UNIVERSIDADE FEDERAL DO AMAZONAS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA

DESENVOLVIMENTO DE BEBIDAS FUNCIONAIS COM PROPRIEDADES ANTIOXIDANTES E PREBIÓTICAS A BASE DE PANC

JOSIANA MOREIRA MAR

MANAUS – AM
2026

JOSIANA MOREIRA MAR

DESENVOLVIMENTO DE BEBIDAS FUNCIONAIS COM PROPRIEDADES ANTIOXIDANTES E PREBIÓTICAS A BASE DE PANC

Tese apresentada ao Programa Multi-Institucional de Pós-Graduação em Biotecnologia da Universidade Federal do Amazonas, em cumprimento às exigências da etapa de qualificação do curso de Doutorado em Biotecnologia.

Orientadora: Drª Jaqueline de Araújo Bezerra (INPA)
Área de Concentração: Biotecnologia para Área de Agroflorestal

MANAUS – AM
2026

Ficha Catalográfica

Elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

M298d Mar, Josiana Moreira

Desenvolvimento de Bebidas Funcionais com Propriedades Antioxidantes e Prebióticas a Base de PANC / Josiana Moreira Mar. - 2026.

137 f. : il., color. ; 31 cm.

Orientador(a): Jaqueline de Araújo Bezerra.

Tese (doutorado) - Universidade Federal do Amazonas, Programa de Pós-Graduação em Biotecnologia, Manaus-AM, 2026.

1. Plantas Alimenticias Não Convencionais. 2. compostos bioativos. 3. antioxidantes. 4. biodiversidade amazônica. I. Bezerra, Jaqueline de Araújo. II. Universidade Federal do Amazonas. Programa de Pós-Graduação em Biotecnologia. III. Título

---

JOSIANA MOREIRA MAR

DESENVOLVIMENTO DE BEBIDAS FUNCIONAIS COM PROPRIEDADES ANTIOXIDANTES E PREBIÓTICAS A BASE DE PANC

Tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade Federal do Amazonas, como requisito parcial para obtenção do título de Doutor em Biotecnologia.

Aprovada em: 30/04/2026

BANCA EXAMINADORA

Prof. Dr. Jaqueline de Araújo Bezerra
Presidente
Universidade Federal do Amazonas

Prof. Dr. Ires Paula de Andrade Miranda
Membro Titular
Universidade Federal do Amazonas

Prof. Dr. Henrique Duarte Fonseca Filho
Membro Titular
Universidade Federal do Amazonas

Prof. Dr. Rosany Picolotto Carvalho
Membro Titular
Universidade Federal do Amazonas

Prof. Dr. Milena Gaion Malosso
Membro Titular
Universidade Federal do Amazonas

AGRADECIMENTOS

Agradeço a Deus por todas as oportunidades concedidas, pela força nos momentos de fragilidade e por me sustentar diante dos desafios ao longo desta trajetória.

À minha mãe, Ana, ao meu padrasto, Aloísio, à minha sobrinha, Ana Júlia, e à minha irmã, Juliana, por todo o amor, apoio, incentivo e compreensão ao longo da minha vida. Vocês são meu alicerce e minha maior inspiração. Em especial à minha irmã, por sempre me encorajar a persistir e nunca desistir diante das dificuldades.

Ao meu companheiro e parceiro, Francisco Alves, pela compreensão nos momentos mais desafiadores, pela paciência nos períodos de maior pressão e pelo incentivo constante para que eu seguisse firme na realização dos meus sonhos.

À minha orientadora e amiga, Dra. Jaqueline, minha sincera gratidão pela dedicação, paciência, humildade e pelos ensinamentos que ultrapassam o âmbito acadêmico. Seu exemplo de compromisso e amor pela ciência marcou profundamente minha formação. Obrigada por confiar em mim e por todo o apoio ao longo dessa jornada.

Aos meus coorientadores, Dr. Edgar Sanches e Dr. Pedro Campelo, pelo suporte, orientação e contribuições essenciais para a concretização deste trabalho.

Aos amigos do laboratório, eternamente Nanopol, em especial Célio Macalia, Adriano Carolino, Kalil Araújo, Jamilton Boaes e Ronald Zico, pelos momentos inesquecíveis compartilhados. A convivência diária, as trocas de conhecimento e, principalmente, os momentos do cafezinho tornaram essa caminhada mais divertida e especial. A todos os meus amigos do Nectam, especialmente Andreza, Daniel e João Vitor, pela amizade, parceria e companheirismo nas atividades de pesquisa.

À Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), pela concessão da bolsa de estudos por meio do Programa de Apoio à Pós-Graduação Stricto Sensu (POSGRAD), e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pelo apoio financeiro e investimento em recursos para o desenvolvimento desta pesquisa.

Ao Programa de Pós-Graduação em Biotecnologia, pelo suporte institucional e pela contribuição essencial à realização deste trabalho.

À Universidade Federal do Amazonas (UFAM), pela formação acadêmica e pela oportunidade de crescimento profissional e científico.

E a todos que, direta ou indiretamente, contribuíram para a concretização deste sonho, minha sincera gratidão.

EPÍGRAFE

"*Pesquisar é ver o que todo mundo viu e pensar o que ninguém pensou.*"
Albert Szent-Györgyi

RESUMO

A crescente demanda por alimentos funcionais tem impulsionado a busca por fontes naturais de compostos bioativos, especialmente oriundos da biodiversidade amazônica. Nesse contexto, as Plantas Alimentícias Não Convencionais (PANC) destacam-se como alternativas promissoras para o desenvolvimento de produtos com propriedades funcionais e sustentáveis. Esta tese teve como objetivo desenvolver e avaliar bebidas funcionais com propriedades antioxidantes e potencial prebiótico a partir de espécies amazônicas, com ênfase em *Tapirira guianensis* Aubl., *Theobroma speciosum* Willd. ex Spreng. e *Hibiscus acetosella* Welw. ex Hiern. Inicialmente, foram aplicadas tecnologias emergentes, como o ultrassom de alta intensidade e o uso de Solventes Eutéticos Profundos Naturais (NADES), visando otimizar a extração de compostos bioativos. Os resultados demonstraram aumento significativo no teor de compostos fenólicos, antocianinas e na capacidade antioxidante, especialmente em sistemas à base de ácido cítrico, evidenciando maior eficiência e caráter sustentável do processo. A secagem de flores de *Theobroma speciosum* Willd. ex Spreng. foi avaliada, sendo observada melhor preservação dos compostos bioativos a 60 °C, favorecendo sua aplicação em infusões funcionais. Paralelamente, a extração com NADES associada ao ultrassom apresentou elevada eficiência, destacando-se sistemas à base de cloreto de colina e ácido láctico na recuperação de fenólicos e antocianinas. Adicionalmente, investigou-se o efeito do plasma atmosférico frio em folhas de *Hibiscus acetosella* Welw. ex Hiern, demonstrando que essa tecnologia é capaz de modular a composição fenólica e a atividade antioxidante de forma dependente das condições operacionais. O tratamento a 18 kV por 6 minutos promoveu maior atividade antioxidante e maior teor de compostos fenólicos e antocianinas, enquanto condições mais intensas levaram à degradação oxidativa. As análises cromatográficas evidenciaram a presença predominante de compostos fenólicos, como derivados do ácido cafeico, associados diretamente à atividade antioxidante. Além disso, alterações microestruturais indicaram que os tratamentos tecnológicos promovem ruptura celular, facilitando a liberação de compostos bioativos. De forma integrada, os resultados demonstram que a combinação de tecnologias verdes e matérias-primas amazônicas permite o desenvolvimento de bebidas funcionais com elevado valor agregado, contribuindo para a valorização da biodiversidade regional, o avanço da biotecnologia verde e o fortalecimento da bioeconomia amazônica.

Palavras-chave: PANC; compostos bioativos; antioxidantes; ultrassom; NADES; plasma atmosférico frio; biodiversidade amazônica.

ABSTRACT

The growing demand for functional foods has driven the search for natural sources of bioactive compounds, particularly those derived from Amazonian biodiversity. In this context, Non-Conventional Food Plants (PANC) stand out as promising alternatives for the development of products with functional and sustainable properties. This thesis aimed to develop and evaluate functional beverages with antioxidant properties and prebiotic potential from Amazonian species, with emphasis on *Tapirira guianensis* Aubl., *Theobroma speciosum* Willd. ex Spreng., and *Hibiscus acetosella* Welw. ex Hiern. Initially, emerging technologies such as high-intensity ultrasound and the use of Natural Deep Eutectic Solvents (NADES) were applied to optimize the extraction of bioactive compounds. The results demonstrated a significant increase in phenolic compounds, anthocyanins, and antioxidant capacity, especially in citric acid-based systems, highlighting greater efficiency and the sustainable nature of the process. The drying of *Theobroma speciosum* Willd. ex Spreng. flowers was also evaluated, with better preservation of bioactive compounds observed at 60 °C, favoring their application in functional infusions. In parallel, NADES extraction combined with ultrasound showed high efficiency, with choline chloride- and lactic acid-based systems standing out in the recovery of phenolics and anthocyanins. Additionally, the effect of cold atmospheric plasma on *Hibiscus acetosella* Welw. ex Hiern leaves was investigated, demonstrating that this technology can modulate phenolic composition and antioxidant activity in a condition-dependent manner. Treatment at 18 kV for 6 minutes resulted in higher antioxidant activity and increased levels of phenolic compounds and anthocyanins, whereas more intense conditions led to oxidative degradation. Chromatographic analyses revealed the predominance of phenolic compounds, such as caffeic acid derivatives, which were directly associated with antioxidant activity. Furthermore, microstructural changes indicated that the applied technologies promote cell disruption, facilitating the release of bioactive compounds. Overall, the results demonstrate that the combination of green technologies and Amazonian raw materials enables the development of functional beverages with high added value, contributing to the valorization of regional biodiversity, the advancement of green biotechnology, and the strengthening of the Amazon bioeconomy.

Keywords: PANC; bioactive compounds; antioxidants; ultrasound; NADES; cold atmospheric plasma; Amazonian biodiversity.

LISTA DE ABREVIATURAS E SIGLAS

ABTS -2,2′-Azino-bis(3-etilbenzotiazolina-6-ácido sulfônico)

ABTS•$^{+}$ - Radical cátion ABTS

AQ - Tempo de aquisição (Acquisition Time)

AT - Acidez titulável

BHT - Butil-hidroxitolueno

BBFO - Broadband Fluorine Observation

ChCl - Cloreto de colina

COSY-– Correlation Spectroscopy

Deff - Difusividade efetiva

DMSO - Dimetilsulfóxido deuterado

DPPH - 2,2-Difenil-1-picrilhidrazil

DPPH• - Radical 2,2-Difenil-1-picrilhidrazil

ED - Densidade de energia (Energy Density)

FID - Free Induction Decay

FRAP - Ferric Reducing Antioxidant Power

GAE - Equivalente de ácido gálico

GRAS - Generally Recognized As Safe

HAD - Hot Air Drying (secagem por ar quente)

HBA - Hydrogen Bond Acceptor (Aceptor de ligação de hidrogênio)

HBD - Hydrogen Bond Donor (Doador de ligação de hidrogênio)

HMBC - Heteronuclear Multiple Bond Correlation

HSQC - Heteronuclear Single Quantum Coherence

HPLC-DAD - Cromatografia Líquida de Alta Eficiência com Detector de Arranjo de Diodos

MR - Moisture Ratio (Razão de umidade)

NADES - Natural Deep Eutectic Solvents (Solventes Naturais Eutéticos Profundos)

NAP - Potência nominal aplicada

NS - Número de varreduras (Number of Scans)

NMR / RMN - Nuclear Magnetic Resonance / Ressonância Magnética Nuclear)

RG - Receiver Gain

RSME - Root Mean Square Error

SW - Spectral Width (Largura espectral)

TMS - Tetrametilsilano

TPC - Total Phenolic Content (Teor total de compostos fenólicos)

UAE - Ultrasound-Assisted Extraction

US - Ultrasound

USAE - Ultrasound-Assisted Extraction

UV - Ultravioleta

VCX - VibraCell Ultrasonic Processor

SUMÁRIO

1. INTRODUÇÃO 10

2. OBJETIVO 11

2.1 Objetivo Geral 11

2.2 Objetivos Específicos 11

3. REVISÃO BIBLIOGRÁFICA 12

3.1 Alimentos e Bebidas Funcionais 12

3.2 Propriedades Antioxidantes em Bebidas Funcionais 13

3.3 Plantas Alimentícias Não Convencionais (PANC) 16

3.4 Desenvolvimento de Bebidas Funcionais à Base de Espécies Amazônicas 17

3.5 Sustentabilidade e Valorização da Biodiversidade 21

3.6 Aplicação de Espécies Amazônicas no Desenvolvimento de Bebidas Funcionais: *Tapirira guianensis* Aubl. *e Theobroma speciosum* Willd. ex Spreng.. 22

4. REFERÊNCIAS 28

CAPÍTULO 1 33

CAPÍTULO 2 50

CAPÍTULO 3 59

CAPÍTULO 4 66

CAPÍTULO 5 85

CAPÍTULO 6 103

1 INTRODUÇÃO

A busca por fontes naturais de compostos bioativos tem ganhado destaque crescente na ciência e indústria, impulsionada pela demanda por soluções mais saudáveis e sustentáveis em diversas áreas, como alimentos, cosméticos e produtos farmacêuticos (Aware *et al*., 2022). O Brasil, detentor de uma vasta biodiversidade, apresenta um cenário promissor para a descoberta e exploração desses recursos, com espécies vegetais que exibem elevado potencial antioxidante e nutracêutico (Valli *et al*., 2018).

Entre as espécies nativas com grande potencial, destacam-se *Theobroma speciosum* Willd. ex Spreng. Conhecida popularmente como cacauí, embora ainda pouco difundido, é reconhecido por suas flores ricas em compostos fenólicos e capacidade antioxidante, sendo uma fonte promissora para bebidas funcionais e outros produtos (Mar *et al*., 2024; Mar *et al*., 2025), *Tapirira guianensis* Aubl. (pau-pombo) amplamente distribuída nos biomas brasileiros, possui alto potencial antioxidante e nutracêutico devido à presença de alquilfenóis, ésteres alquílicos, triterpenos, esteroides, norisoprenoides e flavonoides (Mar *et al.*, 2023) e *Hibiscus acetosella* Welw. ex Hiern que em seu extrato de *Hibiscus acetosella* Welw. ex Hiern apresentou, no espectro de RMN de ^{1}H, sinais característicos do ácido cafeoil-hidroxicítrico, evidenciando elevada retenção de compostos bioativos (Mar *et al.*, 2020).

A extração eficiente desses compostos bioativos é um desafio crucial, uma vez que métodos convencionais frequentemente envolvem solventes orgânicos tóxicos, demandam alto consumo de energia e podem degradar os compostos de interesse (Mungwari *et al.*, 2025). Nesse contexto, as técnicas de extração assistida por ultrassom (EAU) e o uso de Solventes Eutéticos Profundos Naturais (NADES) emergem como alternativas promissoras. Os NADES são solventes ecológicos, de baixa toxicidade, biodegradáveis e de baixo custo, formados a partir de componentes naturais como ácidos orgânicos, açúcares e poliálcoois (Osamede Airouyuwa *et al.*, 2022). A combinação de NADES com EAU tem demonstrado ser eficaz na recuperação de compostos valiosos de plantas, otimizando a eficiência da extração e a estabilidade dos compostos (Abdelrahman *et al.*, 2023).

Estudos recentes têm destacado o uso de solventes alternativos, como os líquidos iônicos naturais (NADES) e a extração assistida por enzimas (EAU), na otimização da obtenção de compostos fenólicos e antocianinas, apontando ganhos relevantes na capacidade antioxidante e na estabilidade dos extratos (Li *et al.*, 2024). Paralelamente, a

tecnologia de ultrassom aplicada a sucos tem se mostrado uma estratégia promissora para aprimorar o perfil químico e a bioacessibilidade de compostos bioativos. Embora alguns estudos relatem reduções em carotenoides, os efeitos positivos sobre o teor de compostos fenólicos e a atividade antioxidante reforçam o potencial dessa abordagem como alternativa tecnológica sustentável (Rodríguez-Rico *et al.*, 2022).

Diante da relevância desses recursos naturais e da necessidade de métodos de extração mais sustentáveis e eficientes, este trabalho visa investigar a otimização da extração de compostos bioativos de *Theobroma speciosum* Willd. ex Spreng., *Tapirira guianensis* Aubl. e *Hibiscus acetocella* Welw. Ex Hiern utilizando abordagens inovadoras. Serão exploradas as condições ideais de extração e a caracterização dos extratos obtidos, buscando maximizar o rendimento e a qualidade dos compostos, e assim, contribuir para o desenvolvimento de produtos funcionais e para o avanço do conhecimento sobre a biodiversidade brasileira.

2 OBJETIVO

2.1 Objetivo Geral

Avaliar as propriedades funcionais de bebidas elaboradas a partir das Plantas Alimentícias Não Convencionais (PANCs) das espécies de *Tapirira guianensis* Aubl., *Theobroma speciosum* Willd. ex Spreng. e *Hibiscus acetocella* Welw. Ex Hiern com o intuito de ampliar o conhecimento acerca de seus aspectos químicos e funcionais.

2.2 Objetivos Específicos

- Obter o perfil químico das bebidas elaboradas a partir das espécies de *Tapirira guianensis* Aubl., *Theobroma speciosum* Willd. ex Spreng. e *Hibiscus acetosella* Welw. ex Hiern com ênfase em compostos bioativos;
- Determinar a atividade antioxidante das bebidas, utilizando diferentes métodos espectrofotométricos;
- Avaliar a presença e o teor de compostos fenólicos, antocianinas, flavonoides e carotenoides nas formulações;
- Investigar a estabilidade dos compostos bioativos durante o processamento e armazenamento das bebidas;
- Analisar o potencial funcional das bebidas, relacionando a composição química com suas propriedades biológicas.

3 REVISÃO BIBLIOGRÁFICA

3.1 Alimentos e Bebidas Funcionais

Os alimentos funcionais são definidos como alimentos que, além de fornecerem os nutrientes essenciais, contêm compostos bioativos capazes de exercer efeitos benéficos à saúde e contribuir para a prevenção de doenças crônicas não transmissíveis. Martirosyan *et al.* (2022) apontam que a ciência dos alimentos funcionais envolve a identificação de compostos bioativos alimentares que influenciam benefícios à saúde. Essa definição ressalta que não basta haver um alimento natural ou enriquecido é necessário demonstrar, por meio de pesquisa, que o componente bioativo tem efeito fisiológico relevante.

Sob a perspectiva conceitual e regulatória, a categoria "alimentos funcionais" enfrenta desafios de definição universal. Segundo Scrinis (2008) um alimento pode ser considerado 'funcional' se for satisfatoriamente demonstrado que afeta beneficamente uma ou mais funções-alvo do corpo, além dos efeitos nutricionais adequados. Essa definição, aceita por organismos como o *International Life Sciences Institute* (ILSI), destaca que o alimento funcional não se limita à nutrição básica, mas cumpre função fisiológica ou metabólica adicional. Tal distinção é fundamental para a pesquisa, formulação e rotulagem desses produtos.

No âmbito das bebidas funcionais, observa-se que esta categoria específica de alimentos funcionais tem ganhado destaque devido à aceitação do consumidor e à versatilidade como veículo de compostos bioativos. Conforme Panou e Karabagias (2025), as bebidas funcionais representam uma interface entre nutrição, ciência dos alimentos e saúde pública, sendo promissoras tanto para a indústria quanto para a pesquisa acadêmica.

Em termos de composição, as bebidas funcionais utilizam polifenóis, flavonoides, carotenoides, fibras solúveis, prebióticos e probióticos. Os prebióticos estimulam o crescimento de microrganismos benéficos no intestino, enquanto os probióticos são microrganismos vivos que contribuem para o equilíbrio da microbiota intestinal. Observa-se que os ingredientes funcionais mais difundidos são ácidos graxos poliinsaturados (PUFAs), probióticos/prebióticos/simbióticos e antioxidantes Esse tipo de evidência torna claro que, no desenvolvimento de formulações funcionais, há uma convergência entre nutrição, bioatividade e tecnologia de alimento (Granato *et al.*, 2020).

O desenvolvimento de bebidas funcionais implica desafios tecnológicos importantes. A literatura aponta que fatores como estabilidade dos compostos bioativos,

interação entre ingredientes, aceitabilidade sensorial e biodisponibilidade fazem diferença para a eficácia final. Dinçay (2020) destaca que a definição de alimentos funcionais deve considerar também eficácia em benefícios de saúde clinicamente comprovados e documentados para a prevenção, gestão ou tratamento de doenças crónicas. Isso significa que formulações devem ir além da criação do produto e considerar evidências significativas de benefício.

Quanto à aceitação do consumidor, fatores como sabor, textura, conveniência de consumo e percepção de saúde influenciam fortemente o sucesso de bebidas funcionais. A revisão de Panou e Karabagias (2025) salienta que as bebidas funcionais podem combinar atributos como reidratação do corpo, recuperação de energia perdida, melhoria da saúde do coração, melhoria da imunidade e do sistema digestivo. Assim, além de funcionalidade, a experiência de consumo e a percepção do usuário são parte essencial da equação.

Entretanto, mesmo com a crescente produção científica e comercialização de bebidas funcionais, ainda existe lacuna significativa de evidências clínicas concretas para muitas formulações. Pesquisas apontam para a necessidade de pesquisa rigorosa e sistemática no delineamento de alimentos funcionais (Martirosyan *et al.*, 2022). Esse quadro evidencia que, embora a tecnologia avance, a comprovação cientifica deve acompanhar para assegurar credibilidade e segurança.

Desta forma, o conceito de alimentos e bebidas funcionais representa uma convergência entre ingestão dietética, bioatividade e saúde. No caso das bebidas funcionais, a conveniência e o potencial de difusão tornam-nos veículos aptos para entregar compostos funcionais, porém sua eficácia depende de formulação adequada, aceitação pelo consumidor, estabilidade dos compostos e evidência científica sólida. Esta abordagem integrada é essencial para que tais produtos realmente contribuam para a promoção da saúde.

Finalmente, a aplicação dessa categoria no desenvolvimento de bebidas funcionais insere-se em um contexto mais amplo de inovação em alimentos, onde a valorização de ingredientes bioativos, matrizes vegetais alternativas e atenção à sustentabilidade se combinam com a funcionalidade.

3.2 Propriedades Antioxidantes em Bebidas Funcionais

As bebidas funcionais têm se destacado como uma das categorias mais dinâmicas dentro do mercado de alimentos e suplementos, sendo formuladas não apenas para

promover hidratação, mas também para fornecer compostos bioativos capazes de modular processos metabólicos e oxidativos no organismo humano. Esses produtos são frequentemente enriquecidos com vitaminas, minerais, compostos fenólicos e outros antioxidantes naturais, com o objetivo de reduzir o estresse oxidativo e contribuir para a prevenção de doenças crônicas não transmissíveis, como diabetes, câncer e enfermidades cardiovasculares (Granato *et al.*, 2010). O conceito de funcionalidade, nesse contexto, ultrapassa a mera composição nutricional, abrangendo também o impacto fisiológico mensurável decorrente do consumo regular dessas bebidas.

Entre os principais compostos antioxidantes presentes em bebidas funcionais destacam-se os polifenóis, flavonoides, ácidos fenólicos, carotenoides e catequinas. O chá verde (*Camellia sinensis*), por exemplo, contém epigalocatequina galato (EGCG), uma das substâncias mais estudadas por sua potente atividade antioxidante e capacidade de modular vias de sinalização relacionadas à inflamação e apoptose celular (Cabrera *et al.*, 2006). Evidências recentes indicam que o uso de maltodextrinas com baixo equivalente de dextrose (DE) como carreador é eficaz na preservação dos compostos fenólicos e flavonoides de *Clidemia japurensis* DC. e *Clidemia hirta* (L.) D.Don, assegurando maior eficiência de encapsulação e estabilidade oxidativa (Mar *et al*., 2020).

Os sucos de frutas ricas em compostos fenólicos,ROBUSTA como a romã (*Punica granatum*), a uva e o açaí, também têm sido amplamente investigados. Gil *et al*. (2000) observaram que o suco de romã apresenta capacidade antioxidante significativamente superior à do vinho tinto e do chá verde, medida pelos métodos ABTS e DPPH. Essa elevada capacidade antioxidante é atribuída principalmente à presença de punicalaginas e ácido elágico, compostos responsáveis por neutralizar espécies reativas de oxigênio e nitrogênio. Estudos clínicos sugerem que o consumo regular de suco de romã pode reduzir a oxidação de lipoproteínas de baixa densidade (LDL) e melhorar a função endotelial (Aviram e Rosenblat, 2012).

A avaliação das propriedades antioxidantes em bebidas funcionais baseia-se em diferentes metodologias analíticas, entre as quais se destacam os ensaios DPPH, ABTS, FRAP e ORAC. Cada método avalia um mecanismo distinto de ação antioxidante, seja por doação de elétrons, transferência de hidrogênio ou quelante de metais (Thaipong *et al*., 2006). Estudos comparativos demonstram que bebidas elaboradas com frutas vermelhas, *goji berry* e laranja vermelha apresentam os maiores índices de capacidade antioxidante total, principalmente devido à alta concentração de antocianinas e flavonóis (J. Silva *et al.*, 2024).

A matriz alimentar e o processamento tecnológico exercem papel determinante na conservação ou degradação dos compostos antioxidantes. Fatores como temperatura, exposição à luz, oxigênio e pH podem alterar a estabilidade de polifenóis e vitaminas sensíveis (Manach *et al*., 2004). Além disso, a adição de açúcares e conservantes artificiais pode afetar a biodisponibilidade desses compostos, reduzindo o impacto fisiológico esperado. Por esse motivo, estudos recentes buscam otimizar o processamento de bebidas funcionais utilizando tecnologias suaves, como a pasteurização a frio e a microfiltração, visando preservar a integridade dos antioxidantes (Manach *et al*., 2004).

A formulação sinérgica tem se mostrado uma estratégia eficaz para aumentar a capacidade antioxidante global de bebidas funcionais. Combinações de extratos de frutas com ervas (como hibisco, gengibre e cúrcuma) e vitaminas antioxidantes (C e E) potencializam a ação biológica por meio de efeitos aditivos ou sinérgicos (Granato *et al.*, 2017). Além disso, o uso de encapsulamento de compostos bioativos, como polifenóis e flavonoides, em nanopartículas ou microcápsulas, tem se destacado como alternativa para aumentar a estabilidade e a biodisponibilidade desses antioxidantes no organismo (Flores *et al.*, 2015).

Embora os resultados *in vitro* sejam amplamente positivos, a tradução desses achados para efeitos clínicos consistentes ainda é um desafio. Revisões sistemáticas apontam que o consumo regular de bebidas ricas em polifenóis pode reduzir marcadores de estresse oxidativo e inflamação, mas os efeitos variam conforme dose, tempo de intervenção e características individuais dos participantes (Del Rio *et al.*, 2013). Ensaios clínicos bem controlados são, portanto, fundamentais para validar alegações funcionais e garantir segurança e eficácia no consumo prolongado.

Do ponto de vista da saúde pública, incentivar o consumo de bebidas funcionais com propriedades antioxidantes pode contribuir para estratégias preventivas contra doenças crônicas degenerativas. Em síntese, as bebidas funcionais constituem uma categoria promissora de alimentos capazes de fornecer antioxidantes naturais com potencial de promover a saúde humana. O avanço nas técnicas de processamento, formulação e análise antioxidante permite o desenvolvimento de produtos mais estáveis e eficazes (Flores *et al.*, 2015). Entretanto, a comprovação dos efeitos fisiológicos desses compostos requer uma abordagem multidisciplinar, integrando química analítica, biologia molecular, nutrição e ciência dos alimentos. O fortalecimento dessa base científica é essencial para transformar o conceito de funcionalidade em evidência significativa de benefício à saúde.

3.3 Plantas Alimentícias Não Convencionais (PANC)

As Plantas Alimentícias Não Convencionais (PANC) vêm ganhando destaque como recurso potencial para diversificação alimentar e valorização da biodiversidade vegetal no Brasil. O termo PANC refere-se a hortaliças, frutas, flores ou ervas que possuem partes comestíveis, porém não são amplamente consumidas pela população em geral, ou não têm cultivo em larga escala (Casemiro e Vendramini, 2020).

Essas plantas apresentam grande relevância nutricional visto que diversas revisões apontam que as PANC podem conter concentrações elevadas de vitaminas, minerais e compostos bioativos, comparáveis ou superiores a muitas hortaliças convencionais (Pereira dos Santos *et al.*, 2021). Esse fato reforça seu papel como alternativa alimentar, sobretudo em contextos de insegurança nutricional ou onde há necessidade de alimentos de baixo custo e produzido localmente.

Entretanto, apesar do seu potencial, o consumo e o cultivo de PANC ainda enfrentam desafios significativos. Uma das preocupações centrais refere-se ao seu aspecto toxicológico que embora muitas espécies apresentem baixo grau de toxicidade, algumas necessitam de preparo ou conhecimento específico para seu uso seguro, o que limita sua disseminação (Casemiro e Vendramini, 2020).

