indices for the 1:4 treatment, which was attributed to the intrinsic components of these compounds affecting the final result of the assay. Cell death was clearly observed on the images of the culture plates before the assay was performed (Supplementary Material).

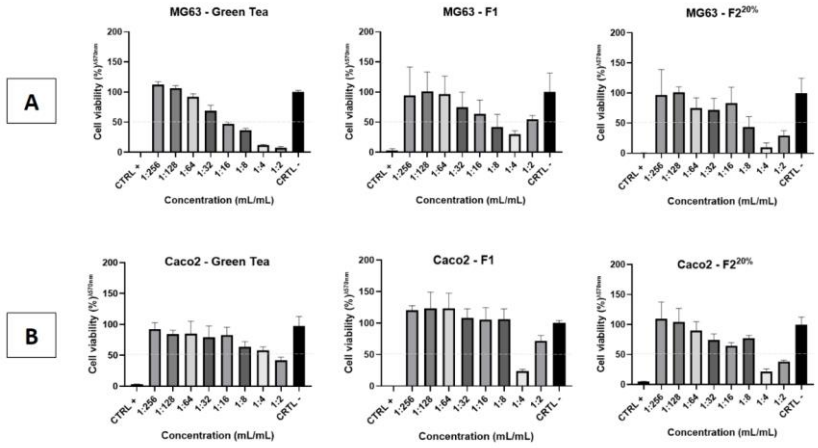


Figure 1. Effects of green tea (GT) or F1 or F2$^{20\%}$ compared to proliferation in medium supplemented with 10% fetal bovine serum (FBS), as a normal growth control (cells without treatment "CTRL-"), on viability cellular and cytotoxicity in MG63 (A) and Caco2 (B) cells. Graphs showing the effects of GT, F1 and F2$^{20\%}$ at 1:2 dilution series 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256, after 48 h by converting MTT into formazan. read at a wavelength of 570 nm. Results are expressed as mean (±SD).

The addition of sugars, the symbiotic culture of bacteria and yeast (SCOBY) and the starter culture for the production of kombucha may explain the increase in cell viability observed in the two cell lines, particularly in the F1 line, especially in the Caco-2 line, where the cells may initially benefit from "nutritive" elements that ensure their survival and metabolism. Another point is the fermentation time and temperature. Some studies that found a higher cytotoxic potential of kombucha compared to green tea had higher fermentation temperatures (25-28°C) than our study (20°C) (10) and longer fermentation times (15).

Currently, Caco-2 cells are known to form microvilli at the apical margin when grown in an ideal culture environment under conditions similar to those in a living organism, which allow the transit of ions and nutrients, and they are often used to test substances with pharmacological potential (16). The higher antiproliferative activity of cell lines treated with kombucha is explained by the large number of bioactive compounds among the phenolic compounds, in particular catechins (which account for about 30% of

the dry weight of green tea leaves) (17), the most abundant substance, and the presence of verbascoside, a phenylethanoid glycoside also found in green tea (10). Catechins and verbascoside are known to have antitumor activity against many cancer cell lines (18,19,20). Catechins have already been shown to have great potential for protection against the development of some cancers by inhibiting enzymes and interrupting processes that lead to disordered growth of cells and thus tumor formation (20,21), while verbascoside, for example, has the ability to inhibit cell growth and viability and promote apoptosis in in vitro and in vivo models of various cancers (20).

Substances formed during fermentation, such as D-saccharinic acid 1,4-lactone (DSL), are known to inhibit the activity of glucuronidase, an enzyme thought to be associated with cancer. Glucuronidase is an enzyme that plays a crucial role in the hydrolysis of glucuronides. These are compounds formed by the conjugation of lipophilic substances with glucuronic acid in the liver, which facilitates their excretion. When glucuronidase hydrolyzes these glucuronides, it releases aglycones, which are the unconjugated forms of the originally metabolized substances. Some of these aglycones can be carcinogenic as they can return to the bloodstream and interact with cellular DNA, potentially causing mutations and contributing to cancer development (23,24,25).

Over time, Cardoso et al. pointed out in their studies that kombucha indeed has a cytotoxic effect on tumor cells of the Caco-2 lineage, recognizing a positive effect of kombucha in reducing the viability of this lineage (10,26). It is worth noting that only the first fermentation of kombucha was examined in these studies. Specifically, in the MG-63 line, a more significant reduction in cell viability was observed in the F220% treatment, which could be directly related to the addition of TMPF in the second fermentation, which increases the presence of various polyphenols in the product, but there are still no studies evaluating the effect of kombucha on osteosarcoma lines.

Recently, mango has attracted a lot of attention in the scientific community due to its remarkable antioxidant, anti-inflammatory, immunomodulatory and anti-cancer properties (27,28). The fruit is known for its rich nutrient content, including vitamin A, vitamin C, essential amino acids, fiber and essential bioactive compounds such as terpenes and phenolic compounds.

Among the phenolic compounds found in mango, mangiferin has been extensively studied for its therapeutic potential in vitro, which may play a role in reducing cell

viability. In a study conducted by Wen et al. (31), the cytotoxic effects of mangiferin were observed in human cell lines Saos-2 and U2OS. In the experiment, cells were administered different doses of 25, 50, 75 and 100 µM mangiferin over a 72-hour period, and a significant decrease in cell viability was observed in direct proportion to the doses administered. In addition to the decrease in cell viability, the researchers also observed a significant decrease in the cells' ability to adhere and invade. This multi-faceted assessment provides valuable insight into the potential of mangiferin as a cytotoxic agent against osteosarcoma and contributes significantly to the understanding of the underlying mechanisms of cytotoxicity of mango and its compounds and their potential impact on the development of new treatment strategies for osteosarcoma.

Colony formation/ clonogenic assay

The figure 2 shows the effects of green tea and kombucha treatment at different fermentation times on MG-63 and Caco-2 cell lines assessed by the clonogenic assay. This method allows analysis of the reproductive viability of cells, more critically looking at the ability of a single cell to form a colony that has doubled and produced 50 or more cells. In this study, the 1:4 and 1:8 concentrations were found to be effective in preventing the growth of clones during the period studied (10 days) for all treatment conditions (Green Tea, F1, and F220%). In the MG-63 cell line, it was observed that the fermented products (F1 and F220%) effectively inhibited clonogenic activity at a concentration of 1:16 ml/ml in addition to the concentrations of 1:4 and 1:8, supporting the idea that fermentation products may play an important role in the control and modulation of elements involved in cell growth and proliferation.