Nas áreas urbanas, as PANC também são vistas como estratégia para hortas comunitárias e espaços de produção agroecológica, contribuindo para metas de desenvolvimento sustentável. Um estudo de 2023 demonstrou que o cultivo de PANC em horta urbana em São Paulo favorece metas da Agenda 2030, especialmente aquelas ligadas à fome zero, cidades sustentáveis e vida terrestre (Dias *et al.*, 2023). Tal perspectiva amplia o papel das PANC além da nutrição, englobando impactos socioambientais e comunitários.

Do ponto de vista etnobotânico e de valorização local, estudos em biomas evidenciam que comunidades possuem saberes tradicionais sobre uso de espécies de PANC, embora o reconhecimento formal e a comercialização sejam limitados. No município de Itaiçaba (CE) foram identificadas 35 espécies de PANC utilizadas localmente, mas muitas vezes sem reconhecimento formal do termo “PANC” ou de seu valor (Sobreira, Sampaio e Souza, 2022). Isso evidencia o potencial ainda não explorado dessas plantas na alimentação regional.

A questão da aceitabilidade sensorial dos produtos que contêm PANCs também exige atenção: uma revisão sistemática de 2025 mostrou que a maioria dos estudos

focados em análise sensorial envolvem folhas de PANC herbáceas, sendo crucial avaliar sabor, aroma e textura para inserção no mercado e aceitação pelo consumidor (Lima *et al.*, 2022). Ou seja, fatores de mercado, cultura alimentar e perfil sensorial devem ser considerados para que as PANC deixem de ser apenas "alternativas" e passem a fazer parte do cotidiano alimentar.

Além disso, há também debates sobre o próprio conceito "não convencional": pesquisas refletem que muitas plantas chamadas de PANC em uma região podem ser convencionais em outra, ou ainda que o termo "convencional" se transforma com o tempo de consumo e difusão (Araújo *et al*., 2025). Esse aspecto destaca que o termo PANC é dinâmico e depende de contextos culturais, geográficos e históricos.

Em síntese, as PANC representam oportunidade multifacetada: nutricional, ambiental, cultural e econômica. No entanto, para que se concretizem como componente relevante da alimentação humana, é necessário que avanços ocorram em pesquisa (nutrição, processamento, mercado), política pública (incentivo, comercialização) e educação alimentar (sensibilização, redes de difusão). A conjugação desses fatores permitirá que as PANC contribuam de fato para diversificação alimentar, segurança nutricional e valorização da biodiversidade vegetal brasileira.

3.4 Desenvolvimento de Bebidas Funcionais à Base de Espécies Amazônicas

O bioma amazônico representa uma fonte promissora de matéria-prima para o desenvolvimento de bebidas funcionais, dada sua biodiversidade e riqueza em espécies com elevado conteúdo de compostos bioativos. Por exemplo, estudos sobre a fruta camu-camu (*Myrciaria dúbia* HBK [McVaugh]) ressaltam que seu alto teor de vitamina C, polifenóis e flavonoides a torna um candidato promissor para aplicações em alimentos funcionais (Salomão-Oliveira e Marinho, 2017).

Na prática, algumas pesquisas no Amazonas estudam bebidas funcionais à base de frutas amazônicas, avaliando propriedades antioxidantes e até atividade prebiótica, com o intuito de valorizar as espécies. Esse tipo de estudo se insere no esforço de agregar valor à cadeia produtiva amazônica e diversificar a oferta de bebidas funcionais.

Tabela 1: Artigos científicos sobre o desenvolvimento de bebidas funcionais à base de espécies amazônicas

Referência (autor, ano)	Título (resumido)	Resumo curto (relevância para bebidas funcionais amazônicas)
Crispino *et al*., 2024	*Development of flavored kombuchas with Amazonian fruits: bioactive compounds evaluation and antioxidant capacity*	Desenvolvimento e caracterização físico-química, bioativos e capacidade antioxidante de kombuchas aromatizadas com frutas amazônicas (ex.: cupuaçu, taperebá). Aplicável diretamente a formulação de bebidas fermentadas funcionais.
Batista *et al.*, 2025	*Fermented Beverages from Amazonian Fruits: Nutritional Characteristics and Bioactive Compounds*	Revisão técnica sobre bebidas fermentadas feitas com frutas amazônicas (açaí, cupuaçu, buriti, camu-camu etc.), discutindo perfil nutricional, compostos bioativos, sensorial e potencial para bioeconomia.
García-Chacón *et al.*, 2023	*Camu Camu (Myrciaria dubia (Kunth) McVaugh): An Amazonian Fruit with Biofunctional Properties−A Review*	Revisão sobre composição fitoquímica, atividade antioxidante e potenciais aplicações do camu-camu, base para formulações (suplementos, sucos funcionais e bebidas envasadas).
Chasquibol *et al.*, 2022	*Design of Functional Powdered Beverages Containing Co-Microcapsules of Sacha Inchi P. huayllabambana Oil and Antioxidant*	Desenvolvimento de bebidas em pó com camu-camu; aborda formulação, estabilidade de compostos bioativos e potencial de aplicação industrial (útil para bebidas instantâneas/enriquecidas).

	Extracts of Camu Camu and Mango Skins Nancy	
Avila-sosa *et al.*, 2019	*Antioxidant Properties of Amazonian Fruits: A Mini Review of In Vivo and In Vitro Studies*	Revisão sobre principais frutas amazônicas ricas em antioxidantes, fornece o fundamento científico (compostos-alvo) para selecionar matérias-primas em bebidas funcionais.
Assis *et al.*, 2025	*Fermentation of Amazonian fruit pulp (bacaba) with distinct probiotics: Impacts on chemical composition, bioaccessibility, and effects on human intestinal microbiota*	Estudo experimental de fermentação de polpa de bacaba com probióticos; avalia composição química, sobrevivência probiótica e efeitos na microbiota — diretamente aplicável à formulação de bebidas probióticas amazônicas.
Silva *et al*., 2024	*Cupuaçu (Theobroma grandiflorum): A Multifunctional Amazonian Fruit with Extensive Benefits*	Artigo sobre cupuaçu: perfil funcional, aplicações alimentares e potencial de uso em iogurtes, sucos e bebidas fermentadas — útil para escolha de matérias-primas e processos.
Martins *et al.*, 2024	*Amazonian fruits: A systematic review of the literature and critical analysis of its trends*	Revisão sistemática centrada em frutos amazônicos e suas aplicações alimentares e tecnológicas, incluindo casos de desenvolvimento de bebidas funcionais.
Natalia *et al*., 2025	*Bioactive and agroindustrial potential of*	Revisão sobre potencial agroindustrial e aplicações de frutos amazônicos (açaí, camu-

	Amazonian fruit species: a review	camu, cupuaçu, bacuri etc.), com discussões sobre incorporação em alimentos e bebidas funcionais.

Um estudo específico envolvendo a fruta camu-camu demonstrou em voluntários fumantes que o consumo diário do suco puro por sete dias reduziu marcadores de estresse oxidativo e inflamação, enquanto suplemento de vitamina C equivalente não apresentou os mesmos efeitos. Esse achado reforça o potencial funcional desse tipo de bebida (Inoue e Komoda, 2008).

Para que bebidas funcionais com espécies amazônicas tenham viabilidade comercial e impacto nutricional, é necessário conhecer profundamente seu perfil fitoquímico e propriedades funcionais. Por exemplo, em trabalho com frutas da família *Myrtaceae* amazônica foram identificados constituintes voláteis (monoterpenos, sesquiterpenos, derivados de ácidos graxos) que influenciam aroma, sabor e, potencialmente, aceitação do produto final (Barroso *et al.*, 2024).

Entretanto, há vários desafios que permanecem. A curta vida de prateleira de frutas frescas amazônicas, o custo de cadeia logística, além da necessidade de formulação adequada (estabilidade dos compostos bioativos, sabor, textura, aceitação sensorial) são barreiras que estudos estão começando a enfrentar.

Em termos de formulação tecnológica, a fermentação de bebidas ou uso de ingredientes amazônicos em matrizes de bebidas funcionalizadas (como shakes, sucos enriquecidos, bebidas lácteas) emerge como via de inovação. Por exemplo, pesquisa com PANC (Plantas Alimentícias Não Convencionais) amazônicas para infusão e bebidas fermentadas demonstra que o uso de espécies regionais pode favorecer arranjos produtivos locais e desenvolvimento de novos bioprodutos (Sousa *et al.*, 2023).

Do ponto de vista de sustentabilidade e bioeconomia, o uso de espécies amazônicas em bebidas funcionais se alinha com tendências de valorização de cadeias locais, agricultura de base extrativista/regenerativa, e economias de impacto (Martinez *et al*., 2021).

Em síntese, o desenvolvimento de bebidas funcionais com espécies amazônicas reúne um tripé de importância: potencial funcional baseado no elevado teor de compostos bioativos de frutas nativas; oportunidade de mercado nacional e internacional com apelo de origem e saúde; e desafio de formulação, processamento e aceitação sensorial que

exige pesquisa interdisciplinar. Para que esse segmento se consolide, será fundamental integrar química analítica, tecnologia de alimentos, inovação de produto e cadeia de abastecimento sustentável.

3.5 Sustentabilidade e Valorização da Biodiversidade

A sustentabilidade, entendida como a conjunção equilibrada entre os vetores ambiental, social e econômico, tornou-se imperativa diante da crise climática, da perda de biodiversidade e da degradação de ecossistemas. No bioma amazônico, por exemplo, a biodiversidade exerce papel central na regulação climática, na provisão de serviços ecossistêmicos e na manutenção de modos de vida tradicionais (Miranda *et al.*, 2023).

Dentro desse quadro, a valorização da biodiversidade, ou seja, o reconhecimento, uso responsável e agregação de valor econômico a espécies, ecossistemas e serviços biológicos, emerge como estratégia vital para promover arranjos produtivos sustentáveis. Um estudo recente destaca que a "bioeconomia" da Amazônia pode gerar emprego local, incrementar o produto nacional bruto e contribuir para a conservação ambiental, desde que apoiada por bases de conhecimento adequadas e infraestrutura capaz de sustentar cadeias de valor de base biótica (Barbosa *et al.*, 2021).

Porém, a efetividade dessa valorização depende de mecanismos de governança multinível que articulem comunidades locais, setor privado, Estado e ciência. Em cadeias de produtos de sociobiodiversidade, como aqueles provenientes de florestas amazônicas, a atuação de organizações da sociedade civil e a coordenação das políticas públicas são essenciais para que os benefícios sejam equitativos e ambientalmente compatíveis (Júnior e Arroyo, 2024).

A cadeia agroalimentar também oferece uma via prática para concretizar a valorização da biodiversidade: a incorporação de espécies sobutilizadas ou nativas em alimentos, bebidas funcionais e cadeias de valor regionais rompe com o modelo agrícola convencional e pode simultaneamente diversificar dietas, gerar renda e conservar ecossistemas (Voglhuber-slavinsky *et al*., 2023).

Entretanto, a sustentabilidade desses arranjos não está garantida automaticamente pois pesquisas apontam que a literatura da bioeconomia tende a enfatizar determinados aspectos ambientais ou econômicos, mas frequentemente deixa de lado a dimensão social ou a equidade distributiva, o que pode comprometer a real sustentabilidade das iniciativas (Lessa *et al.*, 2025).

No contexto amazônico, a intensificação da produção agrícola para proteger floresta e mitigar aquecimento global ilustra essa dualidade: um estudo mostrou que, em tese, aumentar a produtividade poderia reduzir pressão por expansão da fronteira agrícola. Contudo, essas medidas devem ser planejadas com cautela para não agravar desequilíbrios sociais ou ecológico (Marin *et al.*, 2022).

Adicionalmente, a degradação de ecossistemas e a contaminação dos recursos hídricos associadas a atividades humanas (como mineração, desmatamento e queimadas) ameaçam tanto a biodiversidade quanto a segurança hídrica e alimentar. Um levantamento integrado constatou que a conservação da biodiversidade amazônica exige políticas que articulem conservação florestal, manejo sustentável do solo e engajamento das comunidades locais (Benedicto *et al.*, 2025).

Em termos práticos para o desenvolvimento de produtos, a valorização de resíduos agrícolas ou biomassa de frutas nativas aponta para uma economia circular que integra conservação e inovação. Por exemplo, a utilização de biomassa de frutas da Amazônia como insumo para produção de biossurfactantes demonstra como subprodutos podem se tornar valor agregado, alinhando sustentabilidade e valorização de biodiversidade vegetal (Feio *et al.*, 2025).

Desta forma, a valorização da biodiversidade no âmbito da sustentabilidade exige que o uso de recursos naturais não seja apenas exploratório, mas transformado em valor responsável envolvendo ciência, tecnologia, economia local, governança eficaz e equidade social. No caso da Amazônia, esse desafio adquire urgência dada a escala da biodiversidade, a vulnerabilidade dos ecossistemas e a necessidade de modelos produtivos alternativos que articulem conservação e desenvolvimento humano.

3.6 Aplicação de Espécies Amazônicas no Desenvolvimento de Bebidas Funcionais: *Tapirira guianensis* Aubl., *Theobroma speciosum* Willd. ex Spreng. e *Hibiscus acetosella* Welw. ex Hiern

Tapirira guianensis Aubl., popularmente denominada pau-pombo, é uma espécie nativa amplamente distribuída no Brasil, com ocorrência frequente na Mata Atlântica e em ambientes úmidos. Caracteriza-se como uma árvore perenifólia, pioneira e heliófita, que atinge de 8 a 14 metros de altura e apresenta tronco com diâmetro entre 40 e 60 cm. Possui folhas compostas, formadas por quatro a cinco pares de folíolos membranáceos e glabros, variáveis em forma e tamanho, medindo de 4 a 12 cm de comprimento. Além de

sua importância ecológica, a espécie é indicada para reflorestamentos heterogêneos de áreas degradadas e de matas ciliares, especialmente pela produção de frutos amplamente apreciados pela fauna (Santana *et al.*, 2009).

Estudos indicam que suas folhas e cascas apresentam elevados teores de compostos fenólicos e flavonoides, como quercetina, derivados glicosilados de quercetina e galotaninos, associados a atividade antioxidante e a potenciais efeitos cardiovasculares (Rodrigues *et al.*, 2017).



Figura 1: Frutos de *T. guianensis* Aubl.
Fonte: Própria

A avaliação da atividade antioxidante dos extratos de folhas de *T. guianensis* Aubl. revelou valores expressivos em ensaios DPPH, TEAC e ORAC, com resultados comparáveis ou superiores ao chá verde, tradicional referência antioxidante (Patient *et al.*, 2022). Esses achados indicam que os compostos bioativos presentes na espécie podem atuar como agentes funcionais em formulações alimentares, especialmente em bebidas, conferindo proteção frente ao estresse oxidativo.

Além da ação antioxidante, pesquisas também demonstraram que extratos de *T. guianensis* Aubl. apresentam efeito vasodilatador, mediado pelo eixo NO/cGMP-eNOS, com potencial para aplicações funcionais relacionadas à saúde cardiovascular (Rodrigues *et al*., 2017). Ensaios *in vitro* e *in vivo* apontam ainda efeitos citotóxicos seletivos em células tumorais, indicando perspectivas promissoras para uso em produtos nutracêuticos ou suplementos funcionais. O uso do extrato de sementes de *T. guianensis* Aubl. como

fonte natural de compostos bioativos com propriedades citoprotetoras, antiproliferativas, antioxidantes e antimaláricas mostra-se promissor, ressaltando-se a necessidade de estudos adicionais in vivo para melhor elucidar seus mecanismos de ação e garantir sua segurança (Crispim, 2025).

Do ponto de vista de formulação de bebidas funcionais, *T. guianensis* Aubl. pode ser incorporada em sucos, infusões, bebidas fermentadas ou extratos concentrados, visando aumentar o conteúdo de antioxidantes, agregar valor sensorial e promover diferenciais de mercado associados à biodiversidade amazônica. A escolha do material vegetal, método de extração e processamento da bebida são fatores críticos para maximizar a estabilidade dos compostos bioativos e a aceitação sensorial do produto final (Costa *et al*., 2023; Oliveira *et al*., 2018).

Adicionalmente, a sustentabilidade da cadeia de produção deve ser considerada. A exploração de *T. guianensis* Aubl. deve respeitar critérios de manejo florestal sustentável, garantindo o fornecimento contínuo de matéria-prima sem comprometer os ecossistemas amazônicos. O uso dessa espécie em bebidas funcionais também contribui para a valorização da biodiversidade local e para a bioeconomia regional, criando oportunidades de desenvolvimento socioeconômico associadas à conservação ambiental (Barbosa *et al.*, 2021).

Em síntese, *T. guianensis* Aubl. representa uma alternativa promissora para o desenvolvimento de bebidas funcionais à base de espécies amazônicas, com suporte científico quanto ao seu perfil fitoquímico e propriedades antioxidantes e funcionais. Pesquisas futuras devem focar na otimização de técnicas de extração, estabilidade de compostos bioativos em matrizes líquidas, avaliação de efeitos funcionais em modelos biológicos e aceitação sensorial do consumidor, consolidando sua viabilidade tecnológica e mercadológica.

A espécie *Theobroma speciosum* Willd. ex Spreng., da família Malvaceae, conhecido como cacauí, é uma espécie frutífera nativa da Floresta Amazônica, onde ocorre principalmente em áreas de terra firme não sujeitas a inundações (Varella *et al.*, 2018). Constitui uma alternativa promissora de planta amazônica pouco explorada, com elevado potencial para o desenvolvimento de produtos funcionais. Estudos fitoquímicos recentes demonstraram que frutos, sementes e flores comestíveis desta espécie contêm elevados teores de ácidos orgânicos, flavonoides, carotenoides e compostos fenólicos, com reconhecida atividade antioxidante e antimicrobiana (Coyago-cruz *et al*., 2025).

É raramente cultivada e seus frutos, na maior parte, são extraídos de plantas da natureza para consumo. Além doa frutos (polpa e castanha0 também são comestíveis, suas flores cruas como saladas, chás e geleias (Kinupp, 2014).



Figura 2: Flores de *T. speciosum* Willd. ex Spreng.
Fonte: Própria

Em investigações conduzidas por Coyago-cruz *et al*. (2025), sementes e mucilagem de *T. speciosum* apresentaram altos conteúdos de ácido málico, ácido tartárico e naringenina, associadas a significativa atividade antioxidante em ensaios DPPH e ABTS. Esses achados evidenciam que a mucilagem e extratos da espécie podem servir como fontes viáveis de compostos bioativos para incorporação em alimentos e bebidas funcionais, oferecendo benefícios antioxidantes e potencial valor agregado.

Além disso, *T. speciosum* Willd. ex Spreng diferencia-se de espécies mais consumidas da região amazônica, como *T. grandiflorum* (cupuaçu), destacando-se pelo perfil único de compostos bioativos e potencial de nicho de mercado. Em particular, extratos aquosos das flores comestíveis apresentaram elevado teor de fenólicos totais (~640 mg GAE/g) e forte capacidade antioxidante em ensaios DPPH, ABTS e FRAP, indicando que flores e mucilagem podem ser exploradas em formulações funcionais de alto valor agregado (Moreira *et al.*, 2021).

Do ponto de vista tecnológico, *T. speciosum* Willd. ex Spreng pode ser incorporada em bebidas funcionais na forma de extratos, concentrados ou ingredientes ativos em sucos, infusões e bebidas fermentadas. Entretanto, fatores críticos devem ser considerados, incluindo a estabilidade dos compostos bioativos frente a calor, pH, oxigênio e armazenamento, bem como a aceitabilidade sensorial do produto final. Estratégias como microencapsulação ou uso de carreadoras podem ser adotadas para maximizar a preservação dos bioativos e a viabilidade comercial (Moreira *et al.*, 2021).

A utilização de *T. speciosum* Willd. ex Spreng em bebidas funcionais também contribui para a valorização da biodiversidade amazônica e para a bioeconomia regional, promovendo diversificação produtiva, geração de renda local e conservação de recursos florestais. A exploração de espécies nativas pouco estudadas reforça a inserção da ciência de alimentos funcionais em cadeias de produção sustentáveis, com potencial de inovação tecnológica e impacto socioambiental positivo.

Desta forma, a espécie *Theobroma speciosum* Willd. ex Spreng constitui uma fonte promissora de compostos bioativos para bebidas funcionais, combinando forte respaldo científico com alinhamento estratégico aos princípios de sustentabilidade, biodiversidade e inovação tecnológica. Sua exploração na formulação de alimentos e bebidas reforça a integração entre ciência, tecnologia e valorização de recursos amazônicos (Coyago-Cruz *et al*., 2025; Mar *et al*., 2021).

A espécie *Hibiscus acetocella* Welw. Ex Hiern popularmente conhecida por vinagreira roxa é um arbusto de caule semi-lenhoso pertencente à família das malváceas. Usada tradicionalmente como alimentícia e medicinal sendo as partes usadas, folhas, flores e seus cálices jovens, possui propriedades antioxidantes, antifúngicas, antibacterianas e entre outras (Kinupp, 2014).



Figura 3: Folhas de *Hibiscus acetosella* Welw. ex Hiern.
Fonte: Própria

Hibiscus acetosella Welw. Ex Hiern tem sido alvo de diversos estudos científicos que destacam seu perfil fitoquímico e atividades biológicas relevantes. Pesquisas com extratos de folhas identificaram uma variedade de compostos fenólicos, com destaque para ácido cafeico e antocianinas, que estão fortemente associados à capacidade antioxidante observada em ensaios ABTS. Variedades com folhas mais escuras apresentaram maior atividade antioxidante, indicando correlação entre teor de antocianinas e potencial bioativo das plantas (Lyu *et al*., 2020).

Além da atividade antioxidante, os extratos de *Hibiscus acetosella* Welw. Ex Hiern também demonstraram potencial antibacteriano em testes in vitro contra bactérias como Staphylococcus aureus e Pseudomonas aeruginosa, sugerindo propriedades funcionais promissoras para aplicações alimentares e farmacêuticas (Lyu *et al*., 2020).

Estudos mais recentes voltados às flores comestíveis da espécie reforçam a presença de compostos bioativos, incluindo derivados de quercetina, miricetina e antocianinas, que conferem elevada capacidade de captura de radicais livres em ensaios DPPH e ABTS, além de demonstrar ausência de citotoxicidade em linhas celulares testadas, o que sugere seu potencial nutracêutico e seguro para uso alimentar (dos Santos Silva *et al.*, 2023).

4. Referências

Abdelrahman, R., Hamdi, M., N. Baba, W., M. Hassan, H., & Maqsood, S. (2023). Synergistic combination of natural deep eutectic solvents and green extraction techniques for the valorization of date palm leaves: Optimization of the solvent-biomass interaction. *Microchemical Journal*, *195*(7), 109503. https://doi.org/10.1016/j.microc.2023.109503

Araújo, I. O. de, Martins, T. M. L., Leão, K. V., & Navarro, R. D. (2025). Análise Sensorial de Produtos Alimentícios Contendo Plantas Alimentícias Não Convencionais (PANC): Uma Revisão Sistemática. *Fronteira: Journal of Social, Technological and Environmental Science*, *14*(1), 111–123. https://doi.org/10.21664/2238-8869.2025v14i1.p111-123

Assis, B. B. T., Cabral, L., & Silva, F. A. (2025). Fermentation of Amazonian fruit pulp (bacaba) with distinct probiotics: Impacts on chemical composition, bioaccessibility, and effects on human intestinal microbiota. *Food Research International Journal*, *209*(4), 1–16. https://doi.org/10.1016/j.foodres.2025.116326

Avila-sosa, R., Montero-rodríguez, A. F., Aguilar-alonso, P., Vera-lópez, O., Lazcano-hernández, M., Morales-medina, J. C., & Navarro-cruz, A. R. (2019). Review Article Antioxidant Properties of Amazonian Fruits: A Mini Review of In Vivo and In Vitro Studies. *Oxidative Medicine and Cellular Longevity*, *2019*(1), 1–11. https://doi.org/10.1155/2019/8204129

Aviram, M., & Rosenblat, M. (2012). Pomegranate protection against cardiovascular diseases. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 1–20. https://doi.org/10.1155/2012/382763

Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P. R., Rane, M. R., Gurav, R. G., & Jadhav, J. P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany*, *151*, 512–528. https://doi.org/10.1016/j.sajb.2022.05.028

Barbosa, M. de O., Rivas, A. A. F., & Oliveira, L. A. de. (2021). Bioeconomia: Um novo caminho para a sustentabilidade na Amazônia? *Research, Society and Development*, *10*(1), 1–16.

Barroso, A. S., Massing, L. T., Suemitsu, C., Helena, R., Mour, V., Figueiredo, P. L. B., & Maia, G. S. (2024). Volatile Constituents of Some Myrtaceous Edible and Medicinal Fruits from the Brazilian Amazon. *Foods*, *13*(1), 1–19.

Batista, B. N., Correia, A. C., & Jord, A. M. (2025). Fermented Beverages from Amazonian Fruits: Nutritional Characteristics and Bioactive Compounds. *Beverages*, *11*(1), 1–29.

Benedicto, S. C. De, Longo, R. M., Helena, D., Ferreira, L., Sugahara, C. R., Ribeiro, A. Í., Castañeda-ayarza, J. A., & Henrique, L. (2025). Integrating Strategies Aimed at Biodiversity and Water Resource Sustainability in the Amazonian Region. *Sustainability*, *17*(1), 1–21.

Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial Effects of Green Tea—A Review. *Journal of the American College of Nutrition*, *25*(2), 79–99. https://doi.org/10.1080/07315724.2006.10719518

Casemiro, Í. D. P., & Vendramini, A. L. do A. (2020). Unconventional food plants in Brazil: what does Nutrition know about this topic? *Demetra: Alimentação, Nutrição & Saúde*, *15*(1), e42725. https://doi.org/10.12957/demetra.2020.42725

Chasquibol, N., Alarc, R., Gonzales, B. F., Sotelo, A., Landoni, L., Gallardo, G., & Carmen, M. P. (2022). Design of Functional Powdered Beverages Containing Co-Microcapsules of Sacha Inchi P. huayllabambana Oil and Antioxidant Extracts of

Camu Camu and Mango Skins. *Antioxidants*, *11*(1420), 1–17.
Coyago-cruz, E., Salazar, I., Guachamin, A., Alomoto, M., Cerna, M., Mendez, G., Heredia-moya, J., & Vera, E. (2025). Bioactive Compounds, Antioxidant, and Antimicrobial Activity of Seeds and Mucilage of Non-Traditional Cocoas. *Antioxidants 2025, 14*(299), 1–23.
Crispino, A. N. A. C. S., Silva, L. F. D. A., Lima, M. F. T., & Oliveira, J. A. R. D. E. (2024). Development of flavored kombuchas with Amazonian fruits: bioactive compounds evaluation and antioxidant capacity. *An Acad Bras Cienc*, *96*(1), 1–12. https://doi.org/10.1590/0001-3765202420230356
Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, *18*(14), 1818–1892. https://doi.org/10.1089/ars.2012.4581
Dias, R., Dos Santos, A., De Carvalho, M., Oliveira, A., & Branco, A. (2023). Plantas Alimentícias Não Convencionais (PANC): alternativa para hortas urbanas e metas da Agenda 2030. *Revista Latino-Americana de Ambiente Construído & Sustentabilidade*, *4*(15), 124–140. https://qrcd.org/60cz
Dinçay, A. A. (2020). Bulletin of Biotechnology Risks and benefits of functional foods: an overview. *Review Article Bull Biotechnol*, *1*(2), 56–64.
dos Santos Silva, L. Y., da Silva Ramos, A., Cavalcante, D. N., Kinupp, V. F., da Silva Rodrigues, J. V., Ventura, B. M. L., de Oliveira Mendes, T. A., Sanches, E. A., Campelo, P. H., & de Araújo Bezerra, J. (2023). *Hibiscus acetosella*: An Unconventional Alternative Edible Flower Rich in Bioactive Compounds. *Molecules*, *28*(12), 1–13. https://doi.org/10.3390/molecules28124819
Feio, A. M., César, G., Orsato, A., Leite, K., Mariano, L., Pimentel, S., Alves, J. D. A., Gomes, G. S., Ramos, E. O., Quintella, C. M., Fragoso, S. P., Augusto, J., Bitencourt, P., Cruz, E., & Cerqueira, S. (2025). Valorization of Amazonian Fruit Biomass for Biosurfactant Production and Nutritional Applications. *Biomass*, *5*(60), 1–20.
Flores, F. P., Singh, R. K., Kerr, W. L., Phillips, D. R., & Kong, F. (2015). In vitro release properties of encapsulated blueberry (*Vaccinium ashei*) extracts. *Food Chemistry*, *168*(1), 225–232. https://doi.org/10.1016/j.foodchem.2014.07.059
García-Chacón, J. M., Marín-Loaiza, J. C., & Osorio, C. (2023). Camu Camu (*Myrciaria dubia* (Kunth) McVaugh): An Amazonian Fruit with Biofunctional Properties−A Review. *ACS Omega*, *8*(1), 5169–5183. https://doi.org/10.1021/acsomega.2c07245
Gil, M. I., Tomas-Barberan, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, *48*(10), 4581–4589. https://doi.org/10.1021/jf000404a
Granato, D., Barba, F. J., Bursać Kovačević, D., Lorenzo, J. M., Cruz, A. G., & Putnik, P. (2020). Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. *Annual Review of Food Science and Technology*, *11*, 93–118. https://doi.org/10.1146/annurev-food-032519-051708
Granato, D., Branco, G. F., Nazzaro, F., Cruz, A. G., & Faria, J. A. F. (2010). Functional foods and nondairy probiotic food development: Trends, concepts, and products. *Comprehensive Reviews in Food Science and Food Safety*, *9*(3), 292–302. https://doi.org/10.1111/j.1541-4337.2010.00110.x
Granato, D., Nunes, D. S., & Barba, F. J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of

functional foods: A proposal. *Trends in Food Science and Technology*, *62*(12), 13–22. https://doi.org/10.1016/j.tifs.2016.12.010

Inoue, T., & Komoda, H. (2008). Tropical fruit camu-camu (Myrciaria dubia) has anti-oxidative and anti-inflammatory properties. *Trends in Food Science and Technology, 2(1), 1-15.* https://doi.org/10.1016/j.jjcc.2008.06.004

Júnior, F. S. da C., & Arroyo, J. C. T. (2024). O protagonismo da sociedade civil na sociobiodiversidade do contexto amazônico. *Research, Society and Development*, *13*(1), 1–10.

Lessa, J., Virgolino, F., & Holden, N. M. (2025). Does the bioeconomy literature provide a balanced view of sustainability ? *Sustainability Science*, *20*(3), 1075–1092. https://doi.org/10.1007/s11625-025-01642-6

Li, S., Wang, G., Zhao, J., Ou, P., Yao, Q., & Wang, W. (2024). Ultrasound-Assisted Extraction of Phenolic Compounds from Celtuce (*Lactuca sativa* Var. Augustana) Leaves Using Natural Deep Eutectic Solvents (NADES): Process Optimization and Extraction Mechanism Research. *Molecules*, *29*(10). https://doi.org/10.3390/molecules29102385

Lima, M. S. da C., de Souza, E. B., & Sampaio, V. da S. (2022). Non-Conventional Food Plants (PANC) and the Challenge of Valuing Unknown Wealth: a case study in the municipality of Itaiçaba, Ceará. *Revista Brasileira de Geografia Fisica*, *15*(5), 2164–2177. https://doi.org/10.26848/rbgf.v15.5.p2164-2177

Lyu, J. Il, Ryu, J., Jin, C. H., Kim, D., Kim, J. M., Seo, K., Kim, J., Kim, S. H., Ahn, J., Kang, S., & Kwon, S. (2020). Phenolic Compounds in Extracts of *Hibiscus acetosella* (Cranberry Hibiscus) and Their Antioxidant and Antibacterial Properties. *Molecules*, *25*(1), 1–12.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, *79*(5), 727–747. https://doi.org/10.1093/ajcn/79.5.727

Mar, J. M., Carolino, A. de S., Nunes, R. Z. de A., Soares, J. V. S., & Costa, S. dos S. (2025). Ultrasound-assisted green extraction of antioxidant components from *Theobroma speciosum* flower using natural deep eutectic. In *Perspectivas e inovação na pesquisa em Química* (pp. 124–141).

Mar, J. M., da Fonseca Júnior, E. Q., Corrêa, R. F., Campelo, P. H., Sanches, E. A., & Bezerra, J. de A. (2024). *Theobroma spp.*: A review of it's chemical and innovation potential for the food industry. *Food Chemistry Advances*, *4*(March), 1–17. https://doi.org/10.1016/j.focha.2024.100683

Mar, J. M., Frota, R., Ramos, S., Kinupp, V. F., Sanches, E. A., Campelo, P. H., & Ara, J. De. (2023). Enhancing Bioactive Compound Bioaccessibility in *Tapirira guianensis* Juices through Ultrasound-Assisted Applications. *Process*, *11*(2718), 1–16.

Mar, J. M., Silva, L. S., Rabelo, S., Muniz, M. P., Nunomura, S. M., Correa, R. F., Ferreira, V., Campelo, P. H., Bezerra, J. D. A., & Sanches, E. A. (2020). Encapsulation of Amazonian Blueberry juices: Evaluation of bioactive compounds and stability. *LWT - Food Science and Technology*, *124*(2). https://doi.org/10.1016/j.lwt.2020.109152

Mar, J. M., Silva, L. S., Lita, A. C., Kinupp, V. F., Yoshida, M. I., Moreira, W. P., Bruginski, E., Campos, F. R., Machado, M. B., Souza, T. P., Campelo, P. H., Bezerra, J. A., Sanches, E. A. (2020). Bioactive compounds-rich powders: Influence of different carriers and drying techniques on the chemical stability of the Hibiscus acetosella extract. *Powder Technology, 360 (1)*, 383-391. https://doi.org/10.1016/j.powtec.2019.10.062

Marin, F. R., Zanon, A. J., Monzon, J. P., Andrade, J. F., Silva, E. H. F. M., Richter, G. L., Antolin, L. A. S., Ribeiro, B. S. M. R., Ribas, G. G., Battisti, R., Heinemann, A. B., & Grassini, P. (2022). Protecting the Amazon forest and reducing global warming via agricultural intensification. *Nature Sustainability*, *5*(12), 1018–1031.

Martinez, A. M., Hernandez, M. S., & Cardona, J. E. (2021). Use of amazon fruits in the preparation of alcoholic beverages. *Food Bioscience*, 1(8) 8–11. https://doi.org/10.15171/ijpni.2021.07

Martins, J., Frota, R., Aparecido, E., Campelo, P. H., & Araújo, J. De. (2024). Food Bioscience Amazonian fruits: A systematic review of the literature and critical analysis of its trends. *Food Bioscience*, *62*(8), 1–13. https://doi.org/10.1016/j.fbio.2024.105563

Martirosyan, D., Lampert, T., & Lee, M. (2022). A comprehensive review on the role of food bioactive compounds in functional food science. *Functional Food Science*, *3*(2), 64–78. https://doi.org/10.1007/978-981-16-6369-7_69

Miranda, R., Schwaninger, M., Lucena, A., Logullo, Y., Belderrain, M. C. N., Carvalho, T. C. M. B., & Sato, R. C. (2023). Sustainable Amazon: A Systemic Inquiry with Native Populations. *Sustainability*, *15*(7517), 1–24.

Moreira, J., Souza, L., Picanço, W., & Bezerra, A. (2021). Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds. *Food Chemistry*, *356*(March), 1–5. https://doi.org/10.1016/j.foodchem.2021.129723

Mungwari, C. P., King'ondu, C. K., Sigauke, P., & Obadele, B. A. (2025). Conventional and modern techniques for bioactive compounds recovery from plants: Review. *Scientific African*, *27*(November 2024), e02509. https://doi.org/10.1016/j.sciaf.2024.e02509

Natalia, L., Álvarez, C., García-chacón, J. M., Francisco, J., & González-miret, M. L. (2025). Bioactive and agroindustrial potential of Amazonian fruit species: a review. *Cogent Food & Agriculture*, *11*(1). https://doi.org/10.1080/23311932.2025.2451062

Osamede Airouyuwa, J., Mostafa, H., Riaz, A., & Maqsood, S. (2022). Utilization of natural deep eutectic solvents and ultrasound-assisted extraction as green extraction technique for the recovery of bioactive compounds from date palm (*Phoenix dactylifera* L.) seeds: An investigation into optimization of process parameters. *Ultrasonics Sonochemistry*, *91*(12), 106233. https://doi.org/10.1016/j.ultsonch.2022.106233

Panou, A., & Karabagias, I. K. (2025). Composition, Properties, and Beneficial Effects of Functional Beverages on Human Health. *Beverages*, *11*(2), 3–5. https://doi.org/10.3390/beverages11020040

Patient, A., Jean-Marie, E., Robinson, J.-C., & Martial, K. (2022). Polyphenol Composition and Antioxidant Activity of *Tapirira guianensis* Aubl. (Anarcadiaceae) Leaves. *Plants*, *11*(326), 1–14.

Pereira dos Santos, V. L., Rodrigues, I. C. G., Alberini, R. D. C., Garcia, I. D. F., & Berté, R. (2021). Plantas Alimentícias Não Convencionais (Pancs): Uma Revisão. *Revista Brasileira de Ensino de Ciências e Matemática*, *5*(1), 462–470. https://doi.org/10.5335/rbecm.v5i1.11341

Rodrigues, A. M. G., Guimarães, D. O., Konno, T. U. P., Tinoco, L. W., Barth, T., Aguiar, F. A., Lopes, N. P., Leal, I. C. R., Raimundo, J. M., & Muzitano, M. F. (2017). Phytochemical Study of *Tapirira guianensis* Leaves Guided by Vasodilatory and Antioxidant Activities. *Molecules*, *22*(1), 1–12. https://doi.org/10.3390/molecules22020304

Rodríguez-Rico, D., Sáenz-Esqueda, M. de los Á., Meza-Velázquez, J. A., Martínez-

García, J. J., Quezada-Rivera, J. J., Umaña, M. M., & Minjares-Fuentes, R. (2022). High-Intensity Ultrasound Processing Enhances the Bioactive Compounds, Antioxidant Capacity and Microbiological Quality of Melon (*Cucumis melo*) Juice. *Foods*, *11*(17), 1–16. https://doi.org/10.3390/foods11172648

Salomão-Oliveira, A., & Marinho, H. A. (2017). Bioactive Compounds and Antioxidant Activity of Camu-Camu (*Myrciaria dubia* (Kunth) Mc Vaugh) Grown on a Non-Flooded Land Ecosystem. *Journal of Food and Nutrition Research*, *5*(12), 941–946. https://doi.org/10.12691/jfnr-5-12-10

Santana, W. M. S., Silva-Mann, R., Ferreira, R. A., De Fátima Arrigoni-Blank, M., Blank, A. F., & Poderoso, E. J. C. M. (2009). Morfologia de flores, frutos e sementes de pau-pombo (*Tapirira guianensis* Aublet. - Anacardiaceae) na região de São Cristóvão, SE, Brasil. *Scientia Forestalis/Forest Sciences*, *37*(81), 47–54.

Scrinis, G. (2008). Functional foods or functionally marketed foods? a critique of, and alternatives to, the category of "functional foods." *Public Health Nutrition*, *11*(5), 541–545. https://doi.org/10.1017/S1368980008001869

Silva, C. V. A. da, Salimo, Z. M., Souza, T. A. de, & Reyes, D. E. (2024). Cupuaçu (*Theobroma grandiflorum*): A Multifunctional Amazonian Fruit with Extensive Benefits. *Food Research International Journal*, *192*(3), 1–17. https://doi.org/10.1016/j.foodres.2024.114729

Silva, J., Hussein, Z., Chiavelli, L., & Santos, O. (2024). Antioxidant Potential Optimization of Mixed Vegetable Juice: A Chemometric Approach. *Journal of the Brazilian Chemical Society*, *35*(9), 1–11. https://doi.org/10.21577/0103-5053.20240060

Sousa, N. S. O., Souza, E. S., Canto, E. S. M., Silva, J. P. A., Carneiro, L. M., & Souza, J. V. B. (2023). Amazonian fermentations : an analysis of industrial and social technology as tools for the development of bioeconomy in the region. *Brazilian Journal of Biology, 2023*, *83*(e276493), 1–9.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*(6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003

Valli, M., Russo, H. M., & Bolzani, V. da S. (2018). The potential contribution of the natural products from Brazilian biodiversity to bioeconomy. *Anais da Academia Brasileira de Ciencias*, *90*(1), 763–778. https://doi.org/10.1590/0001-3765201820170653

Varella, T. L., Rossi, A. A. B., de Souza, M. D. A., da Silveira, G. F., Cochev, J. S., de Toledo, J. J., & da Silva, C. J. (2018). Populational structure and spatial distribution of *Theobroma speciosum* Willd. Ex Spreng in northern of mato grosso state. *Ciência Florestal*, *28*(1), 115–126.

Voglhuber-slavinsky, A., Lemke, N., Macpherson, J., Schöbel, P., Moller, B., Bahrs, E., & Helming, K. (2023). Valorization for Biodiversity and Ecosystem Services in the Agri-Food Value Chain. *Environmental Management*, *72*(1), 1163–1188. https://doi.org/10.1007/s00267-023-01860-7

CAPÍTULO 1

Aumento da bioacessibilidade de compostos bioativos em sucos de *Tapirira guianensis* por meio de aplicações assistidas por ultrassom.

Resumo: Este estudo avaliou o perfil químico e os compostos bioativos em amostras de suco de *Tapirira guianensis* Aubl. submetidas aos efeitos do ultrassom de alta intensidade (US). Os sucos de *T. guianensis* Aubl. foram produzidos e processados utilizando diferentes níveis de energia ultrassônica (0; 0,9; 1,8; 2,7 e 3,6 $kJ \cdot cm^{-3}$) por 10 minutos. As amostras tratadas foram avaliadas quanto à acidez total, cor, pH, compostos fenólicos totais, antocianinas, carotenoides, capacidade antioxidante, digestibilidade in vitro, bem como à quantificação de compostos bioativos por HPLC-DAD. O extrato hidrometanólico obtido de *T. guianensis* Aubl. evidenciou a presença de duas principais categorias de polifenóis, especificamente ácidos galóilquínicos e flavonóis. De modo geral, a tecnologia de US promoveu algumas alterações negativas, como a degradação de carotenoides, mas também alterações positivas no perfil químico das bebidas, como aumento do teor de fenólicos, melhora da capacidade antioxidante e elevação do conteúdo de antocianinas. Os efeitos benéficos foram predominantes, indicando potencial para o desenvolvimento de novas bebidas funcionais a partir desse fruto ainda pouco explorado cientificamente.

Palavras-chave: Anacardiaceae; pau-pombo; compostos fenólicos; ácidos galóilquínicos; flavonóis.

processes

MDPI

Article

Enhancing Bioactive Compound Bioaccessibility in *Tapirira guianensis* Juices through Ultrasound-Assisted Applications

Josiana Moreira Mar [1,2], Renilto Frota Corrêa [2], Andrezza da Silva Ramos [2], Valdely Ferreira Kinupp [3], Edgar Aparecido Sanches [4], Pedro Henrique Campelo [5] and Jaqueline de Araújo Bezerra [1,2,*]

1 Postgraduate Program in Biotechnology, Federal University of Amazonas (UFAM), Manaus 69067-005, Brazil; josimoreira54@gmail.com
2 Analytical Center of the Federal Institute of Education, Science and Technology of Amazonas (IFAM), Campus Manaus Centro, Manaus 69020-120, Brazil; reniltofc@yahoo.com.br (R.F.C.); andrezza.ds.ramos@gmail.com (A.d.S.R.)
3 Federal Institute of Education, Science and Technology of Amazonas (IFAM), Campus Manaus Zona Leste, Manaus 69083-000, Brazil; valdely.kinupp@ifam.edu.br
4 Laboratory of Nanostructured Polymers (NANOPOL), Materials Physics Department, Federal University of Amazonas (UFAM), Manaus 69067-005, Brazil; sanchesufam@ufam.edu.br
5 Department of Food Technology, Federal University of Viçosa (UFV), Viçosa 36570-900, Brazil; pedrocampelo@ufv.br
* Correspondence: jaqueline.araujo@ifam.edu.br

Abstract: This study evaluated the chemical profile and bioactive compounds in *Tapirira guianensis* juice samples under high-intensity ultrasound (US) effects. *T. guianensis* juices were produced and processed using the US with varying ultrasound energy (0, 0.9, 1.8, 2.7, and 3.6 kJ·cm^{-3}) for 10 min. The treated juices were evaluated for total acidity, color, pH, total phenolic compounds, anthocyanins, carotenoids, antioxidant capacity, in vitro digestibility, as well as quantification of bioactive compounds by HPLC-DAD. The hydromethanolic extract obtained from *T. guianensis* exhibited the presence of two major categories of polyphenols, specifically galloylquinic acids and flavonols. Overall, US technology was responsible for inducing some negative changes, such as carotenoid degradation, but also some positive changes in the chemical profile of the beverages, such as increased phenolic content, improved antioxidant capacity, and increased anthocyanin content. However, the beneficial effects were prominent, thus opening opportunities to develop new functional beverages using this fruit with limited scientific studies.

Keywords: Anacardiaceae; "pau-pombo"; compounds phenolics; galloylquinic acids; flavonols

check for updates

Citation: Mar, J.M.; Corrêa, R.F.; Ramos, A.d.S.; Kinupp, V.F.; Sanches, E.A.; Campelo, P.H.; Bezerra, J.d.A. Enhancing Bioactive Compound Bioaccessibility in *Tapirira guianensis* Juices through Ultrasound-Assisted Applications. *Processes* **2023**, *11*, 2718. https://doi.org/10.3390/pr11092718

Academic Editors: Cunshan Zhou, Pavel Mokrejš and Chi-Fai Chau

Received: 6 August 2023
Revised: 2 September 2023
Accepted: 7 September 2023
Published: 12 September 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The family Anacardiaceae encompasses 800 species that span across 81 genera, primarily inhabiting tropical and subtropical regions. The *Tapirira* genus encompasses 35 acknowledged species from southern Mexico to various regions in South America. Among these species, four are exclusive to Brazil: *Tapirira obtusa* (Benth.) J. D. Mitch., *T. pilosa Sprague*, *T. retusa* Ducke, and *T. guianensis* Aubl. Among these indigenous species, *T. guianensis* is particularly prominent, displaying extensive distribution across all Brazilian biomes. Residents commonly refer to it as "pau-pombo" or "peito-de-pombo" [1]. In its composition, the species *T. guianensis* exhibits high antioxidant and nutraceutical potential, with the presence of alkylphenols, alkyl esters, triterpenes, steroids, norisoprenoids, and isolated flavonoids [2].

Although frequently found in the forests of French Guiana, this versatile tree has not been extensively utilized and is primarily consumed for its fruits. The widespread cultivation of *T. guianensis* is currently limited, but emerging prospects for its application, such as the production of colorless cosmetics or dietary supplements, hold the potential to support valuable endeavors [2].

Nutraceutical refers to isolated products from herbal remedies, dietary supplements (nutrients), specific diets, and processed foods. Lately, there has been an increased emphasis on the investigation of various plants, especially those possessing medicinal properties, aimed at discovering new and untapped sources of antioxidants. Among these natural antioxidant agents, phenolic compounds have gained significant prominence due to their association with decreased vulnerability to degenerative conditions, notably cardiovascular diseases and cancer [3]. Extraction is the primary stage in the chemical process and plays a crucial role in recovering, identifying, and purifying bioactive compounds from plant materials [4]. Various techniques have been reported for extracting polyphenolic compounds from different plants. These methods include maceration, Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, and US [2].

Recently, ultrasonication (US) has risen as an alternative to conventional solvent extraction methods. This technique augments the recovery of bioactive substances by amplifying mass transfer and aiding the penetration of solvents into the cellular structure [5]. The process of extracting phenolic compounds from plants is influenced by the chosen extraction method and the solvent used. Selecting the appropriate solvent for extracting polyphenolic compounds is crucial, as it directly influences the amount and variety of phenolic compounds obtained. The growing exploration of more effective solvents for extracting polyphenols from plants can catalyze the application of these inherent antioxidant properties in both the pharmaceutical and food sectors [6].

Phenolic compounds, secondary metabolites highly prevalent in plants, have garnered escalating interest from diverse audiences owing to their potent and varied health-enhancing effects in medicinal applications [7]. Phenolic compounds have been widely employed in direct applications to promote health and prevent diseases, spanning pharmaceuticals and nutraceuticals. Furthermore, they are notable in the food industry, acting as antibacterial agents, flavor enhancers, and color additives. Additionally, phenolics are prominent in cosmetic formulations as antioxidants, skin brighteners, fragrance components, and more [8]. As the extraction process is considered the initial and most important step in the identification, recovery, and isolation of bioactive compounds, it plays a crucial role in the development of biotechnological products with added value [5]. The meticulous choice of the extraction method is imperative to ensure precise and dependable analysis of bioactive compounds and to enhance comprehension of the chemical diversity within *T. guianensis* fruits. As a result, the present study delves into the exploration of ultrasound-assisted extraction (USAE) for bioactive compounds. The primary objectives of this study are as follows: (1) To undertake the chemical characterization of *T. guianensis* species under varying extraction power conditions; (2) To scrutinize the impact of ultrasound-assisted extraction (USAE) on the bioactive compound composition of the fruits, (3) To validate physicochemical analyses, including pH, titratable acidity, and color. Anticipated outcomes of this study encompass contributing insight into extraction techniques at different power levels from *T. guianensis* fruits, ultimately offering valuable insights for developing functional juice formulations.

2. Materials and Methods

2.1. Sample Preparation

The fruits of *Tapirira guianensis* were collected from the Manaquiri region (BR319, Km 150) in the Amazonas state. On 14 December 2022, undamaged and infection-free fruits were individually handpicked and promptly transported to the laboratory. Healthy and consistent fruits, characterized by uniform size and color, were chosen for seed extraction. These fruit samples were rapidly frozen at −18 °C and subjected to lyophilization using an Interprise I lyophilizer from Terroni, Brazil. The resulting lyophilized samples were ground and subsequently sieved through a mesh with an approximate size of 10. The fruits were blended in an industrial blender with a 1:1 (w/w) ratio of water. The juice was stored in glass bottles at −18 °C until the analyses were conducted.

2.2. Ultrasound-Assisted Juice Processing

The *T. guianensis* juices (with a density of 0.5 g/mL) were subjected to treatment at five different power amplitudes: 0%, 20%, 40%, 60%, and 80% (corresponding to ultrasonic energy density levels of 0.0, 0.9, 1.8, 2.7, and 3.6 kJ·cm^{-3}), as outlined in Equation (1). These energy density values were determined through preliminary tests, considering the minimum and maximum nominal power of the equipment (Table 1). During the ultrasonic homogenization process, a 100 mL volume of juice was treated using a 25 mm diameter probe, operating at a frequency of 20 kHz and with a nominal power of 750 W (utilizing the VibraCell VCX 750 equipment from Sonics, Shawnee, OK, USA). The treatment duration was 10 minutes and conducted within an ice bath to prevent potential sample overheating [9].

$$\mathrm{ED}\ (\mathrm{J{\cdot}cm})^{-3} = \frac{\mathrm{NAP} \times \mathrm{t}}{\mathrm{V}} \quad (1)$$

where, ED represents energy density; NAP represents applied nominal power (W); t is the processing time (s), and m V is the sample volume (cm^3).

Table 1. Sample treatment parameter.

Treatment US	Power (W)	Time (min)	Initial Temperature (°C)	Final Temperature (°C)
0%	-	-	-	-
20%	150	10	23.4 ± 0.4	57.9 ± 0.5
40%	300	10	23.8 ± 0.2	58.3 ± 0.3
60%	450	10	23.8 ± 0.2	57.5 ± 0.2
80%	600	10	24.0 ± 0.4	56.5 ± 0.2

2.3. Color Parameters

Color parameters, including lightness (L*), red-green (a*), and yellow-blue (b*), were assessed employing a colorimeter (Delta Color 71421, Delta Vista, Porto Alegre, Brazil). To quantify the alteration in color, the color variation (ΔE*) was computed using Equation (2). The divergence in color parameters (Δ) was defined as the dissimilarity between the attributes of juices treated with ultrasound and those left untreated.

$$\Delta \mathrm{E}^* = \sqrt[2]{\Delta \mathrm{L}^{*2} + \Delta \mathrm{a}^{*2} + \Delta \mathrm{b}^{*2}} \quad (2)$$

where, ΔE* = Color variation.

2.4. pH and Titratable Acidity

The pH assessment of the ultrasound-treated juices was conducted using a digital pH meter (K39-0014PA Kasvi, Xangai, China) at ambient temperature. Titratable acidity was determined through titration and expressed in grams of citric acid per 100 mL of juice [9].

2.5. Antioxidant Capacity

The assays were performed as described previously [10]. The assessment of the radical scavenging capacity resulting from various treatment processes of *T. guianensis* juices was carried out employing the DPPH• radical method. A 100 µM portion of methanolic DPPH• solution was prepared. Subsequently, the sample was prepared at 1 mg/mL and mixed with 1900 µL of the methanolic DPPH• radical solution. For comparison, Trolox was utilized as a positive control (ranging from 100 to 2000 µM). The mixture was then kept in darkness at room temperature for 30 min. Absorbance readings were taken at 515 nm using a Microplate Reader (Epoch 2, BioTek, Winooski, VT, USA). The antioxidant capacity was quantified in Trolox equivalents. The assay was conducted in triplicate. The relationship

was determined as y = −0.0004x + 0.7349, with an R^2 value of 0.9969, and the results were expressed in terms of micromolar Trolox Equivalents (μM Trolox/mL).

The $ABTS^{\bullet+}$ scavenging assay involves measuring the discoloration of the $ABTS^{\bullet+}$ solution in the presence of antioxidant extracts. After a reaction time of 6 min of the sample with the radical at a ratio of 1:10, absorbances were measured at 750 nm using a microplate reader. The standard curve was generated using Trolox (y = 0.0003x + 0.7483, R^2 = 0.9987), and the results were expressed in micromolar Trolox Equivalents (μM Trolox/mL).

The Ferric Reducing/Antioxidant Power Assay (FRAP assay) involves the evaluation of the extract's capacity to reduce Fe^{3+} to Fe^{2+} in the presence of a ferric-tripyridyltriazine complex. Following a 30-min interaction between the sample and the FRAP reagent at 37 °C, the absorbance was gauged using a UV spectrophotometer at 593 nm. A standard curve was constructed using $FeSO_4$ (with the relationship y = 0.0009x + 0.1664 and an R^2 of 0.9990). The outcomes were presented in terms of micromolar of Fe (II) equivalents (μM Fe(II)/mL) per gram of the sample.

The β-carotene mixture was composed of linoleic acid (20 μL), Tween 40 (200 μL), a β-carotene/chloroform solution (25 μL; 2 mg/mL), and chloroform (500 μL). Subsequently, the chloroform was evaporated under nitrogen conditions. Next, 25 mL of oxygen-saturated water was introduced and vigorously shaken. The resultant reaction mixture displayed absorbance values ranging from 0.6 to 0.7 at 470 nm (with the relationship y = −0.0003x + 0.8011 and an R^2 of 0.994). For the microplate experiment, 250 μL of this reaction mixture was combined with 10 μL of methanol (as a control), or the equivalent volume was added to *T. guianensis* juice samples, and the mixture was incubated at 45 °C. Absorbance readings were taken immediately and subsequently at 15-min intervals over 120 min. The outcomes were conveyed as the percentage inhibition of β-carotene.

2.6. Phenolics Compounds

The quantification of total phenolic compounds was performed using the Folin-Ciocalteu reagent. The reaction between the sample and the reagent was incubated for 5 min, and after that, sodium bicarbonate (6%) was added and incubated for an additional 90 min. Subsequently, the data interpretation was carried out using a microplate reader at 750 nm. Gallic acid was used as the standard (y = 0.0026x + 0.2738, R^2 = 0.9986). Results were expressed as mg of gallic acid equivalents for g of juice (mg GAE/g) [10].

2.7. Total Anthocyanin Content

The method employed to determine the total anthocyanin content in the species *T. guianensis* was based on the specific pH spectrophotometric approach. This method quantifies the total anthocyanin content expressed in milligrams of anthocyanins per 100 g sample. The Extinction Coefficient followed the methodology of adopting a value of 982 for the unique pH (pH 2.0). The absorbances for this method were evaluated using a microplate reader (BioTek, Elx800), with readings taken at a wavelength of 535 nm [11].

2.8. Carotenoid Content

The quantification of carotenoid content in *T. guianensis* juices was conducted through the spectrophotometric method. In a concise summary, 1 mL of *T. guianensis* juice was combined with 6 mL of distilled water and 5 mL of hexane, followed by vigorous vortexing for 1 min. The resulting supernatant (hexane phase), which contained the lipid fraction, was analysed at 452 nm using a Microplate Reader (BioTek, Elx800) with hexane as the reference. The outcomes were presented in terms of carotenoid content, determined by referring to a calibration curve (y = 0.0045x − 0.0011, R^2 = 0.9998) that had been previously constructed using a β-carotene standard [11].

2.9. Digestibility Assay

To assess the impact of ultrasonic treatment on the bioactive compound bioaccessibility in *T. guianensis* juices, an in vitro digestibility simulation was performed according to the

INFOGEST protocol, with the oral phase excluded. The concentration of antioxidants was analyzed following the digestion step.

2.10. Quantification of Bioactive Compounds by HPLC-DAD

The methodology for quantitative analysis of polyphenols entails the determination of response factors for standard polyphenol derivatives employing a gradient elution system. These relative response factors facilitate polyphenol analysis using only a single reference standard per class.

Each sample (500 µL) was homogenated with 500 µL methanol HPLC grade and filtered using FPTE filter 0.45um. Standard of pyrogallol, Gallic acid, Cyanidin, Cyanidin 3-glicoside, Delphinidin 3-glicoside, Epicatechin and Quercetin were purchased from Sigma Aldrich.

A stock solution of each reference standard was dissolved in methanol (1 mg/mL). Working standard solutions were prepared by 0.015–0.5 mg/mL fold dilution of the stock solution with methanol before HPLC analysis.