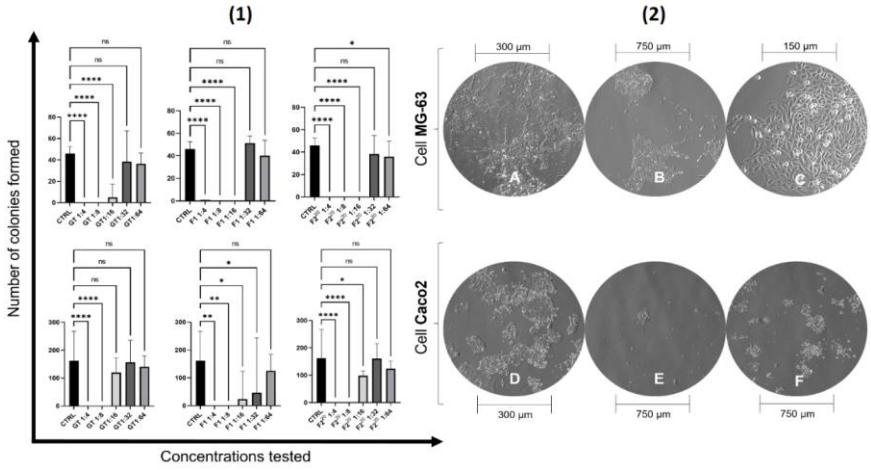


Figure 2. (1) Effects of green tea (GT), F1 or F2$^{20\%}$ on colony forming capacity, compared with cells in medium supplemented with 10% fetal bovine serum (FBS), as normal growth control (cells without treatment "CTRL") in MG-63 and Caco2 cell lines. Results are expressed as mean (±SD). (2) Representative images of the formed colonies, already fixed in both strains: (2A) CTRL MG-63 colony (scale 300 µm/ 10x); (2B): Colony GT MG-63 condition 1:64 (scale 750 µm/ 4x); (2C): Colony F2$^{20\%}$ MG-63 1:64 (Scale 150 µm/20x); (2D): CTRL Caco2 colony (scale 300 µm/ 10x); (2E): Colonia F1 condition 1:16 (scale 750 µm/ 4x) and (2F): Colonia F2$^{20\%}$ condition 1:16 (scale 750 µm/ 4x).

The clonogenicity assay is considered the gold standard for evaluating reproductive cell death and the efficacy of new cancer therapies, as it is a direct measure of the ability of treated cells to continue to divide and form new tumors. Yang (32) describes the application of the clonogenic assay to test therapies and highlights its importance in determining the clonogenic survival of tumor cells. The antioxidant capacity of the polyphenols present in kombucha may be closely related to its efficacy in scavenging free radicals, one of the main mechanisms responsible for the observed antiproliferative effect, complementing the results of the MTT assay (33). These compounds have the ability to modulate various signaling pathways and proteases involved in the mitosis process, including cell proliferation markers such as p53 and p21 (34) and ROS (35), as well as the reduction of Bcl-2 (36). As this modulation has already been described in the literature, the product tested here behaves like a potential strategy for interrupting tumor growth.

Effects of Kombucha on Cell Death Events

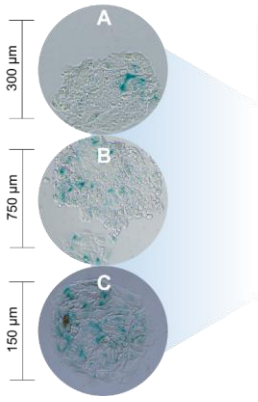
Cell death processes can be divided into apoptosis, autophagy, necrosis, catastrophic mitosis, and senescence based on their morphological and biochemical

characteristics (37,38). Senescence is characterized as an active metabolic process that is essential for aging. It is triggered by a genetic program that involves the deterioration of telomeres and the activation of tumor suppressor genes. Cells that enter senescence lose their ability to proliferate after a certain number of cell divisions (39).

In this study, both cell lines were subjected to the senescence-associated β-Galactosidase staining assay. It was found that in the MG-63 cell line, all treated cells were induced to die by a mechanism unrelated to the senescence process, making the counting of positive colonies for the marker unfeasible. However, in the Caco-2 cell line, the formation of positive colonies for the enzymatic marker was observed with a highly delineated blue spectrum (Figure 3-1). It was found that F1 had the highest tendency to lead the cell to the senescent state under the dilution conditions tested, with representation of >30% β-gal (+) of the total colonies formed. The F220% formulation showed a similar trend to the control group in the most diluted treatment (1:16), with the proportion of β-gal (+) marking below 30% of the total colonies.

It was noted that F1 under the tested dilution conditions showed the highest tendency to lead the cell to the senescent event, with representation of >30% β-gal (+) of the total colonies formed. The F220% formulation showed a similar trend to the control group in the most diluted treatment (1:16), with the proportion of β-gal (+) marking below 30% of the total colonies.

(1)



(2)

		Samples		
		GT	F1	$F2^{20\%}$
1:8	n° Col. Totals	331	484	256
	nº Col. *β-gal (+)*	***52*** *(15,7%)*	***146*** *(30,1%)*	***64*** *(25%)*
1:16	n° Col. Totals	349	498	383
	nº Col. *β-gal (+)*	***125*** *(35,8%)*	***156*** *(31,3%)*	***50*** *(13%)*
CRTL	n° Col. Totals		451	
	nº Col. *β-gal (+)*		**63** *(14,9%)*	

Figure 3. Detection of senescence-associated beta-galactosidase (SA-βgal) in replicative senescence in Caco-2 cell by cytochemical staining. GT = green tea, F1= fermentation one and $F2^{20\%}$ = fermentation two addiction of 20%. The TMPF supplementation increases the percentage of SA-β-gal positive Caco-2. (1) Representative image of colonies stained with the b-gal marker (1A): Scale 300 µm/ 10x; (1B): Scale 750 µm/ 4x; (1C): Scale 150 µm/ 20x. (2) Comparing Caco-2 cultures in the presence of the substances GT or F1 or $F2^{20\%}$ compared to the control culture condition, supplemented with DMEM and SFB. The staining of β-Gal staining in blue highlights the characteristics of senescent cells, such as enlarged cytoplasms and a high presence of cytoplasmic vacuoles.