The mobile phase to polyphenols quantification for HPLC-DAD (Shimadzu Corporation Co., Ltd., Kyoto, Japan) analyses consisted of water (A) and methanol (B) at pH 3 with phosphoric acid. Gradient: 0.0–1.0 min in isocratic mode at 10% (B), 10–40% (B) in 13 min, 40–70% (B) in 6 min, 70–100% (B) in 7 min, followed by elution with 100% methanol for 5 min. A Shimadzu Prominence LC-20AD, equipped with a DGU-20A5 degasser equipped with an SPD-20A (PDA) detector, was employed. The temperature oven was maintained at 30 °C. The Linearity was evaluated by analysis of external standard stock solution from 0.5 to 0.0156 mg/mL (n = 3). The equation parameters (slope and intercept) of each standard curve were used to obtain the sample concentrations. The limits of detection (LOD) and quantitation (LOQ) were calculated from a calibration curve by dividing the standard deviation of the calibration curve by its slope multiplied by 3.3 and 10.0, respectively [12].

Quantification by Relative Response Factor

Quantitative analysis of anthocyanin, flavonol, catechin, gallic acid and pyrogallol derivatives was performed by establishing response factors from quercetin, cyanidin, gallic acid and pyrogallol standard selected as reference [13]. The response factors (RF) for the phenolic derivatives were calculated as a ratio of the concentration in relation to the corresponding area of the standard sample [14]. The relative response factors (RRF) were calculated as the ratio of the RF for each analyte to that of the chosen reference. The quantification of phenolic derivatives content in the sample was carried out according to the following equation:

$$\text{Content } (\%, w/w) = [A_{samp} \times RRF \times Rf \times V_{samp} \times 100]/[W_{samp} \times 1000]$$

where: A_{samp}: area due to the phenolic in the sample (mAU*s); RRF: the average relative response factor of that phenolic derivative to the reference phenolic; Rf: response factor of the phenolic standard [(µg/mL)/mAU*s]; V_{samp}: volume of sample solution (mL); W_{samp}: sample weight (µg).

2.11. Statistical Analysis

A completely randomized experimental design was conducted to evaluate the effect of the US at five levels of energy density (0, 20%, 40%, 60%, and 80%). All results were obtained in triplicate and evaluated using Analysis of Variance (ANOVA). The Duncan's test was applied with a confidence level of 95%.

3. Results and Discussion

3.1. Physicochemical Properties

The color attributes of fruit juice play a crucial role in shaping consumers' perceptions of its overall quality. Furthermore, the attractive hue of the end product can substantially enhance its acceptance levels among consumers.

The color of the juice is primarily dictated by the presence and makeup of anthocyanidins, phenols, and carotenoids within it, which exert a highly beneficial influence on safeguarding probiotic cells [15].

Significant differences were observed among the *T. guianensis* juices produced at different ultrasonic energy densities in terms of ΔE ($p < 0.05$) (Figure 1).

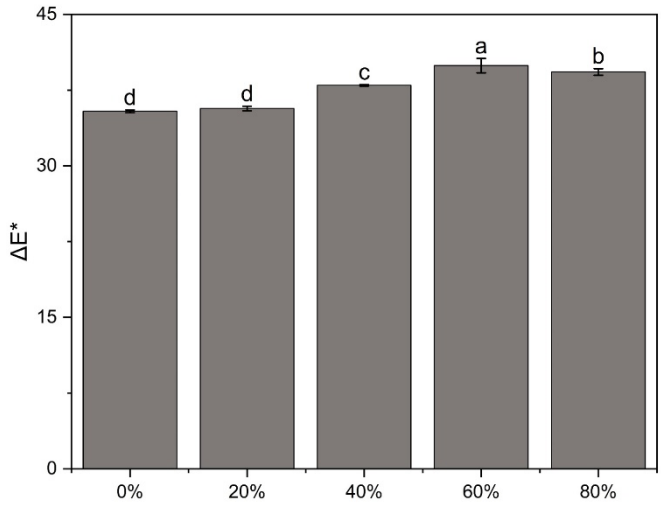


Figure 1. Color parameters for *T. guianensis* juices treated with ultrasound. (a–d) Means with the same letter in the same column are not significantly different according to the Duncan test (p-value > 0.05).

In [16], the L* values were found to vary between 16.4 and 21.9, with the highest values recorded at the 20% mark. This pattern of yellow-orange color is commonly associated with citrus fruit peels, resulting from the breakdown of green chlorophyll and the emergence of the distinctive red-yellow hue.

The a* value, indicating redness, is significant among the color parameters for *T. guianensis* juice. To comprehend the cumulative impacts of the L*, a*, and b* color parameters, ΔE values were computed. The maximum ΔE value was observed at 60% and 80%, primarily due to the elevated b* value. This phenomenon is attributed to the substantial presence of anthocyanins in these samples, as the principal anthocyanin compounds in fruits are recognized for imparting the red color.

Variations in anthocyanin content induced by sonication can be influenced by various factors. Typically, ultrasound treatment enhances the extractive capacity of bioactive compounds, leading to the potential elevation of anthocyanin levels following the exposure of plant tissue to ultrasound waves [15].

Grape juice, rich in anthocyanins, displays a broad spectrum of colors that can be affected by factors including grape ripeness, cultivation practices, and processing methods. Studies suggest that extended sonication treatment (exceeding 2 min) applied to grape juice has the potential to result in a decrease in the L* value [17]. On the other hand, it was discovered that ultrasound treatment during grape must maceration resulted in enhanced grape juice color compared to commercial enzymatic maceration.

Ensuring appropriate pH and acidity levels in food items is crucial, given that these characteristics substantially affect their physical, chemical, and microbial stability. An acidic milieu (with a pH below 4.6) can impede the proliferation of various bacterial strains. Additionally, the degradation of vitamins is influenced by the pH of the processed food [9].

Following the US (ultrasonic-assisted extraction) processing, the pH and total acidity of each sample were measured, revealing no significant differences between the various treatments (Table 2). These findings align with a previous study conducted by [18], which similarly reported no significant shifts in pH and acidity in ultrasonicated "açaí" and "buriti" juices. The consistent pH and acidity levels across the different treatments indicate that these parameters contributed equally to each sample. Consequently, any alterations in the nutritional composition of the beverage cannot be ascribed to variations in pH or acidity.

Table 2. Physicochemical analysis values.

Treatment	pH	Titratable Acidity
0%	4.5 ± 0.2	7.0 ± 0.1
20%	4.7 ± 0.1	7.0 ± 0.1
40%	4.8 ± 0.2	7.5 ± 0.1
60%	4.7 ± 0.2	7.8 ± 0.1
80%	4.8 ± 0.1	7.9 ± 0.2

The observed alterations in the yellow hue of the samples are evident from the decrease in carotenoid content in the analyzed juice samples (as depicted in Figure 2). These outcomes can be attributed to the concept that our perception of color is a psychological response influenced by the visible spectrum emitted or reflected by an object.

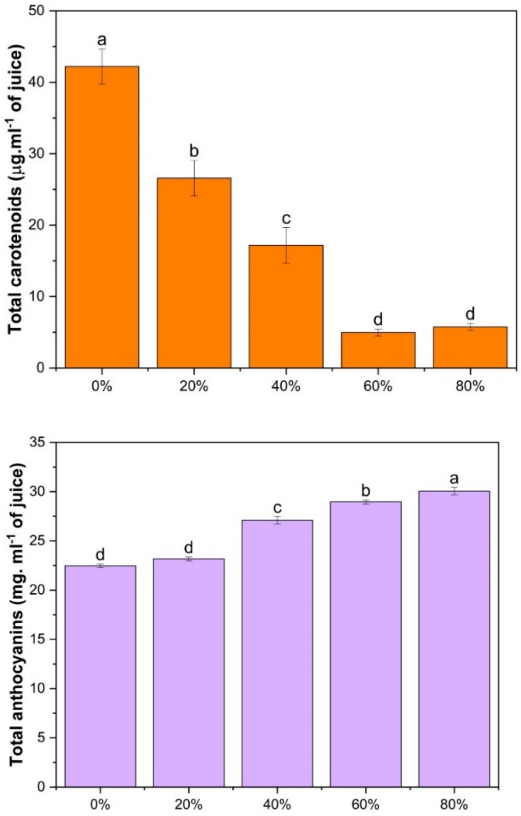


Figure 2. Effect of US energy density on total monomeric anthocyanin and carotenoid content. (a–d) Means with the same letter in the same column are not significantly different according to the Duncan test (p-value > 0.05).

In the context of ultrasound-treated juice, the comprehensive perception of color likely originates from various modifications induced by ultrasound techniques. These encompass potential isomerization reactions of existing anthocyanins, which may be triggered by the formation of free radicals generated during the sonication process [9].

All extracts had negative ΔL* values. The ΔL* value in the color space indicates the blue or yellow hue, ranging from −30 to 30. Negative ΔL* values represent blue. Anthocyanins derived from delphinidin mainly develop shades of blue color. This is apparent from the outcomes of the chemical analysis, which demonstrate a greater prevalence of anthocyanins derived from delphinidin in all the extracts [19].

3.2. Effect of Sonication on Total Carotenoids and Total Anthocyanins

Carotenoids are the pigments responsible for the vibrant colors in fruits and vegetables. They hold significant functions within the human body, serving as provitamin A (in the form of β-carotene), antioxidants, modulators of immune function and carcinogen metabolism, and regulators of cell proliferation and differentiation [15]. Earlier research has established a robust link between the consumption of carotenoids and enhancements in the immune system, along with a diminished susceptibility to numerous cardiovascular diseases, cancer, cataracts, and macular degeneration [20].

The results on the effect of sonication treatments on the total carotenoids of *T. guianensis* juice are presented in Figure 2. A significant reduction (p-value < 0.05) in carotenoid concentration was observed mainly at 60% and 80% (4.9 and 5.7 µg/mL β-carotene equivalents), confirming that high levels of ultrasound treatments can induce chemical decomposition of carotenoids.

The carotenoid levels in the ultrasound-treated Chokanan mango juice [21] and carrot juice [22] decreased according to the results obtained in the present study. This shearing effect of sonication after longer exposure can cause carotenoid isomerization [21], leading to decreased carotenoid levels observed for *T. guianensis* juice.

Upon analyzing the results, it is evident that an increase in ultrasound energy density during juice processing correlates with a rise in total anthocyanin content. The 80% juice sample exhibited the highest number of anthocyanins, reaching 15.05 mg/100 mL. This indicates that sonication power enhances the extraction capacity of anthocyanins. The phenomenon can be explained by the fact that ultrasound treatment facilitates the release of bioactive components from the juice by disrupting and breaking down the material [23]. Moreover, another contributing factor to the increased anthocyanin content is the deactivation of enzymes responsible for anthocyanin degradation by sonication. Specifically, higher-powered sonication contributed to the inhibition of β-D-glucosidase, one of the enzymes primarily responsible for anthocyanin degradation. These enzymes break the covalent bond between the aglycone and the glycosyl residue, resulting in the formation of unstable anthocyanidins. Thus, by deactivating these enzymes, sonication helps preserve the anthocyanins in the juice and prevents their breakdown [15].

3.3. Antioxidant Capacity

Anthocyanins can interrupt oxidative chain reactions by scavenging free radicals, thereby reducing oxidative stress [15]. Given the altered anthocyanin profile after US treatment, an analysis of antioxidant capacity was performed on the samples. In this study, all experimental frequencies contributed positively to antioxidant capacity. As shown in Figure 3, US treatment at 80% exhibited the highest antioxidant potential in the juice. These results demonstrate that the increase in electron-donating antioxidants is important in determining the ultrasound-treated *T. guianensis* juice samples.

Anthocyanins, especially cyanidin and its glycosides, are included in the list of known natural compounds that act as powerful antioxidants. This is due to their unique chemical structure, which is capable of accepting unpaired electrons, such as reactive oxygen/nitrogen species, and inhibiting oxidative stress [24].

Like other "superfruits", Jabuticaba exhibits a potent in vitro antioxidant capacity (DPPH, ABTS). In investigating the effect of ingesting lyophilized extract from *Myrciaria jaboticaba* peels on antioxidant activity in rat plasma, the results revealed an increase in plasma antioxidant capacity (1.7-fold by the TEAC method and 1.3-fold by the ORAC method). Anthocyanins such as cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside appear responsible for the reported effect [25].

The hydroalcoholic extract of *Plinia trunciflora* leaves was evaluated for its antioxidant potential using the FRAP and DPPH methods. It was found to exhibit high activity, primarily due to the presence of cyanidin and cyanidin-3-*O*-glucoside [26].

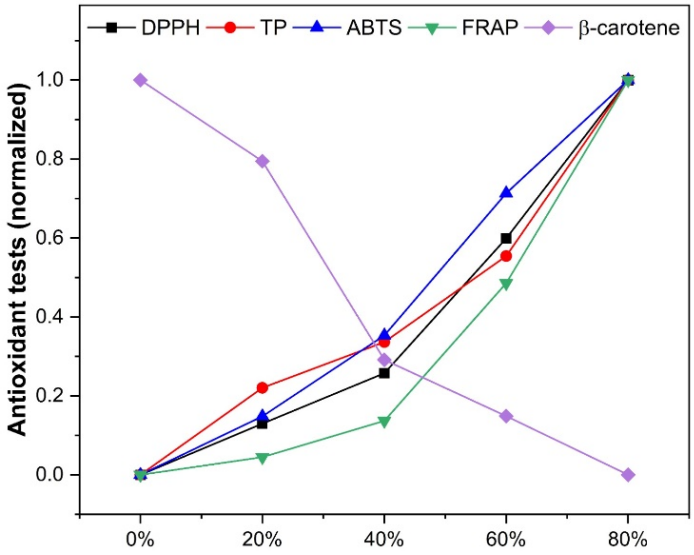


Figure 3. The antioxidant potential of *T. guianensis* juices subjected to high-intensity ultrasound was evaluated through total phenolic compounds, DPPH, ABTS, FRAP, and β-carotene assays.

A positive and significant effect (p-value < 0.05) was observed with the increase in ultrasound energy density. Considering the untreated juice, there was a 41% increase in the content of phenolic compounds (Table 3).

Table 3. Results are expressed as mean ± standard deviations (n = 3).

Treatment	DPPH (μM Trolox/mL)	ABTS (μM Trolox/mL)	FRAP (μM Fe(II)/mL)	Total Phenolics (mg GAE/g)	β-Carotene (% Inhibition of β-Carotene)
0%	1003.9 ± 5.2 ^{e}	1274.3 ± 6.7 ^{e}	1168.2 ± 4.0 ^{e}	466.9 ± 0.2 ^{e}	78.6 ± 1.8 ^{a}
20%	1051.4 ± 8.8 ^{d}	1373.2 ± 0.2 ^{d}	1190.1 ± 4.2 ^{d}	509.3 ± 0.1 ^{d}	66.8 ± 0.3 ^{b}
40%	1098.1 ± 2.9 ^{c}	1509.9 ± 8.4 ^{c}	1234.9 ± 2.2 ^{c}	531.6 ± 0.2 ^{c}	37.9 ± 1.4 ^{c}
60%	1223.1 ± 6.3 ^{b}	1749.9 ± 7.7 ^{b}	1404.9 ± 1.1 ^{b}	573.5 ± 0.3 ^{b}	29.8 ± 2.8 ^{d}
80%	1369.7 ± 6.6 ^{a}	1941.0 ± 12.0 ^{a}	1655.6 ± 4.2 ^{a}	659.3 ± 0.2 ^{a}	21.2 ± 2.9 ^{e}

(a–e) Means with the same letter in the same column are not significantly different.

The results regarding the effects of treatments with *T. guianensis* juice on bioactive compounds are displayed in Table 3. It was observed that ultrasound significantly affected all bioactive compounds. The lowest losses were exhibited in US80% (659.3 ± 0.2) and US60% (573.5 ± 0.3), followed by US40% (531.6 ± 0.2), US20% (509.3 ± 0.1), and US0% (466.9 ± 0.2), respectively.

Physalis minima Linn demonstrated that the leaf extract exhibited the highest total phenolic content value of 1125.42 ± 14.60 mg of gallic acid equivalent (GAE) per gram of plant (dry extract) [27]. Ultrasound-assisted extraction (UAE), utilizing aqueous ethanol as the solvent, was applied for the first time to extract phenolic compounds from *Terminalia chebula* Retz. fruits, where the total phenols yield was 448.7 ± 2.15 mg GAE/g DW, in agreement with the anticipated value (447.8 mg GAE/g DW) [28]. The pulp of the tropical fruit araticum (*Annona crassiflora*) exhibited a total phenolic yield of 433.80 mg GAE/g [29].

Phenolic compounds are typically associated with the cell wall, such as pectin, cellulose, hemicellulose, and lignin residues. Undergoing ultrasound (US), processing can enhance mass transfer rates and create microcavities that may disrupt the cell wall, resulting in the liberation of antioxidant content [9]. Furthermore, augmenting the power during the cavitation process intensifies the breakdown of the cell wall, leading to the generation of

hydroxyl radicals. These radicals play a pivotal role in the hydroxylation of the aromatic ring of phenolic compounds at the ortho, meta, and para positions [30].

The application of the US for extraction purposes has been extensively discussed in the literature. For instance, Ref. [17] demonstrated that using ultrasound at 35 kHz with an ultrasonic bath significantly enhanced the extraction of total phenolic compounds and antioxidant capacity from red grape peel extracts compared to conventional extraction methods (with mechanical agitation, without specified conditions). The ultrasound-assisted approach achieved concentrations 1.8 and 1.6 times higher, respectively. Similarly, Ref. [31] observed a notable increase of 30 to 40% in the total phenolic content and antioxidant capacity of orange peel extracts when employing ultrasound at 25 kHz with 90 W power, using an ultrasonic transducer plate.

A novel study [32] assessed the contrast between ultrasound-assisted extraction (performed using a 25 kHz frequency and 150 W power in an ultrasonic bath) and conventional extraction (employing mechanical agitation without specific conditions) for extracting apple pomace extracts. The researchers observed concentrations 1.2 times higher for phenolic content, 1.4 times higher for proanthocyanidins, and 1.3 times higher for flavan-3-ols in the ultrasound-assisted extraction experiments.

Ref. [33] described a significant effect of ultrasound ($\approx 260 \pm 6$ W/L, with a 3.8 cm^2 surface area ultrasonic probe) on the extraction rate of total phenolic compounds from olive leaves, with a value of 1.9 times higher than conventional extraction (with mechanical agitation, 170 rpm).

3.4. Quantification of Bioactive Compounds by HPLC-DAD

The quantification was carried out using the integration areas of the peaks of the molecules and by interpolation in the calibration curve constructed with a solution of analytical standards similar to each phenolic compound quantified. The identification of different phenolic compounds in this study makes it relevant since the presence of these compounds has a range of bioactivities, as already reported in previous studies.

The total polyphenol content of *T. guianensis* (TG) tea leaf samples was measured in 100% methanol, 75% methanol, 50% methanol, and water extracts, with values of 78.98 ± 0.43 mg GAE/g DW, 29.6 ± 0.97 mg GAE/g DW, 50.72 ± 1.99 mg GAE/g DW, and 56.38 ± 2.72 mg GAE/g DW, respectively [34].

In a recent study, extracts of phenolic compounds with ultrasound revealed that this treatment can enhance the extraction of polyphenols from fruits [35]. The extraction of thermostable bioactive compounds such as gallic acid derivatives can be enhanced up to a certain temperature level. When there is an increase in temperature, the contents tend to decrease. On the other hand, thermolabile molecules such as flavonoids have a disadvantage when there is an increase in US power and temperature [36,37].

The hydromethanolic extract obtained from *T. guianensis* indicated the existence of two primary categories of polyphenols: galloylquinic acids and flavonols. Nonetheless, for an accurate identification of the sugar's composition and the positioning of the flavonoid aglycone and galloylquinic acids, additional validation and structural clarification of the polyphenolics are necessary. These can be accomplished through techniques such as ^{13}C and ^{1}H NMR [34].

The identification and matching of peaks related to phenolic compounds in *T. guianensis* juice samples were carried out by comparing their retention times and absorption spectra with those of established standards (Table 4; Figure 4). A total of thirty-three phenolic compounds were detected in the samples. The flavonol profile of *Tapirira* is complex, with the presence of twelve flavonol derivatives displaying intense signals. Regarding anthocyanins, they were tentatively identified as cyanidin, cyanidin-3-glucoside, and derivatives based on their profiles compared to standard compounds.

Table 4. HPLC-DAD data to characterization and quantification of phenolic compounds from *T. guianensis* using their spectral characteristics in HPLC-DAD by retention time and absorption spectrum (λ max).

RT (min)	Compound	λ (nm)	Tg0 (μg/L ± SD)	Tg20 (μg/L ± SD)	Tg40 (μg/L ± SD)	Tg60 (μg/L ± SD)	Tg80 (μg/L ± SD)
3.40	Py_D	271	131.61 ± 22.96 ^{cd}	112.51 ± 4.92 ^{c}	15.05 ± 2.00 ^{b}	7.49 ± 0.17 ^{a}	7.13 ± 0.95 ^{a}
5.56	Py_D	269	262.44 ± 0.00 ^{c}	259.67 ± 36.73 ^{c}	58.16 ± 2.81 ^{a}	185.69 ± 82.07 ^{b}	344.98 ± 7.19 ^{d}
7.00	Pyrogallol	266	305.47 ± 23.22 ^{d}	299.14 ± 31.08 ^{d}	58.29 ± 2.03 ^{c}	41.28 ± 0.49 ^{b}	37.51 ± 1.65 ^{a}
7.19	Py_D	271	283.59 ± 30.55 ^{a}	970.90 ± 46.60 ^{c}	445.79 ± 62.41 ^{b}	391.60 ± 94.42 ^{b}	400.38 ± 59.61 ^{b}
7.69	Gallic acid	269	155.15 ± 20.64 ^{c}	252.33 ± 98.24 ^{d}	79.49 ± 1.73 ^{b}	57.63 ± 5.35 ^{a}	397.76 ± 32.28 ^{e}
8.24	G_D	266	162.13 ± 11.55 ^{c}	171.18 ± 3.45 ^{c}	68.18 ± 5.24 ^{b}	36.00 ± 3.31 ^{a}	213.78 ± 23.98 ^{d}
8.79	G_D	266	279.79 ± 54.56 ^{c}	121.30 ± 33.36 ^{a}	255.88 ± 6.47 ^{c}	201.26 ± 7.33 ^{b}	381.02 ± 37.53 ^{d}
9.30	G_D	277	147.91 ± 22.54 ^{c}	186.01 ± 19.33 ^{d}	126.18 ± 2.90 ^{b}	97.69 ± 8.37 ^{a}	127.99 ± 21.63 ^{b}
10.51	G_D	524	297.19 ± 12.00 ^{b}	296.38 ± 19.52 ^{b}	253.04 ± 10.12 ^{a}	267.44 ± 23.28 ^{a}	354.20 ± 32.39 ^{c}
11.28	Cy_D	524	206.80 ± 26.98 ^{b}	248.32 ± 1.04 ^{c}	275.16 ± 65.35 ^{b}	167.94 ± 38.66 ^{a}	189.03 ± 28.47 ^{a}
11.84	Cy_D	510	114.81 ± 4.42 ^{b}	112.07 ± 1.74 ^{a}	132.78 ± 9.13 ^{c}	133.78 ± 27.80 ^{d}	137.80 ± 1.05 ^{e}
12.15	Cy_D	361	84.09 ± 44.08 ^{a}	117.24 ± 17.65 ^{b}	118.00 ± 10.64 ^{b}	224.39 ± 98.67 ^{c}	252.93 ± 29.21 ^{d}
12.49	F_D	514	255.28 ± 14.72 ^{b}	244.24 ± 32.68 ^{b}	308.06 ± 57.02 ^{cd}	93.61 ± 3.43 ^{a}	299.62 ± 36.59 ^{c}
12.55	Cyanidin	356	6.38 ± 0.35 ^{a}	184.73 ± 11.76 ^{d}	11.56 ± 0.01 ^{b}	108.95 ± 11.92 ^{c}	178.72 ± 27.81 ^{d}
12.89	F_D	354	342.35 ± 13.01 ^{a}	351.02 ± 25.50^{a}	374.17 ± 2.95 ^{a}	320.11 ± 24.87 ^{a}	503.43 ± 70.09 ^{b}
13.29	Cy_D	514	36.45 ± 1.76 ^{a}	38.83 ± 3.60 ^{a}	64.82 ± 15.70 ^{c}	52.93 ± 4.15 ^{b}	65.44 ± 1.86 ^{d}
13.37	Delphinidin 3-glucoside	354	71.44 ± 0.00 ^{a}	72.08 ± 3.01 ^{a}	134.07 ± 5.07 ^{b}	<LOD	<LOD
13.47	F_D	524	401.85 ± 24.74 ^{c}	429.96 ± 44.34 ^{d}	243.77 ± 61.69 ^{b}	227.63 ± 17.70 ^{b}	197.29 ± 1.19 ^{a}
13.75	Cy_D	524	0.45 ± 0.00 ^{a}	0.59 ± 0.12 ^{a}	181.27 ± 0.13 ^{b}	228.76 ± 42.64 ^{d}	208.86 ± 7.59 ^{c}
14.52	Cy_D	351	0.33 ± 0.00 ^{a}	0.46 ± 0.05 ^{b}	0.60 ± 0.02 ^{c}	0.40 ± 0.03 ^{b}	<LOD
15.73	Cth_D	300	4.68 ± 0.86 ^{a}	11.73 ± 2.02 ^{b}	281.92 ± 57.42 ^{e}	64.31 ± 5.19 ^{c}	95.59 ± 2.35 ^{d}
16.32	Cth_D	300	30.33 ± 4.04 ^{b}	25.53 ± 0.00^{a}	44.52 ± 8.48 ^{c}	25.52 ± 0.02 ^{a}	66.13 ± 0.08 ^{d}
16.53	Cth_D	368	36.12 ± 5.12 ^{a}	76.14 ± 1.14 ^{c}	448.44 ± 18.14 ^{d}	39.23 ± 0.27 ^{a}	58.92 ± 5.02 ^{b}
18.24	F_D	357	6.61 ± 0.09 ^{a}	8.39 ± 0.84 ^{b}	12.74 ± 0.65 ^{c}	11.79 ± 0.42 ^{c}	11.27 ± 1.13 ^{c}
19.48	F_D	360	134.52 ± 5.91 ^{a}	150.60 ± 14.98 ^{b}	208.32 ± 9.97 ^{d}	208.50 ± 14.59 ^{d}	205.83 ± 15.07 ^{c}
19.78	F_D	357	25.25 ± 1.04 ^{a}	29.91 ± 3.62 ^{b}	42.68 ± 2.04 ^{c}	42.75 ± 2.58 ^{c}	42.28 ± 3.75 ^{c}
20.12	F_D	366	2606.37 ± 60.95 ^{c}	2357.27 ± 184.14 ^{b}	1680.91 ± 38.82 ^{a}	1717.23 ± 51.18 ^{a}	1791.89 ± 75.83 ^{a}
20.60	F_D	361	21.93 ± 2.43 ^{a}	26.81 ± 2.95 ^{b}	41.61 ± 2.11 ^{cd}	40.32 ± 2.60 ^{cd}	39.89 ± 2.01 ^{c}
20.94	F_D	366	17.08 ± 1.07 ^{a}	22.06 ± 2.30 ^{b}	33.49 ± 1.76 ^{de}	33.06 ± 1.30 ^{c}	32.42 ± 2.26 ^{d}
22.34	F_D	366	21.23 ± 0.69 ^{a}	22.67 ± 1.71^{b}	33.92 ± 1.15 ^{c}	55.33 ± 3.98 ^{d}	62.15 ± 3.84 ^{e}
22.71	F_D	371	8.05 ± 0.10 ^{a}	9.09 ± 0.58 ^{b}	12.40 ± 0.06 ^{c}	21.05 ± 0.54 ^{d}	24.99 ± 0.72 ^{e}

RT = retention time; Cy_D = Cyanidin derivative; F_D = Flavonol derivative; Cth_D = Catechin derivative; G_D = Gallic acid derivative; Py_D = Pyrogallol derivative; ANOVA: minimal detectable difference = 117.57; F > Fcritical; Tukey: confidence = 95%; Means with the same letter in the same line are not significantly different. % RSD values = <16–30>; R^2 = <0.997–0.999>.

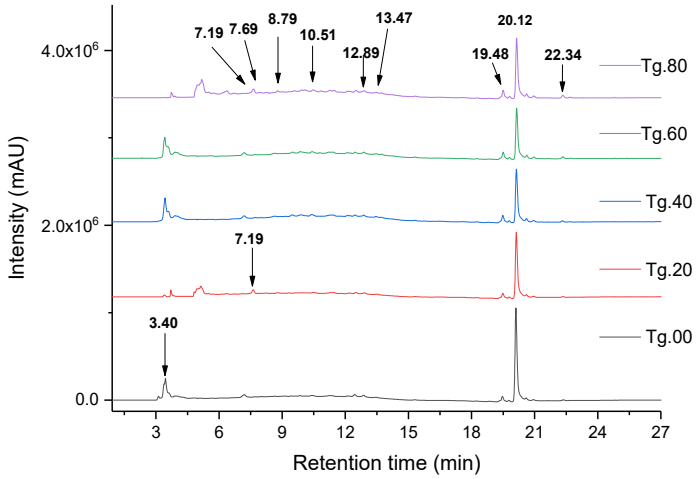


Figure 4. HPLC chromatograms of polyphenols in *Tapirira guianensis* juices. Peak assignment in Table 1.

The polyphenolic value in the samples was significantly different from each other (ANOVA: minimal detectable difference = 117.57; $F > F_{critical}$; Tukey: confidence = 95%). The increase in the process of US value did not favor the extraction of pyrogallol or pyrogallol derivatives. The extractive content of gallic acid increased as the 'process' increased, except to 40, 60 and 80. The process with 'US80' was more efficient to extract the derivatives of Gallic acid.

The stability of gallic acid was observed to be impacted by a range of ultrasonic parameters, encompassing factors like solvent type and concentration, ultrasonic power, frequency, temperature, and extraction duration in the simulated extraction setup. These results underline the importance of not only assessing extraction yield but also accounting for potential phenolic compound degradation during ultrasonic polyphenol extraction. In the ultrasonic extraction sector, achieving optimum outcomes necessitates meticulous optimization of extraction parameters to enhance yield while mitigating the risk of phenolic compound degradation induced by ultrasound [38].

Process variation was not crucial to improving the extraction of some cyanidin derivatives. The Delphinidin 3-glucoside content was below the limit of detection to process in 60 and 80. However, process 40 was more efficient to increase the yield. For flavonol at 13.47 min, as the process value is decreased, the efficiency of the extraction process increases only to 80. In turn, the flavonoid of 12.89 had its yield increased by 80. Figure 5 shows that process 40 was efficient for extracting catechin derivatives, and processes 20, 40 and 60 were more efficient for glycosylated cyanidin derivatives.

An initial exploration was undertaken to analyze the kinetics of extracting overall polyphenols and their fractions to optimize extraction duration through sonication. Methanol was chosen as the preferred solvent owing to its efficiency in extracting catechin, epicatechin, and epigallocatechin [30].

When subjected to various processing methods, the concentrations of delphinidin and petunidin exhibited a decline. Among the techniques used, ultrasound treatment showed the least impact, resulting in reductions of delphinidin by 52.56% and petunidin by 73.82% in just 1 min [39]. Ref. [40] also found that anthocyanin structure impacted their thermal stability. Moreover, the microwave treatment had the most influence on the degradations of delphinidin and petunidin, while the ultrasonic treatment had the lowest impact.

When the degradation kinetics of cyanidin-3-glucosylrutinoside before and after exposure to ultrasonic treatment were compared, Ref. [41] found that the rate constant k increased and the half-life $t_{1/2}$ decreased with increasing ultrasonic power. In addition, the greatest degree of degradation of cyanidin-3-glucosylrutinoside was 44.2% and was obtained at 600 W for 60 min.

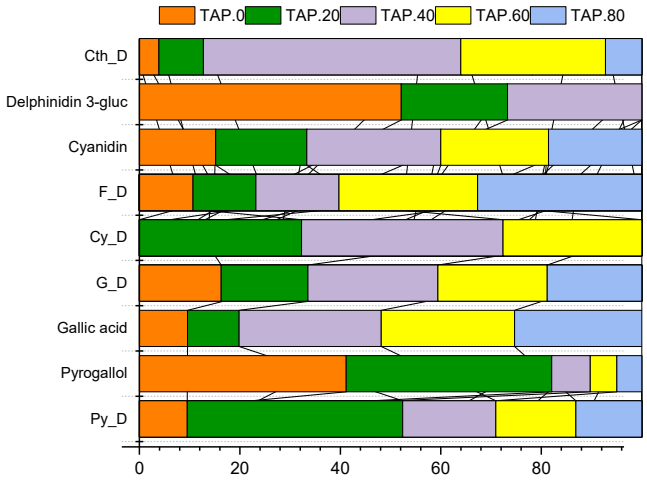


Figure 5. The graphic content of phenolic compounds (Cth_D = Catechin derivative; F_D = Flavonol derivative; Cy_D = Cyanidin derivative; G_D = Gallic acid derivative; Py_D = Pyrogallol derivative) in each experiment of the ultrasound method (TAP = *Tapirira* juice and the subsequent numbers represent ultrasound power or time).

The diverse nature of flavonols led to varied responses in different processes concerning the percentage of extraction of these flavonoids. Among the processes studied, processes 20 and 80 demonstrated the highest extractive potential overall.

Regarding the extraction of phenolic compounds from grape seeds defatted by US and solvent (S) using maceration (M) and (US), a T-Test analysis showed significant effects of maceration on grape seeds defatted by ultrasound (US-M). Specifically, this process had a significant impact on the content of total polyphenols, total tannins, total anthocyanins, cinnamic acids, flavonols, and antioxidant activity ($p < 0.05$) [30].

The utilization of ultrasound extraction significantly boosted the bioactive component content and overall extraction efficiency. Notably, the duration and power amplitude of ultrasound treatment exerted a noteworthy influence on the content of specific bioactive compounds, underscoring their crucial role in the extraction process [23].

4. Conclusions

Based on the findings of the current study, the utilization of ultrasound (US) technology brought about notable modifications in the nutritional composition of *T. guianensis* juices. Some observed changes were negative (such as β-carotene degradation), while others were positive and beneficial (including increased phenolic content, enhanced antioxidant activity, and elevated anthocyanin levels). Notably, ultrasound power emerged as a crucial determinant of these nutritional alterations. The treatment employing the maximum power (US 80%) seemed to be the optimal approach to mitigate the adverse effects of excessive ultrasound processing.

Furthermore, the content of extracted gallic acid displayed an increase with the progression of the process duration, except for the 40, 60, and 80 durations. The 'US80' extraction process showcased enhanced efficiency in extracting gallic acid derivatives. A pivotal contribution of this study lies in the identification and quantification of over 30 phenolic compound derivatives within *T. guianensis* juice sourced from the Amazon fruit cultivar, utilizing High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD).

As a result, this research offers a method to enhance the extraction of bioactive compounds previously identified in earlier studies through the application of ultrasound. Nonetheless, further investigations are warranted, with a particular focus on mitigating potential unfavorable ultrasound effects, alongside conducting sensory analyses involving consumers.

Author Contributions: J.M.M.: Data curation, Formal analysis, Investigation, Methodology, Writing—original draft. R.F.C.: Formal analysis, Writing—review & editing. A.d.S.R.: Formal analysis, Investigation, Methodology, Writing—original draft. V.F.K.: Resources. E.A.S.: Investigation, Methodology, Resources, Writing—review & editing. P.H.C.: Investigation, Methodology, Writing—review & editing. J.d.A.B.: Conceptualization, Data curation, Project administration, Resources, Supervision, Writing-review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FAPEAM (Fundação de Amparo à Pesquisa do Estado do Amazonas, Process number 01.02.016301.03199/2021-02–PROSPAM/FAPEAM, Process number 01.02.016301.03335/2021-56, Edital n. 010/2021-CT&I áreas prioritárias, Edital n. 013/2022–Produtividade-CT&I), Support Program for Strict Sensu Graduate Graduation—Posgrad UFAM/FAPEAM and CNPq (Conselho Nacional de Desenvolvi- mento Científico e Tecnológico, grant number 311522/2020-3) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Data Availability Statement: Data sharing is not applicable.

Acknowledgments: The authors wish to acknowledge to Fundação de Amparo à Pesquisa do Estado do Amazonas—FAPEAM (Process number 01.02.016301.03199/2021-02–PROSPAM/FAPEAM, Process number 01.02.016301.03335/2021-56, Edital n. 010/2021—CT&I áreas prioritárias, Edital n. 013/2022–Produtividade-CT&I), Support Program for Strict Sensu Graduate Graduation—Posgrad UFAM/FAPEAM, Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPq (Process number 311522/2020-3) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for financial support, fellowships and Analytical Centers at IFAM-CMC and UFAM for infrastructure.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Da Silva, E.P.; David, J.M.; David, J.P.; Trindade Garcia, G.H.; Silva, M.T. Chemical Composition of Biological Active Extracts of *Tapirira guianensis* (Anacardiaceae). *Quím. Nova* **2020**, *43*, 1216–1219. [CrossRef]
2. Vural, N.; Algan, Ö.; Abdülkadir, C.M.; Ertan, A.R. Determination of the Various Extraction Solvent Effects on Polyphenolic Profile and Antioxidant Activities of Selected Tea Samples by Chemometric Approach. *J. Food Meas. Charact.* **2020**, *14*, 1286–1305. [CrossRef]
3. Diem, Q.; Elisa, A.; Tran-nguyen, P.L. ScienceDirect Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Limnophila aromatica*. *J. Food Drug Anal.* **2014**, *22*, 296–302. [CrossRef]
4. Kyung, I.; Mi, H.; Hee, M.; Ho, D.; Jin, H. Simultaneous Determination of 15 Phenolic Compounds and Caffeine in Teas and Mate Using RP-HPLC/UV Detection: Method Development and Optimization of Extraction Process. *Food Chem.* **2015**, *172*, 469–475. [CrossRef]
5. Kumar, K.; Srivastav, S.; Singh, V. Ultrasound Assisted Extraction (UAE) of Bioactive Compounds from Fruit and Vegetable Processing by-Products: A Review. *Ultrason. Sonochem.* **2021**, *70*, 105325. [CrossRef]
6. Peixoto, L.S.; Freitas, R.M.; Gomes, J.; Ferreira, T.R.L. Composto Bioativos Do Bioma Do Pantanal Uma Revisão (Bioactive Compounds from the Pantanal Biome: A Review). *Res. Soc. Dev.* **2022**, *11*, e348111335554. [CrossRef]
7. Panwar, D.; Saini, A.; Panesar, P.S.; Chopra, H.K. Unraveling the Scientific Perspectives of Citrus By-Products Utilization: Progress towards Circular Economy. *Trends Food Sci. Technol.* **2021**, *111*, 549–562. [CrossRef]
8. Guimarães, J.T.; Silva, E.K.; Ranadheera, C.S.; Moraes, J.; Raices, R.S.L.; Silva, M.C.; Ferreira, M.S.; Freitas, M.Q.; Meireles, M.A.A.; Cruz, A.G. Effect of High-Intensity Ultrasound on the Nutritional Profile and Volatile Compounds of a Prebiotic Soursop Whey Beverage. *Ultrason. Sonochem.* **2019**, *55*, 157–164. [CrossRef]
9. Ordóñez-santos, L.E.; Martínez-girón, J.; Arias-jaramillo, M.E. Effect of Ultrasound Treatment on Visual Color, Vitamin C, Total Phenols, and Carotenoids Content in Cape Gooseberry Juice. *Food Chem.* **2017**, *233*, 96–100. [CrossRef]
10. Moreira, J.; Souza, L.; Picanço, W.; Bezerra, A. Edible Flowers from *Theobroma speciosum*: Aqueous Extract Rich in Antioxidant Compounds. *Food Chem.* **2021**, *356*, 129723. [CrossRef]
11. Oliveira, A.F.A.; Mar, J.M.; Santos, S.F.; da Silva Júnior, J.L.; Kluczkovski, A.M.; Bakry, A.M.; Bezerra, J.d.A.; Nunomura, R.d.C.S.; Sanches, E.A.; Campelo, P.H. Non-Thermal Combined Treatments in the Processing of Açai (*Euterpe oleracea*) Juice. *Food Chem.* **2018**, *265*, 57–63. [CrossRef] [PubMed]
12. Ramos, A.S.; Souza, R.O.S.; Paula, A.; Boleti, D.A.; Bruginski, E.R.D.; Lima, E.S.; Campos, F.R.; Machado, M.B. Chemical Characterization and Antioxidant Capacity of the Araçá-Pera (*Psidium acutangulum*): An Exotic Amazon Fruit. *Food Res. Int. J.* **2015**, *75*, 315–327. [CrossRef] [PubMed]
13. Barbosa Lima, L.G.; Montenegro, J.; De Abreu, J.P.; Barros Santos, M.C.; Pimenta do Nascimento, T.; Da Silva Santos, M.; Ferreira, A.G.; Cameron, L.C.; Larraz Ferreira, M.S.; Teodoro, A.J. Metabolite Profiling by UPLC-MSE, NMR, and Antioxidant Properties of Amazonian Fruits: Mamey Apple (*Mammea americana*), Camapu (*Physalis angulata*), and Uxi (*Endopleura uchi*) Larissa. *Mol. Nutr. Food Res.* **2000**, *25*, 342.

14. Wang, H.; Provan, G.J.; Helliwell, K. HPLC Determination of Catechins in Tea Leaves and Tea Extracts Using Relative Response Factors. *Food Chem.* **2003**, *81*, 307–312. [CrossRef]
15. Wang, J.; Xie, B.; Sun, Z. Quality Parameters and Bioactive Compound Bioaccessibility Changes in Probiotics Fermented Mango Juice Using Ultraviolet-Assisted Ultrasonic Pre-Treatment during Cold Storage. *LWT* **2021**, *137*, 110438. [CrossRef]
16. Anticona, M.; Blesa, J.; Lopez-Malo, D.; Frigola, A.; Esteve, M.J. Effects of Ultrasound-Assisted Extraction on Physicochemical Properties, Bioactive Compounds, and Antioxidant Capacity for the Valorization of Hybrid Mandarin Peels. *Food Biosci.* **2021**, *42*, 101185. [CrossRef]
17. Corrales, M.; Fernández, A.; Butz, P.; Tauscher, B. Extraction of Anthocyanins from Grape Skins Assisted by High Hydrostatic Pressure. *J. Food Eng.* **2009**, *90*, 415–421. [CrossRef]
18. De Souza Carvalho, L.M.; Lemos, M.C.M.; Sanches, E.A.; da Silva, L.S.; de Araújo Bezerra, J.; Aguiar, J.P.L.; das Chagas do Amaral Souza, F.; Alves Filho, E.G.; Campelo, P.H. Improvement of the Bioaccessibility of Bioactive Compounds from Amazon Fruits Treated Using High Energy Ultrasound. *Ultrason. Sonochem.* **2020**, *67*, 105148. [CrossRef]
19. Vidana Gamage, G.C.; Choo, W.S. Hot Water Extraction, Ultrasound, Microwave and Pectinase-Assisted Extraction of Anthocyanins from Blue Pea Flower. *Food Chem. Adv.* **2023**, *2*, 100209. [CrossRef]
20. Krinsky, N.I.; Johnson, E.J. Carotenoid Actions and Their Relation to Health and Disease. *Mol. Asp. Med.* **2005**, *26*, 459–516. [CrossRef]
21. Shaheer, C.A.; Hafeeda, P.; Kumar, R.; Kathiravan, T.; Kumar, D.; Nadanasabapathi, S. Effect of Thermal and Thermosonication on Anthocyanin Stability in Jamun (*Eugenia jambolana*) Fruit Juice. *Int. Food Res. J.* **2014**, *21*, 2194.
22. Jabbar, S.; Abid, M.; Hu, B.; Wu, T.; Muhammad, M.; Lei, S.; Zhu, X.; Zeng, X. Quality of Carrot Juice as in Fl Uenced by Blanching and Sonication Treatments. *LWT-Food Sci. Technol.* **2014**, *55*, 16–21. [CrossRef]
23. Dadan, M.; Grobelna, A.; Witrowa-Rajchert, D. The Impact of Ultrasound-Assisted Thawing on the Bioactive Components in Juices Obtained from Blue Honeysuckle (*Lonicera caerulea* L.). *Ultrason. Sonochem.* **2022**, *89*, 106156. [CrossRef] [PubMed]
24. Xie, L.; Su, H.; Sun, C.; Zheng, X.; Chen, W. Recent Advances in Understanding the Anti-Obesity Activity of Anthocyanins and Their Biosynthesis in Microorganisms. *Trends Food Sci. Technol.* **2018**, *72*, 13–24. [CrossRef]
25. Wu, S.B.; Long, C.; Kennelly, E.J. Phytochemistry and Health Benefits of Jaboticaba, an Emerging Fruit Crop from Brazil. *Food Res. Int.* **2013**, *54*, 148–159. [CrossRef]
26. Sacchet, C.; Mocelin, R.; Sachett, A.; Bevilaqua, F.; Chitolina, R.; Kuhn, F.; Boligon, A.A.; Athayde, M.L.; Roman Junior, W.A.; Rosemberg, D.B.; et al. Antidepressant-Like and Antioxidant Effects of *Plinia trunciflora* in Mice. *Evid.-Based Complement. Altern. Med.* **2015**, *2015*, 601503. [CrossRef]
27. Mulyadi, H.A.; Sianturi, O.R.; Mastuti, R.; Rosyidah, M.; Mastuti, R.; Rosyidah, M. Total Phenolic Content, Flavonoid Concentration and Antioxidant Activity of Indigenous Herbs, *Physalis minima* Linn. *J. Phys. Conf. Ser.* **2019**, *1349*, 012088. [CrossRef]
28. Sheng, Z.; Zhao, J.; Muhammad, I.; Zhang, Y. Optimization of Total Phenolic Content from *Terminalia chebula* Retz. Fruits Using Response Surface Methodology and Evaluation of Their Antioxidant Activities. *PLoS ONE* **2018**, *14*, e0202368. [CrossRef]
29. Nascimento, A.L.A.A.; Brandi, I.V.; Durães, C.A.F.; Lima, J.P.d.; Soares, S.B.; Mesquita, B.M.A.d.C. Chemical Characterization and Antioxidant Potential of Native Fruits of the Cerrado of Northern Minas Gerais. *Braz. J. Food Technol.* **2020**, *23*, e2019296. [CrossRef]
30. Da Porto, C.; Porretto, E.; Decorti, D. Comparison of Ultrasound-Assisted Extraction with Conventional Extraction Methods of Oil and Polyphenols from Grape (*Vitis vinifera* L.) Seeds. *Ultrason. Sonochem.* **2013**, *20*, 1076–1080. [CrossRef]
31. Khan, M.K.; Abert-Vian, M.; Fabiano-Tixier, A.-S.; Dangles, O.; Chemat, F. Ultrasound-Assisted Extraction of Polyphenols (Flavanone Glycosides) from Orange (*Citrus sinensis* L.) Peel. *Food Chem.* **2010**, *119*, 851–858. [CrossRef]
32. Virot, M.; Tomao, V.; Bourvellec, C.L.; Renard, C.M.C.G.; Chemat, F. Ultrasonics Sonochemistry Towards the Industrial Production of Antioxidants from Food Processing By-Products with Ultrasound-Assisted Extraction. *Ultrason. Sonochem.* **2010**, *17*, 1066–1074. [CrossRef] [PubMed]
33. González-Centeno, M.R.; Comas-Serra, F.; Femenia, A.; Rosselló, C.; Simal, S. Effect of Power Ultrasound Application on Aqueous Extraction of Phenolic Compounds and Antioxidant Capacity from Grape Pomace (*Vitis vinifera* L.): Experimental Kinetics and Modeling. *Ultrason. Sonochem.* **2015**, *22*, 506–514. [CrossRef] [PubMed]
34. Patient, A.; Jean-Marie, E.; Robinson, J.C.; Martial, K.; Meudec, E.; Levalois-Grützmacher, J.; Closs, B.; Bereau, D. Polyphenol Composition and Antioxidant Activity of *Tapirira guianensis* Aubl. (Anarcadiaceae) Leaves. *Plants* **2022**, *11*, 326. [CrossRef]
35. Cruz, J.M.d.A.; Ramos, A.S.; Corrêa, R.F.; Sanches, E.A.; Campelo, P.H.; Kinupp, V.F.; Bezerra, J.d.A. Thermal Treatment and High-Intensity Ultrasound Processing to Evaluate the Chemical Profile and Antioxidant Activity of Amazon Fig Juices. *Processes* **2023**, *11*, 408. [CrossRef]
36. Carlos, A.L.E.; Priego-Capote, F.; De Castro, M.D.L. Comparative Study of the Effect of Sample Pretreatment and Extraction on the Determination of Flavonoids from Lemon (*Citrus limon*). *PLoS ONE* **2016**, *11*, e0148056. [CrossRef]
37. Mehaya, F.M.; Mohammad, A.A. Thermostability of Bioactive Compounds during Roasting Process of Coffee Beans. *Heliyon* **2020**, *6*, e05508. [CrossRef]
38. Zhang, Q.A.; Shen, H.; Fan, X.H.; Shen, Y.; Wang, X.; Song, Y. Changes of Gallic Acid Mediated by Ultrasound in a Model Extraction Solution. *Ultrason. Sonochem.* **2015**, *22*, 149–154. [CrossRef]
39. Wang, F.; Li, H.; Qin, Y.; Mao, Y.; Zhang, B.; Deng, Z. Effects of Heat, Ultrasound, and Microwave Processing on the Stability and Antioxidant Activity of Delphinidin and Petunidin. *J. Food Biochem.* **2019**, *43*, e12818. [CrossRef]

40. Keppler, K.; Humpf, H.U. Metabolism of Anthocyanins and Their Phenolic Degradation Products by the Intestinal Microflora. *Bioorg. Med. Chem.* **2005**, *13*, 5195–5205. [CrossRef]
41. Sun, Y.; Qin, Y.; Li, H.; Peng, H.; Chen, H.; Xie, H.-R.; Deng, Z. Rapid Characterization of Chemical Constituents in Radix Tetrastigma, a Functional Herbal Mixture, before and after Metabolism and Their Antioxidant/Antiproliferative Activities. *J. Funct. Foods* **2015**, *18*, 300–318. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

CAPÍTULO 2

Aprimorando a eficiência de extração de compostos bioativos de *Tapirira guianensis* por meio de extração assistida por ultrassom e solvente eutético profundo à base de cloreto de colina.

Resumo: Nas últimas décadas, surgiu uma nova classe de solventes conhecida como solventes eutéticos profundos naturais. Neste estudo, avaliou-se a eficácia de diferentes solventes eutéticos profundos naturais, preparados por síntese assistida por ultrassom, na extração de compostos bioativos de frutos de *Tapirira guianensis* Aubl. Um total de sete sistemas de solventes eutéticos profundos naturais, combinando cloreto de colina com diferentes doadores de ligação de hidrogênio (ácidos carboxílicos, álcoois e açúcares), foi analisado quanto ao teor total de compostos fenólicos e à atividade antioxidante. Os resultados demonstraram que os solventes eutéticos profundos naturais à base de ácidos carboxílicos foram os mais eficientes, sendo os melhores resultados obtidos com cloreto de colina/ácido cítrico/glicerol (Stap7) > cloreto de colina/ácido cítrico (Stap1) > cloreto de colina/ácido lático (Stap2), apresentando teores totais de fenólicos de 715,7 ± 1,1; 648,5 ± 1,2; e 630,7 ± 0,7 mg EAG/g, respectivamente. A extração ultrassônica com misturas eutéticas a 40% (v/v) demonstrou que as combinações com ácido cítrico foram mais eficazes, produzindo extratos com elevadas concentrações de polifenóis e potencial antioxidante. A atividade antioxidante, avaliada pelos ensaios com 2,2-difenil-1-picrilhidrazil (DPPH), 2,2′-azino-bis(3-etilbenzotiazolina-6-ácido sulfônico) (ABTS) e poder antioxidante de redução do ferro (FRAP), apresentou tendência semelhante. A análise espectroscópica por ressonância magnética nuclear identificou ácido gálico e 5-hidroxivanilina, fornecendo detalhes estruturais dos extratos. A análise de macro e micronutrientes revelou alguns elementos abaixo dos limites de detecção. Portanto, esses extratos podem ser utilizados diretamente no desenvolvimento de aplicações, sem necessidade de isolamento adicional.

Palavras-chave: ultrassom; NADES; compostos antioxidantes; solventes eutéticos.

Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.493
CC BY

Enhancing the extraction efficiency of beneficial compounds from *Tapirira guianensis* fruit using ultrasound-assisted extraction and choline chloride deep eutectic solvent

Josiana Moreira MAR[1,4], Adriano de Souza CAROLINO[2], Kidney de Oliveira Gomes NEVES[3], Marcos Batista MACHADO[3], Renilto Frota CORRÊA[4], Daniel de Queiroz ROCHA[4], Valdely Ferreira KINUPP[4], Edgar Aparecido SANCHES[2], Pedro Henrique CAMPELO[5], Jaqueline de Araújo BEZERRA[1,4*]

Abstract

In recent decades, a new class of solvents known as natural deep eutectic solvents has emerged. In this study, the efficacy of different natural deep eutectic solvents, prepared by ultrasound-assisted synthesis, was evaluated for extracting bioactive compounds from *Tapirira guianensis* fruits. A total of seven natural deep eutectic solvents systems, combining choline chloride with various hydrogen bond donors (carboxylic acids, alcohols, and sugars), were analyzed for total phenolic content and antioxidant activity. The results showed that carboxylic acid-based natural deep eutectic solvents were the most efficient, with the best results obtained using choline chloride/citric acid/glycerol (Stap7) > choline chloride/citric acid (Stap1) > choline chloride/lactic acid (Stap2), yielding total phenolic contents of 715.7 ± 1.1, 648.5 ± 1.2, and 630.7 ± 0.7 mg GAE/g, respectively. Ultrasonic extraction with 40% (v/v) eutectic mixtures showed that combinations with citric acid were more effective, producing extracts with high concentrations of polyphenols and antioxidant potential. The antioxidant activity, measured by 2,2-Diphenyl-1-picrylhydrazyl, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and ferric-reducing antioxidant power assays, showed a similar trend. Spectroscopic analysis (nuclear magnetic resonance) identified gallic acid and 5-hydroxyvanillin, providing structural details of the extracts. Macro- and micronutrient analysis revealed some elements below detection limits. Therefore, these extracts can be used directly for application development without additional isolation.

Keywords: ultrasound; NADES; antioxidant compounds; eutectic solvents.

Practical Application: Utilization of natural deep eutectic solvents (NADES) and ultrasound to efficiently extract bioactive compounds from *Tapirira guianensis* fruits.

1 INTRODUCTION

The species *Tapirira guianensis* is widely distributed in all Brazilian biomes. In its composition, this species presents a high antioxidant and nutraceutical potential, containing polyphenols, specifically galloylquinic acids and flavonols (Mar et al., 2023).

Foods and beverages supply essential macro- and micronutrients—such as carbohydrates, proteins, lipids, vitamins, and minerals—which support key biological functions including energy metabolism, oxygen transport, bone health, blood clotting, and fluid balance (Fiorentini et al., 2022).

Conventional phytochemical extraction methods are time- and energy-consuming, often using non-generally recognized as safe (GRAS) solvents, making them costly and environmentally unfriendly. Even with GRAS solvents like ethanol or water, the extracts typically need further processing, limiting their efficiency and sustainability (Ferreira et al., 2020). In recent decades, a new class of solvents known as natural deep eutectic solvents (NADES) has emerged. These solvents are composed of primary plant metabolites, including sugars (such as glucose, fructose, sucrose, trehalose, and maltose), sugar alcohols (such as sorbitol, glycerol, 1,2-propanediol, and xylitol), organic acids (such as lactic, citric, maleic, malic, oxalic, and ascorbic acids), amino acids (such as proline, glycine, and alanine), and amines (such as betaine and choline) (Fuad & Nadzir, 2021).

Some NADES, though natural, may exhibit cytotoxic effects. Several preparation methods exist, including stirring with heating, lyophilization, evaporation, and microwave-assisted

Received: April 8, 2025.
Accepted: April 22, 2025.
[1]*Universidade Federal do Amazonas, Postgraduate Program in Biotechnology, Manaus, Amazonas, Brazil.*
[2]*Universidade Federal do Amazonas, Laboratory of Nanostructured Polymers, Manaus, Amazonas, Brazil.*
[3]*Universidade Federal do Amazonas, Nuclear Magnetic Resonance Laboratory, Analytical Center, Manaus, Amazonas, Brazil.*
[4]*Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Center for Studies in Science and Technology of the Amazon – Nucléo de Estudos Científicos e Tecnologia da Amazônia, Manuas, Amazonas, Brazil.*
[5]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*
*Corresponding author: jaqueline.araujo@ifam.edu.br
Conflict of interest: nothing to declare.
Funding: Fundação de Amparo à Pesquisa do Estado do Amazonas—FAPEAM (Edital n. 013/2022–Produtividade-CT&I), Support Program for Strict Sensu Graduate Graduation—Posgrad UFAM/FAPEAM, Conselho Nacional de Desenvolvimento Científico e Tecnológico––CNPq (process number 311522/2020-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior––CAPES, and PDPG-Consolidação-3-4/Programa de Desenvolvimento da Pós-Graduação (PDPG) Emergencial de Consolidação Estratégica dos Programas de Pós-Graduação Stricto Sensu acadêmico (number 2214/2022; process number 88881.707227/2022-01).

synthesis. Recently, ultrasound-assisted synthesis has emerged as a more sustainable, faster, and more efficient alternative (Gamela et al., 2020). Ultrasound-assisted extraction (UAE) shows promise, where ultrasonic energy is employed to facilitate the analyte extraction process from the matrix (Santana et al., 2020).

This study developed an UAE method using NADES to recover micro- and macronutrients from *T. guianensis* fruits. It identified the most effective system for extracting phenolic compounds and antioxidant activity and characterized key compounds via ^{1}H NMR and multivariate analysis. The results support eco-friendly applications in food and pharmaceutical industries.

1.1 Relevance of the work

This study highlights the relevance of natural deep eutectic solvents (NADES), combined with ultrasound assistance, for the efficient extraction of bioactive compounds from *Tapirira guianensis* fruits. Among the seven systems tested, those based on citric acid (especially STap7) showed higher total phenolic content and antioxidant activity. The presence of phenolic acids, such as gallic acid, was confirmed by nuclear magnetic resonance (NMR), and the extracts revealed potential for direct applications. These results reinforce the effectiveness of NADES in the extraction and bioaccessibility of beneficial compounds, highlighting their promising use in sustainable and functional formulations.