Apoptosis analysis by flow cytometry using apoptosis markers (Annexin V FITC) and cell death markers (PI) showed that green tea caused a decrease in cell viability (Figure 4, Q4) with statistical significance in both MG-63 and Caco-2 cell lines after 72 hours of treatment with kombucha and green tea compared to untreated cells (CTRL). In MG-63, the viability under CTRL was 96.07% ± 1.04, while under green tea it was 69.93 ± 2.73; in Caco-2 it was 91.53 ± 2.88 under CTRL and 74.10 ± 0.17 under green tea. Figure 4 and Tables 1 and 2 show the data on viability, early and late apoptosis, and cell necrosis observed in the analysis. It can also be seen that the kombucha formulations had a lower percentage of early apoptosis (Figura 4, Q3) for both cell lines compared to green tea, which is the same as the control group (untreated cells). When comparing the control group (untreated cells) with the Kombucha formulation for the die-off rate (Figura 4, Q1), an increase in die-off rate was observed in the MG-63 cell line for both fermentation times, but with statistical significance only for F1. In the Caco-2 cell line, no significant change in the death rate was observed for any of the treatments carried out. Regarding late apoptosis, F220 stood out among the fermented formulations by significantly increasing the rates compared to the control group in both cell lines.

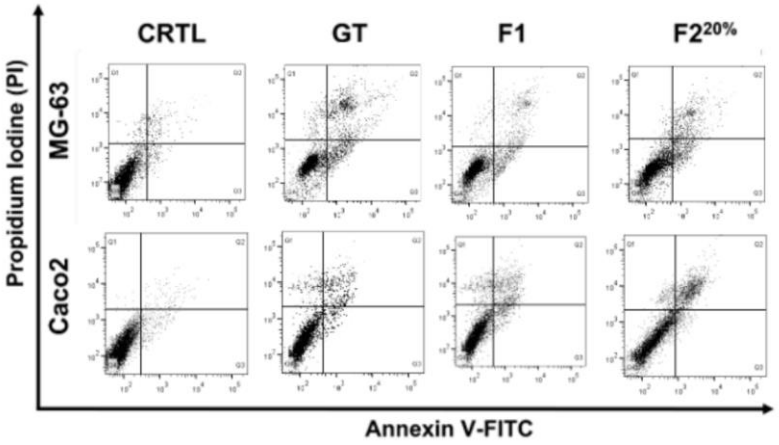


Figure 4. Effect of green tea, F1 and F2$^{20\%}$ on the apoptotic profile of Lineage MG-63 and Caco-2 cells. The results of flow cytometric analysis are shown below after treatment for 72h with untreated cell (CTRL). Significant differences between untreated cells and those incubated with the respective extracts at relative rate were compared by one-way ANOVA, followed by post-test Tukey (*p < 0.05)

Table 1: Apoptosis rate of MG63 Cell Line treated with GT, F1 and F2$^{20\%}$.

Treatment	Viable Cells	Initial Apoptosis	Late Apoptosis	Necrosis
Control	96,07 ± 1,04	2,88 ± 0,38	1,18 ± 0,14	0,29 ± 0,18
GT	69,93 ± 2,73***	10,31 ± 0,61***	16,80 ± 0,45***	3,89± 2,18*
F1	85,30 ± 2,55*	3,37 ± 0,78	6,12 ± 1,71**	1,35 ± 0,45*
F2 20%	81,57 ± 2,35***	4,03 ± 0,01	13,85 ± 0,95***	3,06± 0,04

Results are expressed as a percentage of total cells MG63. Significant differences between untreated cells (Control) and cells treated with green tea (GT), first fermentation kombucha (F1) and second fermentation kombucha added with 20% Tommy mango peel flour (F2$^{20\%}$). One-way ANOVA test with multiple comparisons of the Tukey test compared to the respective control group (* p < 0.05; ** p < 0.01; *** p < 0.001).

Table 2: Apoptosis rate of Caco-2 Cell Line treated with GT, F210 and F2$^{20\%}$.

Treatment	Viable Cells	Initial Apoptosis	Late Apoptosis	Necrosis
Control	94,8 ± 0,60	1,90 ± 0,24	2,35 ± 0,23	0,94 ± 0,41
GT	74,10 ± 0,17***	9,22 ± 0,44***	14,27 ± 0,60***	2,44 ± 0,86*
F1	91,53 ± 2,88	3,48 ± 1,62	4,21 ± 1,10	0,77 ± 0,19
F2 20%	82,83 ± 1,50***	7,31 ± 0,83**	8,56 ± 1,51 **	1,28± 0,57

Results are expressed as a percentage of total cells Caco-2. Significant differences between untreated cells (Control) and cells treated with green tea (GT), first fermentation kombucha (F1) and second fermentation kombucha added with 20% Tommy mango peel flour ($F2^{20\%}$). One-way ANOVA test with multiple comparisons of the Tukey test compared to the respective control group (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Looking at the overall results, it can be seen that adenocarcinoma cells died, but not significantly by the apoptosis process (Table 2), while senescence activity was observed in the same cells (Figure 3). The opposite was observed in osteosarcoma cells, which showed no senescence but apoptosis (Table 1). These comprehensive results are not found in the literature and should be investigated for their signaling pathways.

The kombucha developed for the study was produced under the fermentation conditions of a factory in a mountain town in Brazil with a fermentation temperature of 20°C. According to the literature, the ideal fermentation temperature for kombucha is at least 25°C. This processing characteristic is an important point to emphasize when analyzing the results, as it has been observed that lower temperatures negatively affect the fermentation process and the composition of organic acids, and also affect the degradation of polyphenols contained in green tea (40).

In view of this critical control point, TMPF was added to the recipe to improve the antioxidant and antitumor potential of the drink. In addition to the temperature and hygienic conditions under which the fermentation process takes place, kombucha fermentation depends on the health and quality of the scoby culture, which in any kombucha beverage is influenced by a variety of characteristics and the concentration of tea used. Compared to non-fermented tea, the enhanced beneficial activities of kombucha suggest that some changes are related to the origin of the microbial community present during the fermentation process. Another parameter that may influence the antitumor activity of kombucha is the fermentation time. Jayabalan et al (2008) found that the concentration of total phenolic compounds gradually increased with the fermentation time of kombucha, suggesting that a longer fermentation time may increase the antitumor potential of the beverage. Various scientific findings show that resistance to apoptosis is one of the most characteristic features of most malignant tumors (41). In the present study, kombucha samples, especially those subjected to a second fermentation after the addition of a by-product rich in bioactive compounds, such as mango peel (F220%), significantly promoted apoptosis in both MG-63 and Caco-2 cell lines. In conjunction with the bioactive compounds found in mango, particularly in the peel, such as β-carotene, several

studies suggest that this molecule may inhibit the growth of adenocarcinoma cells by inducing cell cycle arrest and apoptosis and is responsible for the reduction of cyclin A, an important regulator of cell cycle and tumor progression (42).