2 MATERIALS AND METHODS

2.1 Preparation of natural deep eutectic solvents

Choline chloride (ChCl), used as the hydrogen bond acceptor (HBA), was dried at 40°C for 30 min, and then mixed with one or more hydrogen bond donors (HBDs) in specific molar ratios. The mixture was heated at ~80°C with stirring for 30 min, and then diluted with distilled water (30% v/v) to form a colorless, uniform, non-crystallizing solution. The NADES names and molar ratios are shown in Table 1 (Lin et al., 2022).

2.2 Ultrasound-assisted natural deep eutectic solvents extraction

The fruits of *T. guianensis* were collected from Manaus, state of Amazonas (3°6′26″S, 60°1′34″W, SISGEN: A2FF2EEss), carefully washed with distilled water, freeze-dried, and ground for later use. The lyophilized fruits of *T. guianensis* (0.5 g) were mixed with each NADES at a solid–liquid ratio of 1:15 (w/v) as described previously (Silva, Pauletto et al., 2020).

Table 1. Natural deep eutectic solvents abbreviations and molar ratios.

Serial number	Natural deep eutectic solvents	Molar ratio
STap1	Choline chloride: citric acid	1:1
STap2	Choline chloride: lactic acid	1:1
STap3	Choline chloride: malic acid	1:1
STap4	Choline chloride: glucose	1:1
STap5	Choline chloride: glycerol	1:2
STap6	Choline chloride: citric acid:glucose	1:1:1
STap7	Choline chloride:citric acid: glycerol	1:1:1

Natural deep eutectic solvents (30%, v/v).

All NADES were prepared by sonication and heating at 50°C using power amplitudes equivalent to 40% (ultrasonic energy density level of 1.8 kJ·cm^{-3}). The treatment duration was 10 min, and it was performed in an ice bath to prevent sample overheating (Santos-Martín et al., 2023). During the ultrasonic homogenization process, the NADES system was treated using a 25-mm diameter probe, operating at a frequency of 20 kHz and with a nominal power of 750 W (VibraCell VCX 750 da Sonics, Shawnee, OK, EUA) (Mar et al., 2023). Each system was followed by filtration, and the solution was centrifuged to remove small sample particles. The resulting concentrate was collected and stored at 18°C (Lin et al., 2022).

2.3 Nuclear magnetic resonance chemical profile

An aliquot of 20 mg of each extract from the fruits of *T. guianensis* was dissolved in 520 µL of DMSO-d_6 with tetramethysilane (TMS) (0.05% v/v), sonicated in an ultrasonic bath for 10 min, and transferred to a 5-mm NMR tube. The nuclear magnetic resonance (NMR) spectra were acquired at the Nuclear Magnetic Resonance Laboratory (NMRLab) of the Federal University of Amazonas (UFAM) using a Bruker Avance IIIHD NMR spectrometer (Bruker, Billerica, Massachusetts, USA), operating at 11.7 T, equipped with a 5-mm BBFO Plus SmartProbe™ with Z-axis gradient. The pulse sequence used was zgpr with the following acquisition parameters: time domain (TD) data points of 32k, spectral width (SW) of 10 kHz, 90° pulse (P1) of 10.0 µs, relaxation delay (D1) of 2.0 s, acquisition time (AQ) of 1.64 s, receiver gain (RG) value of 64, number of scans (NS) equal to 32, free induction decay (FID) resolution of 0.60 Hz, center frequency (O1) set at 1,562.04 Hz, and suppression power (PLW9) of 8.1292 e^{-005}. Phase and baseline corrections of the spectra were performed manually using TopSpin 3.6.3 software. The chemical shift (in ppm) of the ^{1}H NMR spectra was referenced to the methyl signal of TMS at δ 0.0 and coupling constants (*J*) were recorded in Hz. correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) NMR experiments were conducted to verify the ^{1}H–^{1}H and ^{1}H–^{13}C correlations of the main compounds observed in the ^{1}H NMR spectra.

2.4 Antioxidant capacity assessment

The antioxidant activity based on DPPH$^{\bullet}$ radical and ABTS$^{\bullet+}$ cation radical and ferric-reducing antioxidant power (FRAP) were performed based on a previous report with modifications on an Epoch 2 Biotek microplate reader (Mar, Silva, Moreira et al., 2021).

2.5 Determination of total phenolic content of extracts

Total phenolic compounds were quantified using the Folin–Ciocalteu method. After a 5-min reaction with the reagent, 6% sodium bicarbonate was added, and the mixture was incubated for 90 min. Absorbance was measured at 750 nm using a microplate reader. Gallic acid was used as the standard ($y = 0.0029x + 0.0208$; $R^2 = 0.9915$), and results were expressed as milligrams

of gallic acid equivalents per milliliter of sample (mg GAE/mL), presented as mean ± standard deviation (Mar et al., 2023).

2.6 Digestibility assay in vitro

The concentration of bioactive compounds after digestion was assessed using the international in vitro digestibility protocol. In the gastric phase, tea infusions were mixed with simulated gastric fluid (SGF), pepsin, $CaCl_2$, HCl, and ultrapure water, adjusted to pH 3.0, and stirred at 200 rpm, 37°C for 2 h. In the intestinal phase, the gastric product was combined with simulated intestinal fluid (SIF), $CaCl_2$, lipase, pancreatin, bile extract, and water, adjusted to pH 7.0, and stirred under the same conditions. After digestion, the samples were centrifuged and stored for antioxidant analysis. All enzymes used were high-purity reagents from Sigma-Aldrich. Results were expressed as mean±standard deviation (Mar, Silva, Rabello et al., 2021).

2.7 Determination of macro- and microminerals by inductively coupled plasma optical emission spectroscopy

Acid digestion of 0.5 g of lyophilized *T. guianensis* fruit powder and extracts was performed using a MultiWave 5000 microwave digestion system with 10 mL of ultrapure HNO_3. The digestion program ramped to 220°C in 5 min, held for 15 min, and cooled to 70°C. Digested samples were diluted to 25 mL and stored at 4°C for inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis. Macro- and microminerals were quantified using an Agilent 5800 ICP-OES, with argon as the nebulizer gas. Calibration curves used certified reference materials, showed $R^2 > 0.999$, and %RSE (relative standard error) within ± 10%, confirming the high accuracy, sensitivity, and reproducibility of the method (Rodrigues et al., 2025).

The limit of detection (LOD) and limit of quantification (LOQ) were determined using calibration curves, with LOD ranging from 0.003 mg/100 g (Cr) to 0.18 mg/100 g (K, Ca, and Mg) and LOQ from 0.01 mg/100 g (Cr) to 0.6 mg/100 g (K, Ca, and Mg), indicating high sensitivity. The precision and accuracy of metal analysis via ICP-OES were confirmed using blanks and fortified samples prepared with standard solutions. The %coefficient of variation (%CV) was ≤ 10% for all analytes, showing good repeatability. Recovery rates ranged from 85 to 115% for blanks and 82 to 114% for fortified samples, all within the accepted range (80 to 120%), confirming the method's reliability (Instituto Nacional de Metrologia, Qualidade e Tecnologia [Inmetro], 2020).

2.8 Fourier transform infrared

To determine the chemical profile of the samples, the Fourier-transform infrared spectrometry with attenuated total reflection (FTIR-ATR) module from Agilent Cary (630 FTIR-ATR) was employed, using a ZnSe ATR crystal. The analysis of the STAP1 to STAP7 samples were conducted in the range of 680 to 4000 cm^{-1}.

2.9 Statistical analysis

The analysis of variance (ANOVA) was performed using the R software (version 3.5.1). Differences between the mean values obtained for each treatment were evaluated at a 5% significance level ($p \leq .05$) using Tukey's test.

3 RESULTS AND DISCUSSION

3.1 Extraction of bioactive compounds from natural deep eutectic solvents of T. guianensis *fruits based on spectrophotometric determination*

This study compared the performance of seven NADES combinations—comprising choline chloride (HBA) and various HBDs (carboxylic acids, alcohols, and sugars)—prepared via ultrasound-assisted synthesis for extracting bioactive compounds from *T. guianensis* fruits. The goal was to identify synergistic effects that enhance biological activity. Extraction efficiency was assessed based on total phenolic content (TPC) and antioxidant activity, with ANOVA used to compare the performance of each system.

The NADES based on carboxylic acids as the HBA component provided higher extraction efficiency for both parameters. As summarized in Table 2, the best results were obtained with the use of the systems choline chloride/citric acid/glycerol (STap7) > choline chloride/citric acid (Stap1) > choline chloride/lactic acid (Stap2), yielding TPCs of 715.7 ± 1.1 mg GAE/g, 648.5 ± 1.2 mg GAE/g, and 630.7 ± 0.7 mg GAE/g, respectively.

The extractions conducted using ultrasound-assisted methods with eutectic mixtures tested as aqueous solutions at 40% (v/v) showed that the combination with citric acid exhibited high efficiency. The results demonstrated extracts with high concentrations of polyphenols and antioxidant potential for NADES systems containing choline chloride/citric acid (Bakirtzi et al., 2016). According to Soukaina et al. (2024), the phenolic compound values ranged from 20.34 to 90.15 mg GAE/g of dry

Table 2. Results are expressed as mean ± standard deviation ($n = 3$).

Samples	DPPH (μM TE/mL)	ABTS (μM TE/mL)	TPC (mg GAE/g)	FRAP (μM Fe (II)/mL)
Stap1	1520.2 ± 7.6^{b}	1899.0 ± 8.8^{c}	648.5 ± 1.2^{c}	1575.4 ± 3.9^{c}
Stap2	1503.5 ± 9.0^{a}	1880.8 ± 8.4^{a}	630.7 ± 0.7^{a}	1522.0 ± 2.8^{a}
Stap3	1477.7 ± 6.3^{c}	1830.8 ± 6.9^{d}	607.5 ± 1.1^{d}	1506.9 ± 2.2^{d}
Stap4	1392.7 ± 5.2^{e}	1731.9 ± 6.9^{f}	447.9 ± 0.9^{f}	1266.2 ± 2.8^{f}
Stap5	1427.7 ± 8.0^{d}	1763.0 ± 8.8^{e}	503.3 ± 1.0^{e}	1375.0 ± 3.4^{e}
Stap6	1098.5 ± 6.6^{f}	1466.3 ± 3.3^{g}	409.5 ± 1.0^{g}	1209.8 ± 3.6^{g}
Stap7	1526.0 ± 6.6^{b}	1928.6 ± 7.7^{b}	715.7 ± 1.1^{b}	1607.2 ± 2.8^{b}
Sdtap1	567.7 ± 7.6^{h}	461.9 ± 8.4^{l}	148.2 ± 1.4^{k}	788.0 ± 2.9^{l}
Sdtap2	582.7 ± 3.8^{g}	367.4 ± 8.4^{n}	118.9 ± 0.9^{m}	709.1 ± 2.9^{n}
Sdtap3	553.5 ± 5.0^{i}	600.8 ± 8.4^{k}	168.8 ± 0.9^{j}	831.3 ± 3.3^{k}
Sdtap4	354.3 ± 7.6^{l}	861.9 ± 6.9^{h}	227.3 ± 1.1^{h}	976.1 ± 3.3^{h}
Sdtap5	421.0 ± 9.0^{j}	701.9 ± 8.4^{j}	168.8 ± 1.1^{j}	886.8 ± 4.0^{j}
Sdtap6	402.7 ± 7.6^{k}	806.3 ± 8.8^{i}	196.5 ± 1.1^{i}	942.0 ± 2.6^{i}
Sdtap7	557.7 ± 5.2^{hi}	403.0 ± 8.8^{m}	129.4 ± 0.9^{l}	763.5 ± 2.9^{m}

Results are expressed as mean ± standard deviation ($n = 3$). Different letters in the same column are significant ($p < .05$). DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TPC: total phenolic content; FRAP: ferric-reducing antioxidant power; μM TE/mL: micromoles of Trolox equivalent per milliliter of sample; μM Fe(II)/mL: micromolar of ferrous sulfate per milliliter of sample; mg GAE/mL: milligram of gallic acid equivalent per milliliter of sample.

weight (DW), indicating significant variation in the efficiency of NADES, with a particular emphasis on systems containing citric acid. According to Barbieri et al. (2020), choline chloride-based eutectic solvents have the ability to stabilize phenolic compounds, primarily due to the formation of hydrogen bonds between phenolic acids.

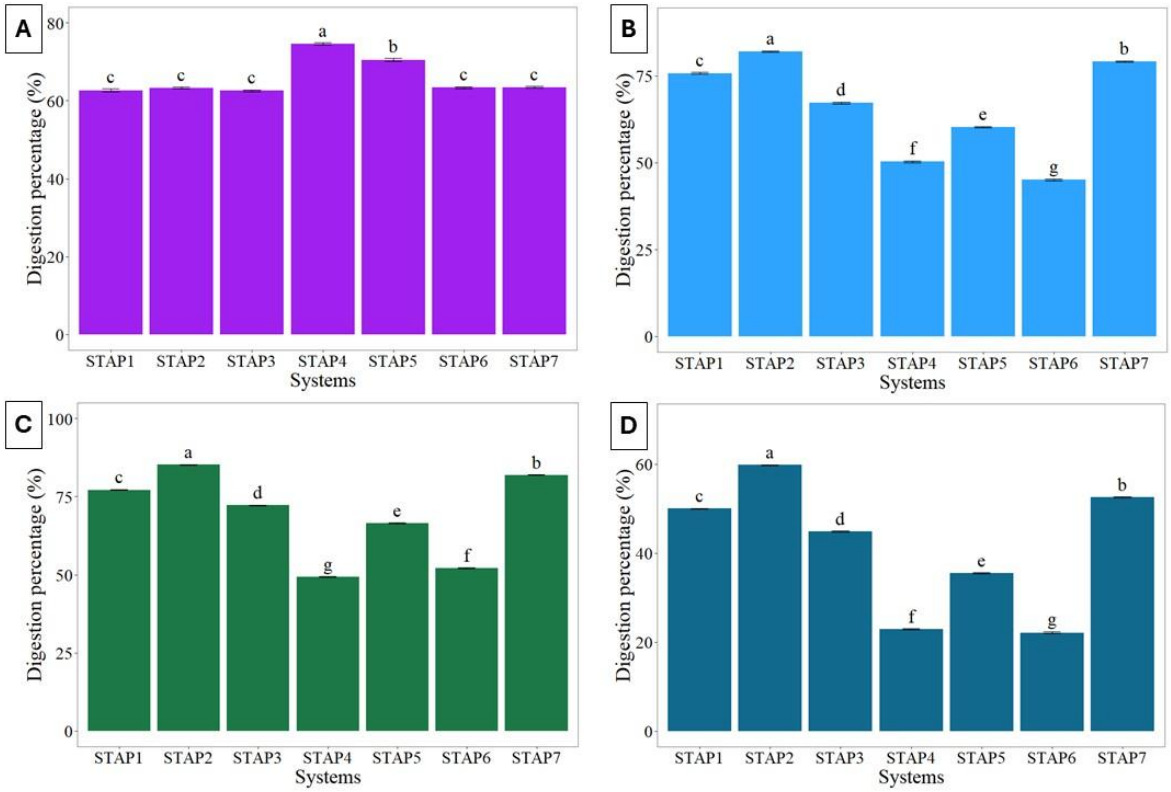
Antioxidant activity, measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and FRAP assays, showed an increasing trend among all *T. guianensis* extracts in the following order: STap7 (1526.0 ± 6.6; 1607.2 ± 2.8) > STap1 (1520.2 ± 7.6; 1575.4 ± 3.9) > STap2 (1503.5 ± 9.0; 1575.4 ± 3.9) (µM Trolox/mL; µM Fe (II)/mL), respectively. Similarly, the DPPH values showed significant differences among the various NADES combinations ($p \leq .05$), indicating a clear influence of the solvents used. These results indicate a similar pattern with the bioactive compounds (TPC) extracted using citric acid-NADES. Previous studies have also reported a strong correlation between polyphenolic content and antioxidant activity (Hsieh et al., 2020).

The in vitro antioxidant capacity values of the *T. guianensis* samples analyzed in this study were assessed using 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt $(ABTS)^{+\bullet}$ assays. As shown in Table 2, it is observed that the antioxidant activity of the extracts obtained with NADES significantly ($p \leq .05$) exceeded that of the aqueous extract (1274.3 ± 6.7 µM Trolox/mL), according to the studies by Mar et al. (2023). These findings are consistent with the trends observed in phenolic compounds, showing greater recovery of TPC in extracts using ChCl. The potential against the $ABTS^{+\bullet}$ radical showed the same trend as the potential against DPPH and FRAP radicals (STap7 > STap1 > STap2).

Bioaccessibility is defined as the fraction of a food/component that is released from the food matrix in the gastrointestinal tract and becomes available for absorption (Paterson et al., 2024). *In vitro* digestion reduced the TPC of STap7 from 715.7 to 129.4 mg GAE/100 g, indicating that 81.9% of the TPC was bioaccessible, while for STap1, the reduced TPC value was 77.1% (Figure 1).

During the gastric and fermentative phases, the release of polyphenols from cashew seeds was more efficient. According to Lima et al. (2014), the TPC in undigested cashew residue was 566.10 mg GAE/100 g DW, which decreased to 105.03 mg GAE/100 g DW after digestion, indicating a bioaccessibility level of only 18.6%. For cashew juice, the phenolic contents were 338.60 mg GAE/100 g DW in undigested samples and 130.60 mg GAE/100 g DW after digestion, resulting in a bioaccessibility of 39%.

The eutectic systems of *T. guianensis* exhibited a reduction in antioxidant activity after the *in vitro* digestion process for the ABTS, DPPH, and FRAP assays, with bioaccessibility ranging from 79.1, 63.4, and 52.5%, respectively, for the sample with the highest antioxidant potential. Cashew showed a decrease in antioxidant activity after *in vitro* digestion and fermentation in the ABTS, DPPH, and FRAP assays, with bioaccessibility ranging from 3.18 ± 0.93 to 17.69 ± 1.58% (Andrade et al., 2022).



Means that do not share the same letters on each graph (A-D) are significantly different.

Figure 1. Bioaccessibility reduction (%) of eutectic solvents. 2,2-Diphenyl-1-picrylhydrazyl (A), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (B), total phenolic content (C), *and* Fe(II) (D) *reduction assays.*

The low antioxidant activity observed at the end of digestion suggests the poor stability of compounds under pH and enzymatic changes. Antioxidants are more reactive in acidic (gastric) conditions and less so in neutral (intestinal) pH. During digestion, compounds may transform into less active forms or new antioxidant metabolites (Scrob et al., 2019).

3.2 Macro- and micronutrient content of natural deep eutectic solvents from T. guianensis *fruits*

The extraction capacity of eutectic solvents is largely influenced by their ability to form hydrogen bonds with analytes (Espino et al., 2016). Reports indicate that NADES act as Lewis acids and, in the presence of electron-donating groups, are capable of extracting inorganic elements from a solution (Huang et al., 2018). After the synthesis of NADES, the performance of seven different systems was evaluated in the extraction of macro- and micronutrients for the determination of K, Ca, Mg, P, Fe, Na, Mn, Cu, Zn, Ba, B, V, and Cr by ICP-OES.

The determination of metal content by ICP-OES revealed that the crude extract exhibited substantially higher concentrations of macroelements such as calcium (Ca), potassium (K), and magnesium (Mg) compared to the extracts obtained using different NADES systems (Table 3). This difference can be attributed to the non-selective nature of conventional extraction, which solubilizes a broader range of compounds from the plant matrix.

In contrast, the NADES systems showed greater selectivity in the extraction of these minerals, with the Stap2 (chloride:lactic acid) system standing out by presenting the highest concentrations among the eutectic solvents tested (Table 2). Although the values obtained with NADES were lower than those of the crude extract, the results indicate that this specific system is particularly efficient in mineral recovery, combining extraction performance with environmental viability and potential for sustainable applications.

It is worth noting that the elements Na, Mn, Cu, Zn, Ba, B, V, and Cr were present below the detection limit (0.05–0.25). Table 3 presents the concentration of different elements (Ca, Fe, K, Mg, and P) in various samples (Stap1 to Stap7), expressed in mg/100 g. Potassium (K) is the predominant element in all samples, with the highest values observed for Stap2 (~20 mg/100 g) and Stap3 (~17 mg/100 g), followed by Stap1, Stap4, and Stap5. Calcium (Ca), iron (Fe), magnesium (Mg), and phosphorus (P) are present in lower concentrations, with values significantly lower than potassium. Statistical differences are indicated by the letters above the bars, where samples with different letters exhibit statistically significant differences.

A comparison with the lyophilized (Table 3) fruit extract reveals significant differences in mineral concentrations. In the extract, potassium (K) is the most abundant element (~950 mg/100 g), followed by calcium (Ca) (~200 mg/100 g), both at much higher levels than in the raw samples. In contrast, iron (Fe) exhibits very low concentrations in the extract, suggesting lower extraction efficiency. Statistical analysis indicates that the extraction process influences the retention and concentration of minerals, particularly K and Ca, which may impact nutrient bioavailability.

Horse chestnut seed flour from the wild pure species contains significantly higher metal levels than the hybrid, with iron at 80.05 mg/100 g versus 1.42 mg/100 g. Potassium, calcium, manganese, nickel, and copper are also more abundant in the pure species (Durante et al., 2021). In pepper seeds, mineral concentrations varied as follows: calcium (1,639–4,861 mg/kg), iron (51–196 mg/kg), potassium (558–13,050 mg/kg), magnesium (553–1,662 mg/kg), manganese (7–46 mg/kg), phosphorus (2,306–3,793 mg/kg), and zinc (8–21 mg/kg) (Gamela et al., 2020).

Potassium is essential for regulating cellular osmotic pressure, membrane transport, and enzyme activation, with a recommended daily intake of 782 mg. Calcium, crucial for bones, muscles, and teeth, has a recommended intake ranging from 400 to 1500 mg/day, depending on age (Agostini-Costa et al., 2017). Magnesium is an essential element for the body due to its role in metabolism and as a constituent and activator of many enzymes, particularly those associated with the conversion of high-energy phosphate compounds. The daily requirement is 300–500 mg (Bezerra et al., 2019). Phosphorus is essential for metabolism, with a daily requirement of 800–1,200 mg, found mainly as phosphate in free or bound forms. Iron, needed at 1.5–2.2 mg/day, is present in hemoglobin and myoglobin, playing a key role in oxygen transport (Gamela et al., 2019). The elemental content of various eutectic solvents used to extract compounds from dried licorice roots (*Glycyrrhiza glabra L.*) was measured using ICP-OES. In the sucrose/lactic acid NADES system, high concentrations were found for Ca (12,570 ± 364 mg/kg), Fe (146 ± 2 mg/kg), K (2,470 ± 64 mg/kg), and Mg (2,552 ± 63 mg/kg), while Cu and Li showed the lowest levels (Shikov et al., 2022).

Table 3. Concentration values of Ca, Fe, K, Mg, and P obtained from samples and raw extract of *T. guianensis* fruits. Results are expressed as mean ± standard deviation.

	Elements				
Samples	**Ca (mg/100 g)**	**Fe (mg/100 g)**	**K (mg/100 g)**	**Mg (mg/100 g)**	**P (mg/100 g)**
Raw extract	186.3 ± 6.2	2.7 ± 2.7	1092.6 ± 43.0	119.92 ± 5.2	27.6 ± 1.2
Stap1	2.8 ± 0.2	-	14.0 ± 0.3	1.6 ± 0.0	-
Stap2	3.6 ± 0.3	1.0±0.1	19.6 ± 0.1	2.4 ± 0.1	0.4 ± 0.1
Stap3	3.3 ± 0.1	-	16.7 ± 0.5	1.5 ± 0.1	0.3 ± 0.0
Stap4	1.6 ± 0.1	1.4 ± 0.1	11.7 ± 0.2	1.2 ± 0.0	0.2 ± 0.0
Stap5	1.4 ± 0.1	-	10.8 ± 0.1	1.0 ± 0.0	0.2 ± 0.0
Stap6	1.0 ± 0.0	-	7.8 ± 0.1	0.2 ± 0.0	-
Stap7	1.4 ± 0.2	-	8.3 ± 0.2	0.7 ± 0.2	-

3.3 Fourier-transform infrared spectroscopy

In this study, the individual components of carboxylic acid-based natural deep eutectic solvents (CA-NADES) and CA-NADES synthesized with the molar ratios showing the best extraction potential were investigated. The objective was to determine the intermolecular interactions between the functional groups of the HBA and HBD.

Pure choline chloride has several functional groups, but few of them coexist after the formation of deep eutectic solvents (DESs). Vibrational bands at 3,200 cm^{-1} and 1,200–880 cm^{-1} are associated with hydroxyl or amino groups (the former corresponds to N—H stretching and the latter to symmetric C—N^+ stretching). Vibrational bands at 1,485–1,420 cm^{-1} indicate the presence of alkyl groups, with the CH_2 bending at 1485 cm^{-1} being the most prominent group detected in all choline chloride-based DESs (Hayyan et al., 2015).

The spectra black, red, and blue represent the pure HBDs: citric acid, lactic acid, and malic acid, respectively. The 3,280–2,500 cm^{-1} range indicates OH stretching, while 1,750–1,660 cm^{-1} corresponds to C=O groups in carboxylic acids. Peaks from 1,450 to 800 cm^{-1} reflect C–O and C–H stretching and O–H bending. Multiple C=O peaks in citric and malic acids (black and blue, respectively) result from their multiple carboxylic groups (Airouyuwa et al., 2023).

According to Figure 2, which concerns the choline chloride/glucose system, vibrational bands in the region of 3,600–3,000 cm^{-1} correspond to the vibrational stretching of O—H. Bands at 2,920 cm^{-1} refer to C—H stretching, 1,470 cm^{-1} to a combination of C—H and C—O—H bands, 1,485 cm^{-1} to the bending of CH_2 of an alkyl group, and 1,665 cm^{-1} to the stretching of C=O. The intense bands near the 1,000 cm^{-1} region are due to the stretching vibrations of C—O and C—C, while bands at 917 cm^{-1} are attributed to the stretching of C=C (Hayyan et al., 2015).

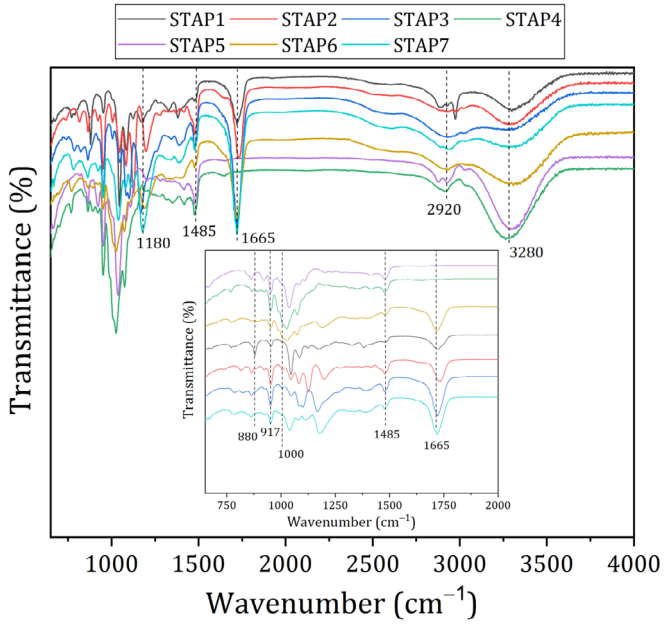


Figure 2. Normalized FTIR-ATR spectra for different DESs, according to their respective natural deep eutectic solvents.

Figure 2 shows the spectrum for the choline chloride/glycerol DES. Vibrational bands at 1,485 cm^{-1} correspond to the CH_2 bending of an alkyl group, and bands near 1,110 cm^{-1}, 1,035 cm^{-1}, and 862 cm^{-1} correspond to functional groups, namely C—O stretching, asymmetric C—C—O stretching, and symmetric C—C—O stretching, respectively (Ibrahim et al., 2006).

The results confirmed the suggested adjustment in the molecular interaction between the synthesized NADES and their respective pure components. These findings are consistent with previous literature (Naseem et al., 2021) and confirm the presence of new hydrogen bonds in the synthesized NADES.

3.4 Chemical profile analysis by nuclear magnetic resonance

In the ^{1}H NMR spectra of the seven extracts from the fruits of *T. guianensis*, a signal at δ 6.93 (s) was identified, characteristic of the hydrogens of gallic acid (Figures 3 and 4), which has already been identified in the leaves of this species (Silva, David et al., 2020).

The structure was confirmed through analysis of the HSQC and HMBC spectra of the extracts, which allowed for the assignment of positions C-2 and C-6 (δ 108.6), C-5 (δ 137.3), and COOH (δ 164.4) (Figure 4). In addition to the signal of gallic acid, the Stap1, Stap2, Stap3, Stap6, and Stap7 samples exhibited signals at δ 7.50 (H-6, *d*, *J* = 3.5 Hz), δ 6.60 (H-2, *d*, *J* = 3.5 Hz), δ 9.54 (H-1′, s), and δ 4.50 (H-1″, s), respectively. The HSQC spectrum analysis allowed for the identification of the C-1' and C-1" positions at δ 177.9 and δ 55.7, respectively (Figure 5).

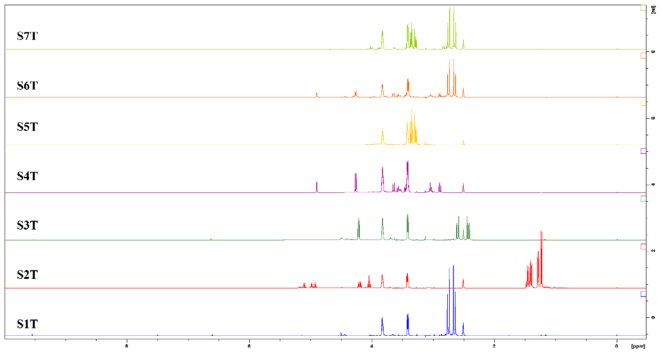
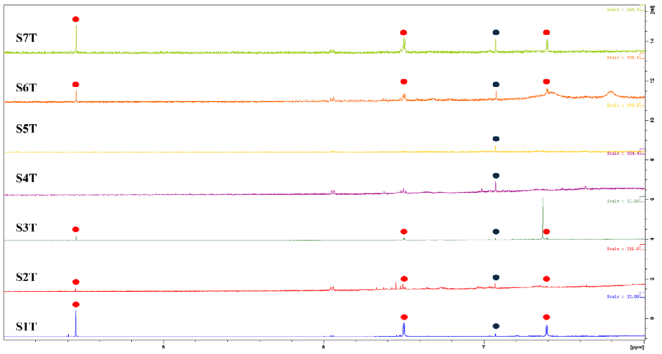


Figure 3. ^{1}H NMR spectra of the seven extracts of *T. guianensis* fruits (500 MHz, DMSO-*d*6).



The red and black circles correspond to the signals of the compounds 5-hydroxyvanillin and gallic acid, respectively.

Figure 4. Expansion of the aromatic region (6.0–10.0 ppm) of the ^{1}H NMR spectra of the seven extracts of *T. guianensis* fruits (500 MHz, DMSO-*d*6).

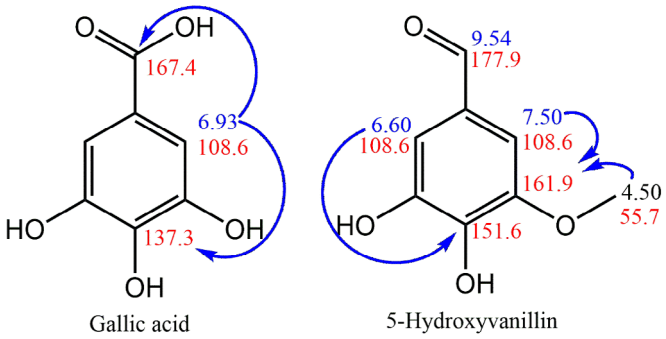


Figure 5. Aromatic compounds identified in the different extracts of *T. guianensis* fruits.

The HMBC correlation map revealed interactions of H-6 with δ 109.6 (C-2), δ 151.6 (C-4), and δ 161.9 (C-5), while H-1″ exhibited a correlation with δ 161.9 (C-5). Therefore, the combined analysis of the one-dimensional (1D) and two-dimensional (2D) NMR spectra allowed the structure of this phenolic compound to be proposed as 5-hydroxyvanillin.

FTIR analysis confirmed key intermolecular interactions in NADES formation, showing vibrational bands linked to hydrogen bonds between HBD and HBA, which stabilize the DESs. New bands in NADES spectra, compared to pure components, indicated additional hydrogen bonds. These molecular changes enhanced the extraction of phenolic compounds, such as gallic acid and 5-hydroxyvanillin, as confirmed by NMR.

4 CONCLUSION

This study evaluated seven ultrasound-assisted NADES for extracting bioactive compounds from *T. guianensis* fruits. Systems using carboxylic acids as HBAs—especially choline chloride/citric acid/glycerol (STap7), choline chloride/citric acid (STap1), and choline chloride/lactic acid (STap2)—achieved the highest TPC and antioxidant activity (DPPH and FRAP assays). STap7 showed the greatest overall efficacy and TPC bioaccessibility, confirming the efficiency of citric acid-based NADES. Mineral analysis indicated effective extraction of Ca, K, and Mg. FTIR and NMR confirmed key phenolic compounds, including gallic acid and 5-hydroxyvanillin. These findings underscore NADES potential for optimizing phenolic extraction and bioaccessibility.

ACKNOWLEDGMENTS

The authors wish to acknowledge Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), Support Program for *Strict Sensu* Graduate Graduation Posgrad (UFAM/FAPEAM), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and fellowships and Analytical Centers at IFAM-CMC and UFAM for infrastructure.

REFERENCES

Agostini-Costa, T. S., Gomes, I. S., Melo, L. A. M. P., Reifschneider, F. J. B., & Ribeiro, C. S. C. (2017). Carotenoid and total vitamin C content of peppers from selected Brazilian cultivars. *Journal of Food Composition and Analysis*, *57*, 73–79. https://doi.org/10.1016/j.jfca.2016.12.020

Airouyuwa, J. O., Mostafa, H., Ranasinghe, M., & Maqsood, S. (2023). Influence of physicochemical properties of carboxylic acid-based natural deep eutectic solvents (CA-NADES) on extraction and stability of bioactive compounds from date (Phoenix dactylifera L.) seeds: An innovative and sustainable extraction technique. *Journal of Molecular Liquids*, *388*, Article 122767. https://doi.org/10.1016/j.molliq.2023.122767

Andrade, J. K., S. Barros, R. G. C., Gualberto, N. C., Oliveira, C. S., Shanmugam, S., & Narain, N. (2022). Influence of in vitro gastrointestinal digestion and probiotic fermentation on the bioaccessibility of gallic acid and on the antioxidant potential of Brazilian fruit residues. *LWT*, *153*, Article 112436. https://doi.org/10.1016/j.lwt.2021.112436

Bakirtzi, C., Triantafyllidou, K., & Makris, D. P. (2016). Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, *3*(3), 120–127. https://doi.org/10.1016/j.jarmap.2016.03.003

Barbieri, J. B., Goltz, C., Cavalheiro, F. B., Toci, A. T., Igarashi-Mafra, L., & Mafra, M. R. (2020). Deep Eutectic solvents applied in the extraction and stabilization of rosemary (rosmarinus officinalis l.) phenolic compounds. *Industrial Crops and Products*, *144*, Article 112049. https://doi.org/10.1016/j.indcrop.2019.112049

Bezerra, M. A., Ferreira, S. L. C., Novaes, C. G., Santos, A. M. P., Valasques, G. S., Cerqueira, U. M. F. M., & Alves, J. P. S. (2019). Simultaneous optimization of multiple responses and its application in Analytical Chemistry – A review. *Talanta*, *194*, 941–959. https://doi.org/10.1016/j.talanta.2018.10.088

Durante, C., Cocchi, M., Lancellotti, L., Maletti, L., Marchetti, A., Roncaglia, F., Sighinolfi, S., & Tassi, L. (2021). Analytical concentrations of some elements in seeds and crude extracts from Aesculus hippocastanum, by icp-oes technique. *Agronomy*, *11*(1), Article 47. https://doi.org/10.3390/agronomy11010047

Espino, M., Ángeles Fernández, M., Gomez, F. J. V., & Silva, M. F. (2016). Natural designer solvents for greening analytical chemistry. *TrAC Trends in Analytical Chemistry*, *76*, 126–136. https://doi.org/10.1016/j.trac.2015.11.006

Ferreira, L. F., Minuzzi, N. M., Rodrigues, R. F., Pauletto, R., Rodrigues, E., Emanuelli, T., & Bochi, V. C. (2020). Citric acid water-based solution for blueberry bagasse anthocyanins recovery: Optimization and comparisons with microwave-assisted extraction (MAE). *LWT*, *133*, Article 110064. https://doi.org/10.1016/j.lwt.2020.110064

Fiorentini, E. F., Llaver, M., Oviedo, M. N., Quintas, P. Y., & Wuilloud, R. G. (2022). State-of-the-art analytical methods based on ionic liquids for food and beverage analysis. *Green Analytical Chemistry*, *1*, Article 100002. https://doi.org/10.1016/j.greeac.2022.100002

Fuad, F. M., & Nadzir, M. M. (2021). Hydrophilic natural deep eutectic solvent : A review on physicochemical properties and extractability of bioactive compounds. *Journal of Molecular Liquids*, *339*, Article 116923. https://doi.org/10.1016/j.molliq.2021.116923

Gamela, R. R., Barrera, E. G., Duarte, Á. T., Boschetti, W., Silva, M. M., Vale, M. G. R., & Dessuy, M. B. (2019). Fast sequential determination of zn, fe, mg, ca, na, and k in infant formulas by high-resolution continuum source flame atomic absorption spectrometry using ultrasound-assisted extraction. *Food Analytical Methods*, *12*(6), 1420–1428. https://doi.org/10.1007/s12161-019-01478-8

Gamela, R. R., Costa, V. C., & Pereira-Filho, E. R. (2020). Multivariate optimization of ultrasound-assisted extraction procedure for the determination of Ca, Fe, K, Mg, Mn, P, and Zn in *Pepper samples* by ICP OES. *Food Analytical Methods*, *13*(1), 69–77. https://doi.org/10.1007/s12161-019-01524-5

Hayyan, M., Abo-Hamad, A., AlSaadi, M. A., & Hashim, M. A. (2015). Functionalization of graphene using deep eutectic solvents. *Nanoscale Research Letters*, *10*, Article 324. https://doi.org/10.1186/s11671-015-1004-2

Hsieh, Y.-H., Li, Y., Pan, Z., Chen, Z., Lu, J., Yuan, J., Zhu, Z., & Zhang, J. (2020). Ultrasonication-assisted synthesis of alcohol-based deep eutectic solvents for extraction of active compounds from ginger. *Ultrasonics Sonochemistry*, *63*, Article 104915. https://doi.org/10.1016/j.ultsonch.2019.104915

Huang, Y., Feng, F., Chen, Z.-G., Wu, T., & Wang, Z.-H. (2018). Green and efficient removal of cadmium from rice flour using natural deep eutectic solvents. *Food Chemistry*, *244*(5), 260–265. https://doi.org/10.1016/j.foodchem.2017.10.060

Ibrahim, M. A., Alaam, M., El-Haes, H., Jalbout, A. F., & Leon, A. (2006). Analysis of the structure and vibrational spectra of glucose and fructose. *Ecletica Quimica*, *31*(3), 15–21. https://doi.org/10.1590/S0100-46702006000300002

Instituto Nacional de Metrologia, Qualidade e Tecnologia. (2020). *Orientação sobre Validação de Métodos Analíticos: Documento de caráter orientativo*. Coordenação Geral de Acreditação. Retrieved July 7, 2025, from https://www.gov.br/cdtn/pt-br/assuntos/documentos-cgcre-abnt-nbr-iso-iec-17025/doq-cgcre-008/view

Lima, A. C. S., Soares, D. J., Silva, L. M. R., Figueiredo, R. W., Sousa, P. H. M., & Menezes, E. A. (2014). In vitro bioaccessibility of copper, iron, zinc and antioxidant compounds of whole cashew apple juice and cashew apple fibre (*Anacardium occidentale L.*) following simulated gastro-intestinal digestion. *Food Chemistry*, *161*, 142–147. https://doi.org/10.1016/j.foodchem.2014.03.123

Lin, S., Meng, X., Tan, C., Tong, Y., Wan, M., Wang, M., Zhao, Y., Deng, H., Kong, Y., & Ma, Y. (2022). Composition and antioxidant activity of anthocyanins from Aronia melanocarpa extracted using an ultrasonic-microwave-assisted natural deep eutectic solvent extraction method. *Ultrasonics Sonochemistry*, *89*, Article 106102. https://doi.org/10.1016/j.ultsonch.2022.106102

Mar, J. M., Corrêa, R. F., Ramos, A. S., Kinupp, V. F., Sanches, E. A., Campelo, P. H., & Bezerra, J. A. (2023). Enhancing bioactive compound bioaccessibility in Tapirira guianensis juices through ultrasound-assisted applications. *Processes*, *11*(9), Article 2718. https://doi.org/10.3390/pr11092718

Mar, J. M., Silva, L. S., Moreira, W. P., Biondo, M. M., Pontes, F. L. D., Campos, F. R., Kinupp, V. F., Campelo, P. H., Sanches, E. A, & Bezerra, J. A. (2021). Edible flowers from Theobroma speciosum: Aqueous extract rich in antioxidant compounds. *Food Chemistry*, *356*, Article 129723. https://doi.org/10.1016/j.foodchem.2021.129723

Mar, J. M., Silva, L. S., Rabello, M. S., Biondo, M. M., Kinupp, V. F., Campelo, P. H., Bruginski, E., Campos, F. R., Bezerra, J. A., & Sanches, E. A. (2021). Development of alginate/inulin carrier systems containing non-conventional Amazonian berry extracts. *Food Research International*, *139*, Article 109838. https://doi.org/10.1016/j.foodres.2020.109838

Naseem, Z., Shehzad, R. A., Ihsan, A., Iqbal, J., Zahid, M., Pervaiz, A., & Sarwari, G. (2021). Theoretical investigation of supramolecular hydrogen-bonded choline chloride-based deep eutectic solvents using density functional theory. *Chemical Physics Letters*, *769*, Article 138427. https://doi.org/10.1016/j.cplett.2021.138427

Paterson, S., Majchrzak, M., Alexandru, D., Di Bella, S., Fernández-Tomé, S., Arranz, E., Fuente, M. A., Gómez-Cortés, P., & Hernández-Ledesma, B. (2024). Impact of the biomass pretreatment and simulated gastrointestinal digestion on the digestibility and antioxidant activity of microalgae Chlorella vulgaris and Tetraselmis chuii. *Food Chemistry*, *453*, Article 139686. https://doi.org/10.1016/j.foodchem.2024.139686

Rodrigues, L. S., Ferreira, D. S., Pereira-Filho, E. R., & Pereira, F. M. V. (2025). Mineral and trace element analysis of non-conventional food plants using ICP OES and chemometric techniques. *Food Chemistry*, *472*, Article 142854. https://doi.org/10.1016/j.foodchem.2025.142854

Santana, A. P. R., Andrade, D. F., Guimarães, T. G. S., Amaral, C. D. B., Oliveira, A., & Gonzalez, M. H. (2020). Synthesis of natural deep eutectic solvents using a mixture design for extraction of animal and plant samples prior to ICP-MS analysis. *Talanta*, *216*, Article 120956. https://doi.org/10.1016/j.talanta.2020.120956

Santos-Martín, M., Cubero-Cardoso, J., González-Domínguez, R., Cortés-Triviño, E., Sayago, A., Urbano, J., & Fernández-Recamales, A. (2023). Biomass and bioenergy ultrasound-assisted extraction of phenolic compounds from blueberry leaves using natural deep eutectic solvents (NADES) for the valorization of agrifood wastes. *Biomass and Bioenergy*, *175*, Article 106882. https://doi.org/10.1016/j.biombioe.2023.106882

Scrob, T., Hosu, A., & Cimpoiu, C. (2019). The influence of in vitro gastrointestinal digestion of brassica oleracea florets on the antioxidant activity and chlorophyll, carotenoid and phenolic content. *Antioxidants*, *8*(7), Article 212. https://doi.org/10.3390/antiox8070212

Shikov, A. N., Shikova, V. A., Whaley, A. O., Burakova, M. A., Flisyuk, E. V., Whaley, A. K., Terninko, I. I., Generalova, Y. E., Gravel, I. V., & Pozharitskaya, O. N. (2022). The Ability of Acid-Based Natural Deep Eutectic Solvents to Co-Extract Elements from the Roots of Glycyrrhiza glabra L. and Associated Health Risks. *Molecules*, *27*(22), Article 7690. https://doi.org/10.3390/molecules27227690

Silva, D. T., Pauletto, R., Cavalheiro, S. S., Bochi, V. C., Rodrigues, E., Weber, J., Silva, C. B., Morisso, F. D. P., Barcia, M. T., & Emanuelli, T. (2020). Natural deep eutectic solvents as a biocompatible tool for the extraction of blueberry anthocyanins. *Journal of Food Composition and Analysis*, *89*, Article 103470. https://doi.org/10.1016/j.jfca.2020.103470

Silva, E. P., David, J. M., David, J. P., Garcia, G. H. T., & Silva, M. T. (2020). Chemical composition of biological active extracts of *Tapirira guianensis* (*Anacardiaceae*). *Quimica Nova*, *43*(9), 1216–1219. https://doi.org/10.21577/0100-4042.20170605

Soukaina, K., Safa, Z., Soukaina, H., Hicham, C., & Bouchra, C. (2024). Choline chloride-based deep eutectic solvents (NADES): Potential use as green extraction media for polyphenols from Mentha pulegium, antioxidant activity, and antifungal activity. *Microchemical Journal*, *199*, Article 110174. https://doi.org/10.1016/j.microc.2024.110174

CAPÍTULO 3

Extração verde assistida por ultrassom de componentes antioxidantes da flor de *Theobroma speciosum* Willd. ex Spreng utilizando solventes eutéticos profundos naturais.

Resumo: Contexto: Este estudo investiga estratégias sustentáveis para a extração de compostos bioativos das flores de *Theobroma speciosum* Willd. ex Spreng utilizando solventes eutéticos profundos naturais (NADES) como alternativas ambientalmente amigáveis aos solventes convencionais. **Métodos:** Foram formulados sete sistemas de NADES (ST1 a ST7), utilizando cloreto de colina (ChCl) como aceptor de ligação de hidrogênio (HBA) e diferentes doadores de ligação de hidrogênio (HBD), incluindo ácido cítrico, ácido lático, ácido málico, glicose e glicerol. Os solventes foram preparados por aquecimento a 50 °C e aplicação de ultrassom a 60% de amplitude. A capacidade antioxidante dos extratos foi avaliada por meio dos ensaios DPPH, ABTS e FRAP. **Resultados:** A maioria dos extratos obtidos com NADES apresentou maior ou semelhante capacidade de sequestro de radicais livres em concentrações mais baixas quando comparada aos solventes tradicionais, especialmente nos ensaios DPPH e ABTS, além de demonstrar eficiente redução de íons férricos no ensaio FRAP. O pH foi identificado como um fator crítico que influencia a eficiência da extração e a estabilidade dos compostos, afetando a solubilidade e a interação entre os NADES e os compostos-alvo. Entre os sistemas testados, o NADES composto por ChCl e ácido lático (ST2) apresentou a maior eficiência na extração de compostos fenólicos e antocianinas. **Conclusão:** Os NADES representam uma alternativa sustentável, de baixa toxicidade e custo acessível para a extração de antioxidantes naturais. Suas propriedades ajustáveis e alto desempenho indicam amplo potencial de aplicação nas indústrias alimentícia, farmacêutica e cosmética, contribuindo para bioprocessos mais sustentáveis e eficientes.

Palavras-chave: Ultrassom; NADES; Componentes antioxidantes.

Original article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.492
CC BY

Influence of experimental temperatures on drying kinetics of *Theobroma speciosum* tea flowers

Josiana Moreira MAR[1,3], Adriano de Souza CAROLINO[2], Renilto Frota CORREA[3], Valdely Ferreira KINUPP[3], Edgar Aparecido SANCHES[2], Pedro Henrique CAMPELO[4], Jaqueline de Araújo BEZERRA[1,4]

Abstract

Drying is a crucial step in ensuring the quality of teas, as it can alter their chemical composition and organoleptic characteristics. In the present study, we examined the effects of different drying temperatures (ranging from 60 to 90°C) using hot air drying (HAD) on *Theobroma speciosum* flowers concerning drying kinetics, color, digestibility, and antioxidant capacity. From the obtained curves, we analyzed the physical aspects of *T. speciosum*, along with the moisture loss throughout the process, and the impact of different temperatures. This revealed that the Midilli mathematical model was the most suitable, where a set of equations was used to predict the analysis method's behavior. The results demonstrated that drying at 60°C was more effective in terms of drying rates, diffusivity, and bioactive compound content, making it a promising option for the pre-treatment of *T. speciosum* flower tea.

Keywords: *T. speciosum*; drying; antioxidants; Midilli mathematical model.

Practical Application: Drying at 60°C preserves bioactive compounds and optimizes the quality properties of tea.

1 INTRODUCTION

Edible flowers have been used in traditional medicine and cuisine worldwide, adding delightful flavors and providing valuable bioactive compounds such as polyphenols and anthocyanins when used in salads, desserts, beverages, and tea blends (Takahashi et al., 2020). Bioactive compounds in edible flowers are potent antioxidants that enhance nutritional value, improve flavor and health, protect against chronic diseases, and prevent oxidative deterioration of food (Zawiślak et al., 2022).

Numerous studies have extensively documented the antioxidant activity and phenolic composition of *Theobroma* species (*T. cacao*, *T. bicolor*, and *T. grandiflorum*) in their pulp juice, along with cupuassu pulp and seeds, as well as cupuassu and cocoa liquors and cocoa powder (Mar et al., 2024). *Theobroma speciosum*, known as "cacauí" in Brazil, is considered a prominent unconventional food plant. The aqueous extract of *T. speciosum* flowers exhibit fractions rich in phenolic compounds, which are directly related to its antioxidant capacity (Moreira Mar et al., 2021).

Optimal drying conditions are essential in food processing to preserve bioactive compounds by preventing enzymatic degradation and microbial growth (Zawiślak et al., 2022). Hot air drying (HAD) is a widely used and more affordable method for industrial drying due to its faster process (Shi et al., 2019).

Dehydration with hot air is common for preserving plant materials. However, high temperatures can degrade flavor, color, and bioactive compounds. Lower drying temperatures help retain nutritional and sensory quality (Marcel et al., 2016). The choice of drying techniques depends on factors such as the type of product, the availability of a dryer, cost considerations, and desired time and energy consumption (Selvakumar, 2023).

Antioxidant properties of tea infusions vary with brewing conditions. Phytochemical release depends on time and temperature, and these factors influence the tea's health benefits. (Zhang et al., 2020). Moreover, the excellence of the tea infusion hinges on a range of factors. These encompass the type of tea, duration of brewing, temperature of the water, ratio of water to tea, and brewing time (Safdar et al., 2016).

This study analyzed moisture removal (MR) in *T. speciosum* flowers using drying models, determined diffusion coefficients, and evaluated the impact of drying air temperature on physicochemical properties, color, phenolic content, and antioxidant capacity before and after digestion.

Received: April 7, 2025.
Accepted: April 22, 2025.
[1]*Universidade Federal do Amazonas, Postgraduate Program in Biotechnology, Manaus, Amazonas, Brazil.*
[2]*Universidade Federal do Amazonas, Laboratory of Nanostructured Polymers, Manaus, Amazonas, Brazil.*
[3]*Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Center for Studies in Science and Technology of the Amazon, Manaus, Amazonas, Brazil.*
[4]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*
*Corresponding author: jaqueline.araujo@ifam.edu.br
Conflict of interest: nothing to declare.
Funding: Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) (Stricto Sensu Postgraduate Support Program – Posgrad Universidade Federal do Amazonas–UFAM, process number 01.02.016301.03335/2021-56, Edital n. 010/2021- CT&I áreas prioritárias, Edital n. 013/2022–Produtividade-CT&I), Support program for Strict Sensu graduate graduation – posgrad UFAM/FAPEAM, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process number 311522/2020-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), PDPG-Consolidação-3-4/Programa de Desenvolvimento da Pós-Graduação (PDPG) Emergencial de Consolidação Estratégica dos Programas de Pós-Graduação Stricto Sensu acadêmico (number 2214/2022; process number 88881.707227/2022-01).

Relevance of the work

This study is relevant as it investigates the impact of different drying temperatures on the quality of *T. speciosum* flowers, with potential use in functional teas. Drying directly affects the preservation of bioactive compounds and sensory characteristics. The research analyzed drying kinetics, mathematical models, and parameters such as antioxidant activity, color, and bioaccessibility. Results showed that drying at 60°C was the most efficient, preserving high levels of functional compounds and ensuring better final product quality. This contributes to the development of a more efficient drying process, with reduced energy consumption and enhanced nutritional and functional value of the product.

2 MATERIALS AND METHOD

2.1 Collection and preparation of flower teas

T. speciosum flowers were collected from Manaus (3°6′26″S, 60°1′34″W; SisGen A26CD5E) and dried in a MYLABOR oven at 60, 70, 80, and 90°C with an air velocity of 850 $m^3 \cdot h^{-1}$. For each treatment, 5 g of flowers was sampled every 10 min to monitor moisture content over drying times from 0 to 240 min. The samples were then stored in a desiccator for chemical analysis. The final dried samples (0.5 g, < 0.15 mm) were used to prepare infusions, following specific mass-to-volume ratios. All procedures were performed in triplicate.

2.2 Drying kinetics and effective diffusivity

In order to establish the drying kinetics of *T. speciosum* flowers and analyze the mathematical dehydration models, the moisture ratio was calculated from the experimental data using Equation 1.

$$MR = \frac{(U(t) - U_{eq})}{(U_i - U_{eq})} \quad (1)$$

The experimentally obtained drying curves were fitted using the following models: the Midilli model (Equation 2) (Midilli et al., 2002), the Henderson and Pabis model (Equation 3), the Page model (Equation 4), and the Lewis model (Equation 5). Parameter estimation was carried out using RStudio® software version 2023.06.1+524 for Windows, where a non-linear regression analysis was conducted using the Non-Linear Regression package.

$$MR = a.\exp(-k.t^N) + b.t \quad (2)$$

$$MR = a.\exp(-k.t) \quad (3)$$

$$MR = \exp(-k.t^N) \quad (4)$$

$$MR = \exp(-k.t) \quad (5)$$

To identify the best-fitting model for the experimental data, performance indices described by Ross (1996) were applied: root mean square error (RMSE), percentage prediction error (%SEP), bias factor (B_f), and accuracy factor (A_f) (Equations 6, 7, 8 and 9). Data processing was conducted using RStudio® version 2023.06.1+524 for Windows.

$$REQM = \sqrt{\frac{\Sigma(obs - pred)^2}{n}}\ t \quad (6)$$

$$\%SP = \frac{100}{mean\ obs}\sqrt{\frac{\Sigma(obs - pred)^2}{n}}\ t \quad (7)$$

$$Bf = 10^{\frac{\Sigma log\frac{pred}{obs}}{n}} \quad (8)$$

$$Af = 10^{\frac{\Sigma\ |log\frac{pred}{obs}|}{n}} \quad (9)$$

The temperature dependence of effective diffusivity can be described by the Arrhenius equation. The activation energy (E_a) and pre-exponential factor (D_o) are determined from the slope and intercept of the ln(D_{eff}) versus 1/T plot (Uribe et al., 2014) (Equation 10).

$$D_{eff} = D_o exp\left(\frac{-E_a}{RT}\right) \quad (10)$$

where:

R: the universal gas constant (8.314 J mol^{-1} K^{-1});

E_a: the activation energy (kJ mol^{-1} and D_o the Arrhenius factor (m^2 s^{-1}))

T: the absolute temperature (K).

2.3 Digestibility assay

The effect of drying on bioaccessibility in *T. speciosum* was evaluated through in vitro digestion (INFOGEST, without oral phase), assessing antioxidant potential before and after gastric and intestinal phases (de Souza Carvalho et al., 2020).

2.4 Antioxidant capacity assessment and total phenolic contents

For the pre- and post-digestibility samples, antioxidant activities were evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) radical scavenging methods, as well as the ferric-reducing antioxidant power (FRAP) and total phenolic content (TPC) (Mar et al., 2021). The TPC was determined using the Folin–Ciocalteu colorimetric method. Results were expressed as mg of gallic acid equivalents (GAE) for 1 mL of tea. These analyses were performed with slight modifications from a previous report using the Epoch 2 Biotek microplate reader (Moreira Mar et al., 2021).

2.5 Color analysis

Color analysis of the samples was conducted using a Delta-Vista spectrophotometer (450G, DeltaColor, Brazil) with LED light, D65 illuminant, and a 10° observer, evaluating the L*, a*, and b* coordinates. A tea sample without temperature influence was used as the white reference (Mar et al., 2020).

2.6 Statistical analysis

Analysis of variance (ANOVA) was conducted in R (v3.5.1), and treatment means were compared using Tukey's test at a 5% significance level ($p \leq .05$).

3 RESULTS AND DISCUSSION

3.1 Drying kinetics modeling

Drying curves for *T. speciosum* flowers at four temperatures, displayed in Figure 1, show that MR changes over drying time. All curves followed an exponential pattern. Equilibrium moisture content, approximately 0.02 g of water per gram of dry matter, was achieved at all temperatures, with quicker attainment at higher temperatures. At 80 and 90°C, the equilibrium moisture content was reached between 150 and 120 min, respectively, while at 60 and 70°C, drying took over 180 min under constant airflow.

Drying *Camellia sinensis* flowers to 0.08 g H_2O/g dry matter took 180 min at 60°C and 60 min at 120°C. Higher temperatures increased vapor pressure, speeding up moisture migration. Thus, drying was faster at elevated temperatures (Shi et al., 2019). Drying *Cynara cardunculus* L. flowers at 35°C took 15 h. Each 10°C increase significantly reduced the drying time, reaching 2.5 h at 65°C; it can be said that higher temperatures greatly accelerated MR (Guiné et al., 2019).