Conclusion

The data presented in this study demonstrate that kombucha has significant potential to reduce cancer cell viability in human osteosarcoma and colorectal adenocarcinoma cell lines by regulating tumor progression through the induction of cell death events evidenced by senescence and apoptosis. It is important to emphasize that the antiproliferative results of kombucha on Caco-2 cells have already been described in the literature, especially in kombucha from the first fermentation, but have not yet been studied in human osteosarcoma cells produced with the addition of by-products in a second fermentation. In conclusion, it is emphasized that a further series of studies should focus on identifying the best production method for kombucha at the interface of technological processing and value addition to act as an antitumor agent and to be designated as an element in the functional food and beverage market. Our results suggest that kombucha is a potential element in the therapeutic treatment window to act as an anti-cancer agent.

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Capítulo 5
Conclusão

Considerando o crescente aumento do câncer no Brasil e no mundo, e o conhecimento de que a dieta é um importante fator modificável para a sua prevenção, entende-se a necessidade de desenvolvimentos de novos produtos alimentícios que atendam a demanda dos ODS da ONU para melhora da saúde, prevenção de DCNT, inovação e sustentabilidade da indústria.

Neste trabalho apresentamos o processo de desenvolvimento de uma kombucha enriquecida com resíduo agroindustrial, nesse caso, a farinha da casca de manga com o objetivo de apresentar um produto que possa atuar na prevenção do câncer.

Discutimos ainda, a importância de se retratar nas pesquisas as condições reais de produção da indústria de alimentos, assim, concluímos que, a temperatura de fermentação mais baixa provoca alterações no conteúdo de fenólicos totais e no potencial antioxidante da kombucha, mas que a adição da farinha da casca da manga pode modular essas alterações sem a necessidade de maior tempo de fermentação em condições de temperatura abaixo de 20°C. Assim, apresentamos uma alternativa eficaz para melhorar a qualidade nutricional de kombuchas produzidas em regiões mais frias.

Nossos dados mostram que ocorrem alterações na composição físico-química em função do processo fermentativo, como previsto observamos a diminuição do pH, sólidos solúveis, aumento de ácido acético e açúcares redutores, porém sem diferença entre a primeira e segunda fermentação. Mostrando a necessidade de mais tempo de fermentação quando em temperaturas mais baixas. Nossos resultados apontam ainda uma possível interferência da baixa temperatura durante a fermentação na composição de fenólicos totais e na capacidade antioxidante, que pode ter sido revertida com a adição da farinha de casca de manga tanto com 10% quanto com 20%. A análise da capacidade antioxidante que mostrou melhor este resultado foi o DPPH.

A partir das análises de sequenciamento de DNA nas amostras da kombucha de primeira e segunda fermentação, encontramos grande diversificação de fungos e bactérias, podendo-se destacar que o maior *Filo* foi o de *firmicutes* e na kombucha de primeira fermentação o gênero predominante foi *Liquorilactobacillus nagelli (72%)* sendo considerado um probiótico. e ainda o número de bactérias não atingiu número suficiente para contagem na segunda fermentação, que ocorre de forma anaeróbica. Em relação ao conteúdo de fungos, a maioria foi de *Brettanomyces/Dekkera bruxellensis* (90%), Uma limitação do estudo foi a falta da quantificação do biofilme de celulose

(*scoby*) que pode direcionar às alterações em decorrência do processo fermentativo ser aeróbico ou anaeróbico e ainda a ausência de quantificação total de unidades formadoras de colônias (UFC).

Observamos que as diferentes células tumorais analisadas apresentam reações diferentes ao tratamento com a kombucha, e que no geral houve atividade antitumoral em decorrência da exposição à bebida, sendo as células de osteossarcoma (MG-63) foram mais sensíveis ao tratamento com a kombucha, especialmente a F2 (com a adição da farinha de casca de manga). Nossos dados apontam para a necessidade de estudar o comportamento de outros tipos de células tumorais frente ao tratamento com a kombucha e ainda estudar as vias da atividade antitumoral.

Tendo em vista o exposto e considerando a boa aceitação das características sensoriais por consumidores e não consumidores de kombucha, e seu desempenho antioxidante, na citotoxicidade e na indução da apoptose, entende-se que a bebida desenvolvida apresenta grande potencial para ser lançada no mercado de alimentos como um produto de ação preventiva para o desenvolvimento do câncer.

Nossos resultados permitem ainda a criação de um padrão de produção, para kombuchas produzidas em regiões de clima mais ameno, que recupera o potencial antioxidante com a adição de farinha de casca de manga sem que haja necessidade de maior tempo de fermentação.

Considerações finais e perspectivas futuras

O Capítulo III gerou um material importante tanto para a indústria de alimentos como para a ciência em geral. Desenvolmo produto com afição de resíduos agroindustriais e evidenciamos a importância de estudar as propriedades dos alimentos nas condições reais de produção na indústria de alimentos e ainda, neste caso, o estudo da kombucha proveniente de segunda fermentação, quando a maioria das pesquisas é realizada com kombucha somente de primeira fermentação, considerando que o consumidor da bebida comercial recebe a mesma com segunda fermentação e saborizada com diferentes ingredientes vegetais, condições essas que impactam na ação do produto no organismo.

Vale destacar também que a avaliação da aceitação da bebida foi feita com consumidores e não consumidores de kombucha, considerando que em virtude das características sensoriais da kombucha serem particulares, a bebida não é bem aceita pela população em geral, mostrando a necessidade de avaliar a aceitação separadamente e ainda observar quais são os pontos de menor pontuação entre os não consumidores a fim de ajustar a formulação para uma melhor aceitação. Para o desenvolvimento e lançamento de um novo produto também se faz necessário o entendimento dos motivos que levam os consumidores a escolher o produto, usando essas informações para modular a composição do produto, custo, escolha da embalagem e para o marketing.

Este, pode ser considerado o capítulo que desvendou o potencial inovador desta pesquisa quando apresentou que a inserção de resíduos agroindustriais (farinha da casca da manga) é uma alternativa viável para melhorar as propriedades funcionais da kombucha fermentada em condições de temperatura mais baixa. Mostrando assim o papel da ciência na solução de problemas reais.

Assim sendo, a aplicação dos conhecimentos obtidos nesta pesquisa como um todo, poderá, a curto prazo, beneficiar pesquisadores da área das Ciências Agrárias e da Ciência de Alimentos, fomentando a aplicação da técnica sob outras matrizes alimentares, agregando valor a elas e contribuindo para sustentabilidade da indústria geradora de resíduos agroindustriais. E, a médio e longo prazo, poderá beneficiar a população que tem uma necessidade urgente de acesso a alimentos disponíveis que de fato atuem na prevenção de doenças crônicas, como o câncer.

Nossa pesquisa apresenta ao mundo acadêmico a necessidade de mais e profundos estudos para desvendar completamente os complexos e diversos fatores que permeiam o processo fermentativo da kombucha.

Já o capítulo IV apresenta grande importância ao avaliar a ação antitumoral da kombucha em células tumorais de diferentes morfologias, sendo as células de adenocarcinoma de cólon, formado a partir de células epiteliais em tecido glandular e as células de osteossarcoma que se originam de tecido conjuntivo. Os resultados apresentados neste capítulo permitem ainda o depósito de patente desta bebida como agente antitumoral.

Perspectivas futuras

A área da ciência de alimentos que atua no desenvolvimento de novos produtos alimentícios é uma grande oportunidade para nutricionistas mostrarem suas habilidades na modulação de alimentos, englobando o conhecimento de tecnologia de alimentos e de bioquímica, fisiologia e recomendações nutricionais. Esta área vem crescendo e permite uma nova forma de atuação deste profissional. Vale destacar a importância da equipe multiprofissional nesta área a fim de agregar conhecimentos diversos para a elaboração de um produto mais adequado às necessidades da população, aceito sensorialmente, com custo acessível e sustentável do ponto de vista do meio ambiente e da indústria.

Considerando a necessidade crescente de unir pesquisadores e indústria, a intenção de continuidade dos estudos para desenvolvimento de novos produtos que atendam às necessidades de inovação, sustentabilidade e de saúde da população, uma parceria entre UNIFASE e UFF foi firmada para dar continuidade aos estudos da kombucha. Este grupo de pesquisa, alocado na UNIFASE/Petrópolis, conta com o uso do laboratório de tecnologia de alimentos e o laboratório de medicina regenerativa e ainda o LABAL/UFF. A equipe é formada por 7 alunos de iniciação científica dos cursos de nutrição e medicina, 2 professoras internas e 2 professores colaboradores externos.

Por fim, que possamos usar a oportunidade de lidar diretamente com a ciência e usá-la para além da curiosidade humana, para a solução de problemas que afligem a humanidade, proporcionando o bem para todos e formando uma grande rede do bem. Por todos e para todos!

ANEXO I – Manuscrito publicado na ***Concillium*** em 31 de maio de 2024.

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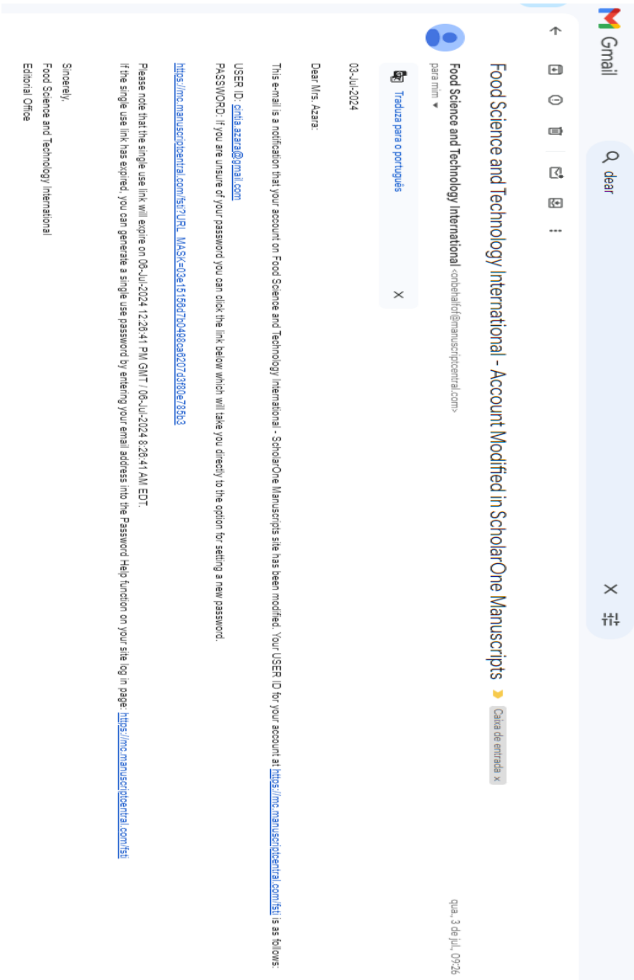
ANEXO II – Carta referente à comprovação de submissão do Artigo apresentado no Capítulo 5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ANEXO III – Material suplementar artigo experimental 2

Supplementary material

Complete list of microorganisms (bacteria and fungi in F1 and fungi in F2 20%) in kombucha. From DNA extraction, the 16S (for bacterial analysis) and ITS (for fungal analysis) genes were amplified. The amplified fragments were then sequenced on the Illumina NextSeq® platform, and the sequences were analyzed with the Qiime software to identify the microorganisms present in the samples and their respective percentages therein.

Table 1: Complete list of microorganisms (bacteria and fungi in F1 and fungi in F2 20%) in kombucha.

Domain	Phylum	Class	Order	Family	Gender	Species	F1	F1 %
Bacteria							197	0,23267350 1
Bacteria	*p__Firmicutes*	*c__Bacilli*	*o__Bacillales*	*f__Bacillaceae*	*g__Bacillus*		28	0,03307034 5
Bacteria	*p__Firmicutes*	*c__Bacilli*	*o__Lactobacillales*	*f__Lactobacillaceae*			6	0,00708650 3
Bacteria	*p__Firmicutes*	*c__Bacilli*	*o__Lactobacillales*	*f__Lactobacillaceae*	*g__Lactobacillus*		13	0,01535408 9
Bacteria	*p__Firmicutes*	*c__Bacilli*	*o__Lactobacillales*	*f__Lactobacillaceae*	*g__Liquorilactobacillus*	*s__Liquorilactobacillus nagelii*	6159 3	72,7464921 8
Bacteria	*p__Firmicutes*	*c__Clostridia*	*o__Clostridiales*	*f__Clostridiaceae*	*g__Clostridium*	*s__Clostridium sp001916075*	3	0,00354325 1

Bacteria	*p__Firmicutes*	*c__Clostridia*	*o__Lachnospirales*	*f__Lachnospiraceae*	*g__Agathobacter*	*s__Agathobacter faecis*	7	0,008267586
Bacteria	*p__Firmicutes*	*c__Clostridia*	*o__Oscillospirales*	*f__Ruminococcaceae*	*g__Gemmiger*		7	0,008267586
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Acetobacter*		10214	12,06358955
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Acetobacter*	*s__Acetobacter fabarum*	413	0,48778759 4
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Acetobacter*	*s__Acetobacter indonesiensis*	91	0,10747862 2
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Acetobacter*	*s__Acetobacter persici*	860	1,01573203 6
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Gluconacetobacter*		36	0,04251901 5
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Gluconobacter*		32	0,03779468
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Komagataeibacter*		*1905*	*2,24996456 7*
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Komagataeibacter*	*s__Komagataeibacter rhaeticus*	*1445*	*1,70666603 7*
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Acetobacterales*	*f__Acetobacteraceae*	*g__Komagataeibacter*	*s__Komagataeibacter saccharivorans*	*7293*	*8,61364388*
Bacteria	*p__Proteobacteria*	*c__Alphaproteobacteria*	*o__Rhizobiales*	*f__Xanthobacteraceae*	*g__Bradyrhizobium*		*96*	*0,11338404 1*
Bacteria	*p__Proteobacteria*	*c__Gammaproteobacteria*	*o__Enterobacterales*	*f__Enterobacteriaceae*			*9*	*0,01062975 4*
Bacteria	*p__Proteobacteria*	*c__Gammaproteobacteria*	*o__Enterobacterales*	*f__Enterobacteriaceae*	*g__Cronobacter*	*s__Cronobacter malonaticus*	*24*	*0,02834601*
Bacteria	*p__Proteobacteria*	*c__Gammaproteobacteria*	*o__Enterobacterales*	*f__Vibrionaceae*	*g__Vibrio*	*s__Vibrio sinensis*	*396*	*0,46770917*

Table 2: Complete list of microorganisms (bacteria and fungi in F1 and fungi in F2 20%) in kombucha.

Dominio	**Filo**	**Classe**	**Ordem**	**Familia**	**Genero**	**Especie**	**F1**	**F2 - 10 %**	**F1 %**	**F2 - 10 % %**

Unassigned							949	56	1,3931093	0,0577563 71
k__Fungi							27	46	0,0396353 55	0,0474427 34
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Cladosporiaceae	g__Cladosporium		82	100	0,1203740 4	0,1031363 77
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Cladosporiaceae	g__Cladosporium	s__Cladosporium_halotolerans	109	18	0,1600093 95	0,0185645 48
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Cladosporiaceae	g__Cladosporium	s__Cladosporium_sphaerospermum	17	0	0,0249555 94	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Capnodiales	f__Neodevriesiaceae	g__Neodevriesia		0	85	0	0,0876659 21
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Dothideales	f__Saccotheciaceae	g__Aureobasidium		6	0	0,0088078 57	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Jahnulales	f__Aliquandostipitaceae	g__Aliquandostipitaceae_gen_Incertae_sedis	s__Aliquandostipitaceae_sp	0	7	0	0,0072195 46
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales				31	0	0,0455072 59	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Dictyosporiaceae	g__Pseudocoleophoma	s__Pseudocoleophoma_sp	7	0	0,0102758 33	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Didymellaceae			66	0	0,0968864 23	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Didymosphaeriaceae	g__Paraconiothyrium	s__Paraconiothyrium_sp	66	0	0,0968864 23	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Didymosphaeriaceae	g__Paraphaeosphaeria		8	0	0,0117438 09	0
k__Fungi	p__Ascomycota	c__Dothideomycetes	o__Pleosporales	f__Didymosphaeriaceae	g__Paraphaeosphaeria	s__Paraphaeosphaeria_arecacearum	6	0	0,0088078 57	0
k__Fungi	p__Asco	c__Dothideo	o__Pleosporales	f__Morosphaeriaceae	g__Rhizopycnis	s__Rhizopycnis_sp	7	0	0,0102	0

	myc ota	mycet es							758 33	
k__ Fun gi	p__ Asco myc ota	c__Do thideo mycet es	o__Pleospor ales	f__Phaeospha eriaceae	g__Phaeos phaeria	s__Phaeos phaeria_ca ricis	6	0	0,0 088 078 57	0
k__ Fun gi	p__ Asco myc ota	c__Do thideo mycet es	o__Pleospor ales	f__Pleosporal es_fam _Incertae_sed is	g__Pleosp orales_gen _Incertae_s edis	s__Pleosp orales_sp	5 0	0	0,0 733 988 05	0
k__ Fun gi	p__ Asco myc ota	c__Do thideo mycet es	o__Pleospor ales	f__Sporormia ceae			3	0	0,0 044 039 28	0
k__ Fun gi	p__ Asco myc ota	c__Do thideo mycet es	o__Pleospor ales	f__Sporormia ceae	g__Wester dykella	s__Wester dykella_re niformis	2 8	0	0,0 411 033 31	0
k__ Fun gi	p__ Asco myc ota	c__Do thideo mycet es	o__Venturial es	f__Venturiale s_fam _Incertae_sed is	g__Venturi ales_gen_ Incertae_se dis	s__Ventur iales_sp	5 9	0	0,0 866 105 9	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Chaetoth yriales	f__Herpotrich iellaceae	g__Exophi ala		2 0	0	0,0 293 595 22	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Chaetoth yriales	f__Trichomer iaceae	g__Knufia	s__Knufia _sp	5 6	0	0,0 822 066 62	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae			6 0	0	0,0 880 785 66	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Asperg illus		1 5	0	0,0 220 196 42	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Asperg illus	s__Asperg illus_conic us	3 8	0	0,0 557 830 92	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Asperg illus	s__Asperg illus_pseu doterreus	9	0	0,0 132 117 85	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Asperg illus	s__Asperg illus_ruber	0	99 80	0	10, 293 010 45
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Penicill ium		1 0 0	0	0,1 467 976 1	0
k__ Fun gi	p__ Asco myc ota	c__Eu rotiom ycetes	o__Eurotiale s	f__Aspergilla ceae	g__Penicill ium	s__Penicil lium_broc ae	0	11	0	0,0 113 450 01

k__Fungi	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Aspergillaceae	g__Penicillium	s__Penicillium_paneum	1252	0	1,837906079	0
k__Fungi	p__Ascomycota	c__Eurotiomycetes	o__Eurotiales	f__Eurotiales_fam_Incertae_sedis	g__Eurotiales_gen_Incertae_sedis	s__Eurotiales_sp	29	0	0,042571307	0
k__Fungi	p__Ascomycota	c__Eurotiomycetes	o__Eurotiomycetes_ord_Incertae_sedis	f__Eurotiomycetes_fam_Incertae_sedis	g__Eurotiomycetes_gen_Incertae_sedis	s__Eurotiomycetes_sp	18	0	0,026423557	0
k__Fungi	p__Ascomycota	c__Leotiomycetes	o__Helotiales				13	0	0,019083689	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Debaryomycetaceae	g__Debaryomyces		35	0	0,051379164	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Debaryomycetaceae	g__Kurtzmaniella	s__Kurtzmaniella_quercitrusa	5	0	0,007339881	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Debaryomycetaceae	g__Lodderomyces	s__Lodderomyces_elongisporus	117	0	0,171753204	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Phaffomycetaceae	g__Wickerhamomyces	s__Wickerhamomyces_anomalus	7	0	0,010275833	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Phaffomycetaceae	g__Wickerhamomyces	s__Wickerhamomyces_pijperi	42	0	0,061654996	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Pichiaceae	g__Dekkera	s__Dekkera_bruxellensis	617442	84506	90,63578045	87,15642694
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Pichiaceae	g__Martiniozyma	s__Martiniozyma_asiatica	72	0	0,105694279	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Pichiaceae	g__Pichia	s__Pichia_deserticola	118	0	0,17322118	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Pichiaceae	g__Pichia	s__Pichia_eremophila	13	0	0,019083689	0
k__Fungi	p__Ascomycota	c__Saccharomycetes	o__Saccharomycetales	f__Pichiaceae	g__Pichia	s__Pichia_mandshurica	0	49	0	0,050536825
k__Fungi	p__Asco	c__Saccharo	o__Saccharomycetales	f__Pichiaceae	g__Pichia	s__Pichia_membranifaciens	108	136	0,1585	0,1402

	myc ota	mycet es							414 19	654 73
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Pichiaceae	g__Pichia	s__Pichia_ sp	0	11 3	0	0,1 165 441 06
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetaceae	g__Zygosa ccharomyc es	s__Zygosa ccharomyc es_bisporu s	8 3	0	0,1 218 420 16	0
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetaceae	g__Zygosa ccharomyc es	s__Zygosa ccharomyc es_parabai lii	0	35	0	0,0 360 977 32
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Candid a		2 1 6	0	0,3 170 828 38	0
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Candid a	s__Candid a_parapsil osis	0	13 7	0	0,1 412 968 37
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Candid a	s__Candid a_tropicali s	1 3	0	0,0 190 836 89	0
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Starme rella	s__Starme rella_apic ola	7 5 0	43 4	1,1 009 820 76	0,4 476 118 77
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Starme rella	s__Starme rella_bacil laris	1 8 7	0	0,2 745 115 31	0
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Saccharo mycetales_fa m_Incertae_s edis	g__Starme rella	s__Starme rella_etche llsii	3 3 7	0	0,4 947 079 46	0
k__ Fun gi	p__ Asco myc ota	c__Sa ccharo mycet es	o__Saccharo mycetales	f__Trigonopsi daceae	g__Trigon opsis	s__Trigon opsis_vari abilis	3 1	0	0,0 455 072 59	0
k__ Fun gi	p__ Asco myc ota	c__So rdario mycet es	o__Amphisp haeriales	f__Pestalotio psidaceae	g__Neopes talotiopsis		1 2	0	0,0 176 157 13	0
k__ Fun gi	p__ Asco myc ota	c__So rdario mycet es	o__Chaetosp haeriales	f__Chaetosph aeriaceae	g__Chlorid ium		0	83	0	0,0 856 031 93
k__ Fun gi	p__ Asco myc ota	c__So rdario mycet es	o__Glomerel lales	f__Plectospha erellaceae	g__Plectos phaerella		4 1	0	0,0 601 870 2	0
k__ Fun gi	p__ Asco myc ota	c__So rdario mycet es	o__Glomerel lales	f__Plectospha erellaceae	g__Plectos phaerella	s__Plectos phaerella_ sp	4 7	0	0,0 689 948 77	0

k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Bionectriaceae			13	0	0,019083689	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Bionectriaceae	g__Paracylindrocarpon	s__Paracylindrocarpon_nabanhensis	17	0	0,024955594	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Hypocreales_fam_Incertae_sedis	g__Sesquicillium	s__Sesquicillium_sp	38	0	0,055783092	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium		135	103	0,198176774	0,106230469
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__Fusarium_equiseti	70	0	0,102758327	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__Fusarium_oxysporum	19	0	0,027891546	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Fusarium	s__Fusarium_sacchari	0	227	0	0,234119576
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Nectriaceae	g__Neocosmospora	s__Neocosmospora_rubicola	23	0	0,033763345	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Ophiocordycipitaceae	g__Purpureocillium		0	3	0	0,003094091
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Hypocreales	f__Ophiocordycipitaceae	g__Purpureocillium	s__Purpureocillium_takamizusanense	28	0	0,041103331	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales				0	8	0	0,0082509 1
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Chaetomium	s__Chaetomium_cupreum	42	0	0,061654996	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Dichotomopilus		31	0	0,045507259	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Humicola		49	0	0,071930829	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Chaetomiaceae	g__Pseudothielavia		54	0	0,079270709	0

k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Sordariales	f__Lasiosphaeriaceae	g__Schizothecium	s__Schizothecium_inaequale	35	0	0,051379164	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Amphisphaeriaceae	g__Microdochium	s__Microdochium_poae	8	0	0,011743809	0
k__Fungi	p__Ascomycota	c__Sordariomycetes	o__Xylariales	f__Sporocadaceae	g__Robillarda	s__Robillarda_sessilis	28	0	0,041103331	0
k__Fungi	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Agaricaceae	g__Lepiota		46	0	0,067526901	0
k__Fungi	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Entolomataceae			12	0	0,017615713	0
k__Fungi	p__Basidiomycota	c__Agaricomycetes	o__Agaricales	f__Tricholomataceae	g__Clitocybe	s__Clitocybe_ulmicola	16	0	0,023487618	0
k__Fungi	p__Basidiomycota	c__Agaricomycetes	o__Cantharellales	f__Ceratobasidiaceae	g__Ceratobasidium	s__Ceratobasidium_niltonsouzanum	4	0	0,005871904	0
k__Fungi	p__Basidiomycota	c__Agaricomycetes	o__Phallales	f__Phallaceae	g__Phallus	s__Phallus_rugulosus	18	0	0,026423557	0
k__Fungi	p__Basidiomycota	c__Malasseziomycetes	o__Malasseziales	f__Malasseziaceae	g__Malassezia	s__Malassezia_restricta	0	89	0	0,091791376
k__Fungi	p__Basidiomycota	c__Microbotryomycetes	o__Sporidiobolales	f__Sporidiobolaceae	g__Rhodotorula	s__Rhodotorula_dairenensis	87	0	0,127713921	0
k__Fungi	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Bulleribasidiaceae	g__Hannaella		9	0	0,013211785	0
k__Fungi	p__Basidiomycota	c__Tremellomycetes	o__Tremellales	f__Bulleribasidiaceae	g__Hannaella	s__Hannaella_sinensis	6	0	0,008807857	0
k__Fungi	p__Basidiomycota	c__Tremellomycetes	o__Trichosporonales	f__Trichosporonaceae	g__Apiotrichum	s__Apiotrichum_scarabaeorum	0	90	0	0,092822274
k__Fungi	p__Basidiomycota	c__Wallemiomycetes	o__Wallemiales	f__Wallemiaceae	g__Wallemia	s__Wallemia_canadensis	14	0	0,020551665	0
k__Fungi	p__Basidiomycota	c__Wallemiomycetes	o__Wallemiales	f__Wallemiaceae	g__Wallemia	s__Wallemia_mellicola	41	0	0,006018702	0

k__Fungi	p__Mucoromycota	c__Mucoromycetes	o__Mucorales	f__Rhizopodaceae	g__Rhizopus	s__Rhizopus_arrhizus	7	0	0,010275833	0
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae					31	91	0,045507259	0,093854103
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Asterales	f__Asteraceae	g__Mikania	s__Mikania_micrantha	12	0	0,017615713	0
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Caryophyllales	f__Amaranthaceae	g__Beta	s__Beta_nana	47	0	0,068994877	0
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Cucurbitales	f__Cucurbitaceae	g__Cayaponia	s__Cayaponia_attenuata	0	48	0	0,049505461
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Ericales	f__Theaceae	g__Camellia		51	0	0,074866781	0
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Ericales	f__Theaceae	g__Camellia	s__Camellia_sinensis	7	18	0,010275833	0,018564548
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Ericales	f__Theaceae	g__Camellia	s__Camellia_sinensis_var._assamica	0	45	0	0,046411337
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Fabales	f__Fabaceae	g__Glycine	s__Glycine_soja	0	4	0	0,004125455
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Gentianales	f__Rubiaceae	g__Sabicea	s__Sabicea_panamensis	0	18	0	0,018564548
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Rosales	f__Urticaceae	g__Cecropia	s__Cecropia_pachystachya	10	0	0,014679761	0
k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Sapindales	f__Anacardiaceae	g__Mangifera		0	198	0	0,204210027

k__Viridiplantae	p__Anthophyta	c__Eudicotyledonae	o__Sapindales	f__Anacardiaceae	g__Mangifera	s__Mangifera_quadrifida	0	181	0	0,186676843
k__Viridiplantae	p__Anthophyta	c__Monocotyledonae	o__Poales	f__Poaceae	g__Paspalum	s__Paspalum_conjugatum	0	40	0	0,041254551

APÊNDICE I – Termo de Concentimento livre e esclarecido Análise Sensorial

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nome:_____ Data: _____

Título: "Análise sensorial da aceitação de kombucha adicionada de farinha de casca de manga"**.**

OBJETIVO DO ESTUDO: O objetivo deste trabalho é produzir uma kombucha com adição de farinha de casca de manga tommy Atkins na segunda fermentação e avaliar a aceitação da bebida.

ALTERNATIVA PARA PARTICIPAÇÃO NO ESTUDO: Você está sendo convidado (a) como voluntário (a) a participar de um teste de análise sensorial de kombucha. A qualquer momento você pode desistir participar da pesquisa, seja antes ou depois da coleta dos dados e retirar seu consentimento. Sua recusa não trará prejuízo em sua relação com o pesquisador ou com a instituição a ele vinculada. Se concordar em participar desse estudo você será solicitado a responder um questionário realizado pessoalmente. O questionário aplicado não afeta a integridade física dos participantes.

PROCEDIMENTO DO ESTUDO: Caso concorde em participar deste estudo, você será solicitado a responder dois questionários uma em relação às características da kombucha e outro sobre sua intenção de compra. O questionário aplicado não afeta a integridade física do participante, no máximo podem provocar um desconforto pelo tempo exigido.

RISCOS: ESSE PRODUTO REPRESENTA UM RISCO PARA PESSOAS ALÉRGICAS OU QUE POSSUEM ALGUMA SENSIBILIDADE A CHÁ VERDE

E/OU MANGA, POR ESSE MOTIVO NÃO DEVE SER CONSUMIDO PELAS MESMAS, IMPOSSIBILITANDO SUA PARTICIPAÇÃO NA PESQUISA. OS DEMAIS INDIVIDUOS QUE PARTICIPARAM DA PESQUISA, PODERÃO SENTIR ALGUM DESCONFORTO GASTROINTESTINAL APÓS A REALIZAÇÃO DAS ANÁLISES POR CONTA DA FORMULAÇÃO DO PRODUTO.

CONFIDENCIALIDADE: As informações a serem obtidas no estudo serão analisadas juntamente com as informações dos entrevistados, não sendo divulgada a identificação de nenhum dos participantes do estudo. Estas informações serão utilizadas pelo pesquisador envolvido no projeto para fins estatísticos.

Fui suficientemente informado (a) a respeito das informações sobre o estudo citado que li ou foram lidas para mim. Concordo voluntariamente em participar deste estudo e poderei retirar o meu consentimento a qualquer momento, sem penalidades, prejuízo.

Contato do responsável pelo estudo: (24) 998819-6819

Em caso de dúvidas quanto à condução ética do estudo, entre em contato com o comitê de ética em pesquisa da fmp/fase/hac.

TEL: (24)2244-6497

EMAIL:CEP@FMPFASE.EDU.BR

PORTAL ELETRÔNICO: HTTP://WWW.FMPFASE.EDU.BR

_____

Assinatura do participante

Cíntia R. P. Azara Doutoranda PPGAN/UNIRIO Responsável pelo estudo	Prof. Dr. Anderson Teodoro – PPGAN/UNIRIO Orientador do Estudo.

APÊNDICE II – Ficha de avaliação sensorial e intenção e compra

<table>
<tr><td>Nome
_____</td><td>Sexo
_____</td><td>Idade
_____</td><td>Data
_____</td></tr>
<tr><td colspan="4">Por favor, prove a amostra e avalie as características a direita de acordo com a escala abaixo:</td></tr>
<tr><td rowspan="3">(9) Gostei extremamente
(8) Gostei muito
(7) Gostei moderadamente
(6) Gostei ligeiramente
(5) Indiferente
(4) Desgostei ligeiramente
(3) Desgostei moderadamente
(2) Desgostei muito
(1) Desgostei extremamente</td><td colspan="2">Atributos:</td><td>Notas:</td></tr>
<tr><td colspan="2">Cor
Aparência geral
Sabor
Textura</td><td>_____
_____
_____
_____</td></tr>
<tr><td colspan="3"></td></tr>
</table>

(ADOLFO LUTZ, 2008)

Julgador nº: _____

1) Avalie a amostra que você provou segunda a sua intenção de compra, utilizando a escala abaixo.

Nota		Amostra	
(5)	Certamente compraria	_____	( )
(4)	Provavelmente compraria		
(3)	Talvez compraria, talvez não compraria	_____	( )
(2)	Provavelmente não compraria		
(1)	Certamente não compraria		( )

(ADOLF LUTZ, 2008)