Table 1 shows the constants, coefficient of determination (R^2), and mean estimated standard errors (SEs) for each model obtained via nonlinear regression. The drying rate constant "k" was higher at 60°C for all models, except for the Midilli model, where the elevated drying air temperature led to a higher drying rate, explaining the observed divergence.

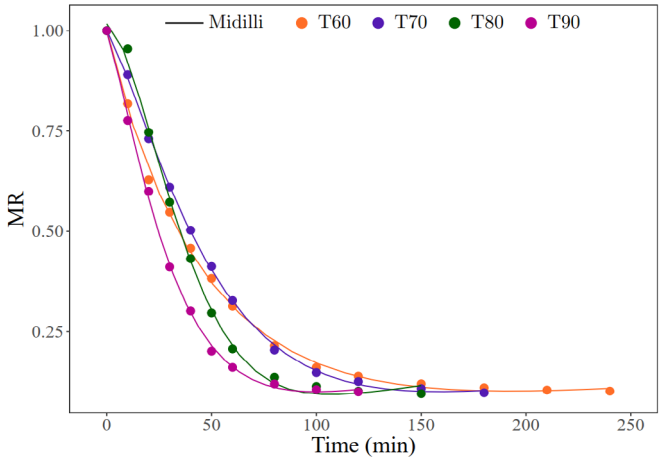


Figure 1. Experimental drying curves modeled by the Midilli equation for samples of *T. speciosum* flowers at different drying temperatures.

Among the four models tested, the Midilli model best fit the drying data of *T. speciosum* flowers, showing the lowest mean errors and highest R^2 values across all temperatures, with relative errors below 10%, indicating a good fit (Guiné et al., 2019).

The Midilli model was found to be the most suitable for describing the drying process in various fruit studies, such as pineapple drying at temperatures of 40, 50, and 60°C. To determine the best-fitting model, performance indicators from Ross (1996) were used, as outlined in Table 2. According to Ross (1996), the Midilli model showed the lowest RMSE and %SEP values, indicating a better fit to the experimental data, followed closely by the Page model. It also had the most favorable bias factor (B_f), closest to the ideal value of 1, reinforcing its predictive accuracy.

Finally, the accuracy factor (A_f) represents the average difference between predicted and observed data. Enhanced model accuracy is indicated by a reduced A_f value. For the temperatures under study, the Midilli model exhibited minimized values.

The Midilli model demonstrated the best fit to the experimental data at all four temperatures studied. Notably, the Page model yielded results comparable to the Midilli model within these temperature ranges.

Table 1. Kinetic and statistical parameters obtained for *T. speciosum* samples.

Model	Temp.	Model constants	R^2	SE
Midilli	60	$n = 9.617\times10^{-1}$ $k = 2.430\times10^{-2}$	0.9985	3.25×10^{-2} 3.19×10^{-3}
	70	$n = 1.240$ $k = 7.626\times10^{-3}$	0.9995	2.96×10^{-2} 8.99×10^{-4}
	80	$n = 1.583$ $k = 2.761\times10^{-3}$	0.9978	8.39×10^{-2} 8.86×10^{-4}
	90	$n = 1.227$ $k = 1.441\times10^{-2}$	0.9990	4.40×10^{-2} 2.27×10^{-3}
Henderson and Padis	60	$a = 0.95911$ $k = 0.01768$	0.9836	3.60×10^{-2} 1.24×10^{-3}
	70	$a = 1.027959$ $k = 0.018017$	0.9920	2.31×10^{-2} 7.61×10^{-4}
	80	$a = 1.085153$ k 0.022798	0.9725	4.84×10^{-2} 1.84×10^{-3}
	90	$a = 1.010499$ $k = 0.028638$	0.9891	3.15×10^{-2} 1.59×10^{-2}
Page	60	$n = 0.791108$ $k = 0.042596$	0.9877	4.69×10^{-2} 8.24×10^{-3}
	70	$n = 1.060223$ $k = 0.013702$	0.992	5.82×10^{-2} 3.24×10^{-3}
	80	$n = 1.345806$ $k = 0.005645$	0.9836	1.37×10^{-1} 2.96×10^{-3}
	90	$n = 0.999999$ $k = 0.028303$	0.9885	7.78×10^{-2} 8.14×10^{-3}
Lewis	60	$k = 0.0187170$	0.9884	9.66×10^{-4}
	70	$k = 0.0174053$	0.9909	5.77×10^{-4}
	80	$k = 0.020768$	0.9702	1.52×10^{-3}
	90	$k = 0.02830$	0.9885	1.18×10^{-3}

Table 2. Performance indicators for each model.

Model	Temp.	Performance indicators	Model	Temp.	Performance indicators
Midilli	60	REQM = 0.0480 %SEP = 13.1800 B_f = 1.0038 A_f = 1.0041	Page	60	REQM = 0.0511 %SEP = 14.0329 B_f = 1.0132 A_f = 1.0010
	70	REQM = 0.0470 %SEP = 10.9160 B_f = 1.0392 A_f = 1.0374		70	REQM = 0.0340 %SEP = 7.9146 B_f = 1.0017 A_f = 1.0153
	80	REQM = 0.0921 %SEP = 21.7566 B_f = 1.0493 A_f = 1.0368		80	REQM = 0.0792 %SEP = 18.7104 B_f = 0.9382 A_f = 1.1152
	90	REQM = 0.1401 %SEP = 37.0891 B_f = 1.0718 A_f = 1.0580		90	REQM = 0.0935 %SEP = 24.7549 B_f = 0.9403 A_f = 1.1158
Henderson and Padis	60	REQM = 0.1401 %SEP = 37.0891 B_f = 1.0718 A_f = 1.0580	Lewis	60	REQM = 0.0582 %SEP = 15.9910 B_f = 0.9953 A_f = 1.0283
	70	REQM = 0.0563 %SEP = 15.4749 B_f = 0.9998 A_f = 1.0218		70	REQM = 0.0333 %SEP = 7.7470 B_f = 1.0080 A_f = 1.0059
	80	REQM = 0.0688 %SEP = 16.2672 B_f = 0.9923 A_f = 1.0307		80	REQM = 0.0685 %SEP = 16.1879 B_f = 1.0038 A_f = 1.0160
	90	REQM = 0.0952 %SEP = 25.2171 B_f = 0.9381 A_f = 1.1192		90	REQM = 0.0935 %SEP = 24.7549 B_f = 0.9403 A_f = 1.1159

It can be observed that, for this model, the average adjusted coefficient of determination was 0.999, and according to Silva et al. (2020), values above 98% confirm a strong fit of the models to the dehydration process. Among them, the Midilli model best described the mass loss behavior during the drying of Viola (*Viola tricolor*)×Wittrockiana (*Viola wittrockiana*) flowers (Midilli et al., 2002).

The variation in *T. speciosum* flowers can be explained by the fact that higher temperatures increase the vapor pressure inside the flowers, resulting in faster removal of moisture from the interior to the surface of the flowers. The D_{eff} values were 4.15×10^{-6}, 4.52×10^{-6}, 4.96×10^{-6}, and 5.27×10^{-6} $m^2.s^{-1}$ in the range of 60–90°C (Figure 2). The D_{eff} values were reported to be 2.68×10^{-9}, 3.86×10^{-9}, 5.27×10^{-9}, and 8.66×10^{-9} $m^2.s^{-1}$ in flowers of *C. sinensis* (Shi et al., 2019).

Higher drying temperatures increased D_{eff} values, as the added heat enhanced water molecule kinetic energy and moisture diffusivity. This behavior followed an Arrhenius-type relationship, with a strong linear correlation (R^2 = 0.996) between the natural logarithm of D_{eff} and the inverse of absolute temperature.

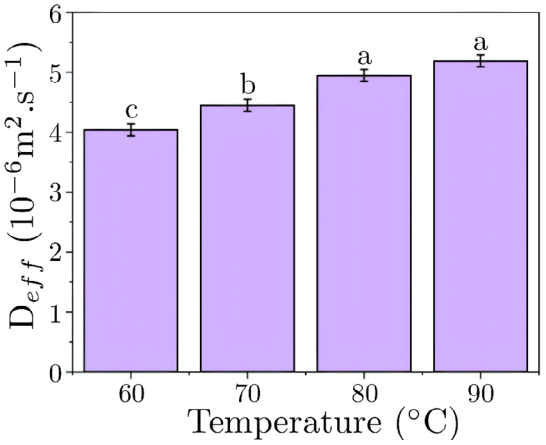


Figure 2. Effective diffusivity in *T. speciosum* flowers.

3.2 Color analysis of fruits

Color parameters of *T. speciosum* flowers under different temperatures are shown in Table 3. As the drying air temperature and air velocity decreased, leading to longer drying times, the L* and b* values decreased, while the a* value increased.

3.3 Antioxidant activity and bioaccessibility

The TPC of dried *T. speciosum* samples decreased with higher drying temperatures, ranging from 515.07 to 130.45 μmolGA/mL before digestion and from 81.10 to 8.92 μmolGA/mL after digestion. Despite this reduction, no significant differences were observed across temperatures ($p < .05$), with the lowest TPC retention at 80°C. Polyphenolics are sensitive to heat, and extended exposure to high temperatures leads to permanent chemical alterations in these compounds (Vashisth et al., 2011) (Table 4).

Higher drying temperatures reduce drying time and may enhance bioactive compound levels. However, optimizing time and temperature is essential to preserve and recover phenolics based on each product's specific composition (Vashisth et al., 2011).

The means of antioxidant activities for *T. speciosum* samples, measured by the DPPH (1,384.75 μM Trolox/mL), ABTS (1,842.11 μM Trolox/mL), and FRAP (1,964.51 μM Fe (II)/mL) assays, showed the highest antioxidant capacity for T60 samples, followed by TP samples (1,199.75 μM Trolox/mL for DPPH, 1,842.10 μM Trolox/mL for ABTS, and 362.63 μM Fe(II)/mL for FRAP), with no significant differences ($p > .05$) compared to the other samples.

The health benefits of tea consumption are primarily based on its antioxidant activity (Liu et al., 2018). The antioxidant capacities of T60 and TP infusions were influenced by infusion conditions, showing significantly higher ABTS and DPPH scavenging activity than T70, T80, and T90. Controlled drying at lower temperatures enhanced the antioxidant capacity, whereas higher drying temperatures reduced it, aligning with previous findings (Kowalska et al., 2021).

The post-digestion bioavailability of antioxidants is highly significant. Many researchers use *in vitro* digestion methods to

Table 3. Color parameters for *T. speciosum* flowers.

Sample	L*	a*	b*	ΔE*
T60°C 	21.767 ± 2.157[a]	8.313 ± 1.432[a]	4.522 ± 1.005[ab]	10.589 ± 4.162[a]
70°C 	23.032 ± 1.391[a]	8.072 ± 1.979[a]	3.743 ± 1.120[b]	13.031 ± 2.685[a]
80°C 	23.239 ± 2.229[a]	9.393 ± 1.473[a]	5.313 ± 1.317[a]	13.431 ± 4.302[a]
90°C 	21.911 ± 2.037[a]	6.734 ± 1.821[b]	4.539 ± 1.453[ab]	10.868 ± 3.931[a]

L*: lightness; a*: the red-green coordinate; b*: the yellow-blue coordinate; ΔE*: the color variation. Means that do not share the same letters in the column (a-b) are significantly different.

Table 4. Values of DPPH, ABTS, and FRAP before and after *in vitro* digestion of *T. speciosum* flower teas.

Samples	DPPH (μM TE/mL)	ABTS (μM TE/mL)	TPC (mg GAE/mL)	FRAP (μM Fe(II)/mL)
TP	1,199.7 ± 10.9[b]	1,842.1 ± 13.5[a]	362.6 ± 1.0[b]	1,509.7 ± 3.6[b]
T60	1,384.7 ± 9.0[a]	1,608.8 ± 6.9[b]	515.1 ± 0.7[a]	1,964.5 ± 2.3[a]
T70	831.4 ± 5.7[d]	1,422.1 ± 10.2[c]	235.3 ± 1.2[d]	1,170.7 ± 2.8[d]
T80	674.7 ± 10.0[e]	1,072.9 ± 7.5[d]	130.4 ± 1.4[e]	790.1 ± 3.9[e]
T90	876.4 ± 8.8[c]	785.4 ± 10.2[e]	262.6 ± 0.8[c]	1,295.6 ± 4.6[c]
TPD	443.1 ± 8.0[i]	243.2 ± 8.4[j]	8.9 ± 0.8[j]	644.1 ± 4.6[j]
T60D	578.1 ± 7.6[g]	604.3 ± 6.7[f]	65.8 ± 1.0[g]	713.0 ± 3.4[g]
T70D	450.6 ± 8.8[i]	485.4 ± 3.8[g]	16.3 ± 1.2[i]	657.5 ± 2.3[i]
T80D	626.4 ± 7.6[f]	340.9 ± 12.0[h]	81.1 ± 1.3[f]	749.7 ± 4.5[f]
T90D	523.9 ± 7.6[h]	546.5 ± 11.7[i]	54.4 ± 1.3[h]	678.6 ± 3.9[h]

Results are expressed as mean ± standard deviation (n = 3); [a-j]Different letters in the same column are significant ($p < .05$); μM TE/mL: micromoles of Trolox equivalent per millimeters of sample; μM Fe(II)/mL: micromolar of ferrous sulfate per millimeters of sample; mg GAE/mL: milligram of gallic acid equivalent per millimeters of sample.

evaluate the stability of antioxidants found in foods, meals, and supplements (Liu et al., 2018). During digestion, the antioxidant potential of T60 and TP samples decreased significantly (58.25–97.54%) due to pH and enzyme conditions, reducing the bioactive compound levels in *T. speciosum* flowers.

Antioxidant capacity is affected by polyphenol levels and pH changes during digestion. As digestion progresses from the gastric to the intestinal phase, structural changes in polyphenols occur, altering their activity. In the intestine's alkaline environment (pH 6–8), polyphenols may degrade or transform into undetected forms with different properties and bioactivities. These changes are consistent with known polyphenol metabolism patterns, as reported in previous studies (Liu et al., 2020).

The antioxidant properties of grapefruit and pineapple juices increased before digestion but declined after pancreatic digestion due to alkaline pH. This suggests antioxidant instability. Structural changes in polyphenols may have affected their detectability, as noted by José Jara-Palacios et al. (2018).

4 CONCLUSIONS

This study evaluated the drying kinetics of *T. speciosum* flowers and compared mathematical models using performance indicators. The Midilli model best fit the data but required four parameters, while the Page model showed similar accuracy with only two. Drying at 60°C led to faster equilibrium, and diffusion coefficients and activation energies followed the Arrhenius equation, indicating increased moisture diffusion with higher activation energy. Samples dried at 60°C retained high levels of bioactive compounds, antioxidant activity, and color quality. Drying for over 180 min at 60°C proved optimal for efficiency and quality, offering valuable insights for processing tea flowers.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) (Stricto Sensu Postgraduate Support Program – Posgrad Universidade Federal do Amazonas–UFAM, process number 01.02.016301.03335/2021-56, Edital n. 010/2021- CT&I áreas prioritárias, Edital n. 013/2022–Produtividade-CT&I), Support program for Strict Sensu graduate graduation – posgrad UFAM/FAPEAM, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process number 311522/2020-3), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), PDPGConsolidação-3-4/Programa de Desenvolvimento da Pós-Graduação (PDPG) Emergencial de Consolidação Estratégica dos Programas de Pós-Graduação Stricto Sensu acadêmico (number 2214/2022; process number 88881.707227/2022-01) for financial support and fellowships and Analytical Centers at IFAM-CMC and UFAM for infrastructure.

REFERENCES

de Souza Carvalho, L. M., Lemos, M. C. M., Sanches, E. A., da Silva, L. S., de Araújo Bezerra, J., Aguiar, J. P. L., das Chagas do Amaral Souza, F., Alves Filho, E. G., & Campelo, P. H. (2020). Improvement of the bioaccessibility of bioactive compounds from Amazon fruits treated using high energy ultrasound. *Ultrasonics Sonochemistry*, *67*(4), Article 105148. https://doi.org/10.1016/j.ultsonch.2020.105148

Guiné, R. P. F., Fontes, L., & Lima, M. J. R. (2019). Drying kinetics and mass transfer properties in the drying of thistle flower. *Brazilian Journal of Food Technology*, *22*(1), Article e2019051. https://doi.org/10.1590/1981-6723.05119

José Jara-Palacios, M., Gonçalves, S., Hernanz, D., Heredia, F. J., & Romano, A. (2018). Effects of in vitro gastrointestinal digestion on phenolic compounds and antioxidant activity of different white winemaking byproducts extracts. *Food Research International*, *109*(4), 433–439. https://doi.org/10.1016/j.foodres.2018.04.060

Kowalska, J., Marzec, A., Domian, E., Galus, S., Ciurzyńska, A., Brzezińska, R., & Kowalska, H. (2021). Influence of tea brewing parameters on the antioxidant potential of infusions and extracts depending on the degree of processing of the leaves of *Camellia sinensis*. *Molecules*, *26*(16), Article 4773. https://doi.org/10.3390/molecules26164773

Liu, X. Q., Deng, Y. X., Dai, Z., Hu, T., Cai, W. W., Liu, H. F., Li, H., Zhu, W. L., Li, B. Y., Wang, Q., & Zhang, S. J. (2020). Sodium tanshinone IIA sulfonate protects against Aβ1–42-induced cellular toxicity by modulating Aβ-degrading enzymes in HT22 cells. *International Journal of Biological Macromolecules*, *151*(1), 47–55. https://doi.org/10.1016/j.ijbiomac.2020.02.040

Liu, Y., Luo, L., Liao, C., Chen, L., Wang, J., & Zeng, L. (2018). Effects of brewing conditions on the phytochemical composition, sensory qualities and antioxidant activity of green tea infusion: A study using response surface methodology. *Food Chemistry*, *269*(2), 24–34. https://doi.org/10.1016/j.foodchem.2018.06.130

Mar, J. M., da Fonseca Júnior, E. Q., Corrêa, R. F., Campelo, P. H., Sanches, E. A., & Bezerra, J. de A. (2024). *Theobroma spp*.: A review of it's chemical and innovation potential for the food industry. *Food Chemistry Advances*, *4*, Article 100683. https://doi.org/10.1016/j.focha.2024.100683

Mar, J. M., Silva, L. S., Rabello, M. da S., Biondo, M. M., Kinupp, V. F., Campelo, P. H., Bruginski, E., Campos, F. R., Bezerra, J. de A., & Sanches, E. A. (2021). Development of alginate/inulin carrier systems containing non-conventional Amazonian berry extracts. *Food Research International*, *139*(10), Article 109838. https://doi.org/10.1016/j.foodres.2020.109838

Mar, J. M., Silva, L. S., Rabelo, S., Muniz, M. P., Nunomura, S. M., Correa, R. F., Ferreira, V., Campelo, P. H., Bezerra, J. D. A., & Sanches, E. A. (2020). Encapsulation of Amazonian blueberry juices: Evaluation of bioactive compounds and stability. *LWT - Food Science and Technology*, *124*(2), Article 109152. https://doi.org/10.1016/j.lwt.2020.109152

Marcel, A., Hubert, M., Bienvenu, M. J., & Pascal, O. (2016). Physico-chemical characteristics and biochemical potential of *Moringa oleifera* Lam. (*Moringaceae*). *Der Pharmacia Lettre*, *8*(18), 43–47. Retrieved from https://africaresearchconnects.com/pt-pt/papel/66097ffd912dd4b33d7b89ff157f2e6bbbd61cd0249a8cb059bd74acf8c139be/

Midilli, A., Kucuk, H., & Yapar, Z. (2002). A new model for single-layer drying. *Drying Technology*, *20*(7), 1503–1513. https://doi.org/10.1081/DRT-120005864

Moreira Mar, J., da Silva, L. S., Moreira, W. P., Biondo, M. M., Pontes, F. L. D., Campos, F. R., Kinupp, V. F., Campelo, P. H., Sanches, E. A., & Bezerra, J. A. (2021). Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds. *Food Chemistry*, *356*(1), Article 129723. https://doi.org/10.1016/j.foodchem.2021.129723

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, *81*(5), 501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

Safdar, N., Sarfaraz, A., Kazmi, Z., & Yasmin, A. (2016). Ten different brewing methods of green tea: comparative antioxidant study. *Journal of Applied Biology & Biotechnology*, *4*(3), 33–40. https://dx.doi.org/10.7324/JABB.2016.40306

Selvakumar, P. (2023). Drying diverse temperatures alters the physico - chemical and phytochemical properties of moringa flowers. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*, *2*(1), 60–66. https://doi.org/10.4103/jpdtsm.jpdtsm_77_22

Shi, L., Gu, Y., Wu, D., Wu, X., Grierson, D., Tu, Y., & Wu, Y. (2019). Hot air drying of tea flowers: effect of experimental temperatures on drying kinetics, bioactive compounds and quality attributes. *International Journal of Food Science and Technology*, *54*(2), 526–535. https://doi.org/10.1111/ijfs.13967

Silva, L. A., Fischer, S. Z., & Zambiazi, R. C. (2020). Proximal composition, bioactive compounds content and color preference of Viola x Wittrockiana flowers. *International Journal of Gastronomy and Food Science*, *22*, Article 100236. https://doi.org/10.1016/j.ijgfs.2020.100236

Takahashi, J. A., Rezende, F. A. G. G., Moura, M. A. F., Dominguete, L. C. B., & Sande, D. (2020). Edible flowers: Bioactive profile and its potential to be used in food development. *Food Research International*, *129*(10), Article 108868. https://doi.org/10.1016/j.foodres.2019.108868

Uribe, E., Lemus-Mondaca, R., Vega-Gálvez, A., Zamorano, M., Quispe-Fuentes, I., Pasten, A., & Di Scala, K. (2014). Influence of process temperature on drying kinetics, physicochemical properties and antioxidant capacity of the olive-waste cake. *Food Chemistry*, *147*, 170–176. https://doi.org/10.1016/j.foodchem.2013.09.121

Vashisth, T., Singh, R. K., & Pegg, R. B. (2011). Effects of drying on the phenolics content and antioxidant activity of muscadine pomace. *Lwt*, *44*(7), 1649–1657. https://doi.org/10.1016/j.lwt.2011.02.011

Zawiślak, A., Francik, R., Francik, S., & Knapczyk, A. (2022). Impact of drying conditions on antioxidant activity of red clover (*Trifolium pratense*), sweet violet (*Viola odorata*) and elderberry flowers (*Sambucus nigra*). *Materials*, *15*(9), Article 3317. https://doi.org/10.3390/ma15093317

Zhang, S., Yang, Y., Cheng, X., Thangaraj, K., Arkorful, E., Chen, X., & Li, X. (2020). Prediction of suitable brewing cuppages of Dahongpao tea based on chemical composition, liquor colour and sensory quality in different brewing. *Scientific Reports*, *10*(1), Article 945. https://doi.org/10.1038/s41598-020-57623-5

CAPÍTULO 4

Extração verde assistida por ultrassom de componentes antioxidantes da flor de *Theobroma speciosum* Willd. ex Spreng utilizando solventes eutéticos profundos naturais.

Resumo: Contexto: Este estudo investiga estratégias sustentáveis para a extração de compostos bioativos das flores de *Theobroma speciosum* Willd. ex Spreng utilizando solventes eutéticos profundos naturais (NADES) como alternativas ambientalmente amigáveis aos solventes convencionais. **Métodos:** Foram formulados sete sistemas de NADES (ST1 a ST7), utilizando cloreto de colina (ChCl) como aceptor de ligação de hidrogênio (HBA) e diferentes doadores de ligação de hidrogênio (HBD), incluindo ácido cítrico, ácido lático, ácido málico, glicose e glicerol. Os solventes foram preparados por aquecimento a 50 °C e aplicação de ultrassom a 60% de amplitude. A capacidade antioxidante dos extratos foi avaliada por meio dos ensaios DPPH, ABTS e FRAP. **Resultados:** A maioria dos extratos obtidos com NADES apresentou maior ou semelhante capacidade de sequestro de radicais livres em concentrações mais baixas quando comparada aos solventes tradicionais, especialmente nos ensaios DPPH e ABTS, além de demonstrar eficiente redução de íons férricos no ensaio FRAP. O pH foi identificado como um fator crítico que influencia a eficiência da extração e a estabilidade dos compostos, afetando a solubilidade e a interação entre os NADES e os compostos-alvo. Entre os sistemas testados, o NADES composto por ChCl e ácido lático (ST2) apresentou a maior eficiência na extração de compostos fenólicos e antocianinas. **Conclusão:** Os NADES representam uma alternativa sustentável, de baixa toxicidade e custo acessível para a extração de antioxidantes naturais. Suas propriedades ajustáveis e alto desempenho indicam amplo potencial de aplicação nas indústrias alimentícia, farmacêutica e cosmética, contribuindo para bioprocessos mais sustentáveis e eficientes.

Palavras-chave: Ultrassom; NADES; Componentes antioxidantes.

CAPÍTULO 8

ULTRASOUND-ASSISTED GREEN EXTRACTION OF ANTIOXIDANT COMPONENTS FROM THEOBROMA SPECIOSUM FLOWER USING NATURAL DEEP EUTECTIC SOLVENTS

https://doi.org/10.22533/at.ed.361112504048

Data de aceite: 19/09/2025

Josiana Moreira Mar
Posgraduate Program in Biotechnology, Federal University of Amazonas, Manaus, Brazil
NECTAM, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Centro, Manaus Brazil
https://orcid.org/0000-0003-4442-6874

Adriano de Souza Carolino
Laboratory of Nanostructured Polymers, Materials Physics Department, Federal University of Amazonas, Manaus 69067-005, Brazil
https://orcid.org/0000-0003-2284-4805

Ronald Zico de Aguiar Nunes
Laboratory of Nanostructured Polymers, Materials Physics Department, Federal University of Amazonas, Manaus Brazil
https://orcid.org/0000-0001-9337-0622

João Vitor Souza Soares
Posgraduate Program in Biotechnology, Federal University of Amazonas, Manaus, Brazil;
NECTAM, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Centro, Manaus Brazil
https://orcid.org/0000-0003-4866-7210

Salomão dos Santos Costa
LPMAT, Laboratório de processamento de materiais tecnológicos, Federal University of Amazonas, Manaus, Brazil
https://orcid.org/0000-0002-4667-0187

Renilto Frota Correa
NECTAM, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Centro, Manaus Brazil
https://orcid.org/0000-0001-6236-2283

Valdely Ferreira Kinupp
Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Zona Leste, Manaus Brazil
https://orcid.org/0000-0002-3892-7288

Edgar Aparecido Sanches
Laboratory of Nanostructured Polymers, Materials Physics Department, Federal University of Amazonas, Manaus Brazil
https://orcid.org/0000-0002-1446-723X

Pedro Henrique Campelo
Department of Food Technology, Federal University of Viçosa, Viçosa Brazil
https://orcid.org/0000-0002-5137-0162

Jaqueline de Araújo Bezerra
NECTAM, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Centro, Manaus Brazil
https://orcid.org/0000-0002-9168-9864

ABSTRACT: Background: This study investigates sustainable strategies for the extraction of bioactive compounds from T. speciosum flowers using natural deep eutectic solvents (NADES) as environmentally friendly alternatives to conventional solvents. **Methods:** Seven NADES systems (ST1 to ST7) were formulated using choline chloride (ChCl) as the hydrogen bond acceptor (HBA) and different hydrogen bond donors (HBD), including citric acid, lactic acid, malic acid, glucose, and glycerol. The solvents were prepared by heating at 50 °C and applying ultrasound at 60% amplitude. The antioxidant capacity of the extracts was evaluated through DPPH, ABTS, and FRAP assays. **Results:** Most NADES extracts showed enhanced or comparable radical scavenging capacity at lower concentrations than traditional solvents, particularly in the DPPH and ABTS assays, while also demonstrating effective ferric ion reduction in the FRAP assay. The pH was identified as a critical factor influencing extraction efficiency and compound stability, affecting the solubility and interaction between NADES and the target compounds. Among the systems tested, the NADES composed of ChCl and lactic acid (ST2) demonstrated the highest extraction efficiency for phenolic compounds and anthocyanins. **Conclusion:** NADES represent a sustainable, low-toxicity, and cost-effective alternative for the extraction of natural antioxidants. Their tunable properties and high performance suggest broad potential for application in food, pharmaceutical, and cosmetic industries, contributing to greener and more efficient bioprocesses.
KEYWORDS: Ultrasound, NADES, Antioxidant Components.

INTRODUCTION

New sources of healthy foods containing bioactive compounds are being investigated as adequate solutions for health and nutrition. The bioactive compounds can help prevent many diseases, including cancers, coughing, inflammatory, cardiovascular and oxidative stress diseases [1].

The phenolic compounds are one of the most popular bioactive compounds that have a wide range of biological activities and are widely used in food, cosmetic and pharmaceutical industries [2]. The extraction of the phenolic compounds from vegetal materials has got urge attention of these last decades. Generally, for the industrial-scale extraction of these bioactive compounds, conventional organic solvents such as hexane, chloroform, alcohols and ethyl acetate are used. However, the use of large quantities of these organic solvents has the drawbacks of having undesirable effects on the environment and leaving unacceptable residues in the extracts [3].

Water is an eco-friendly solvent, but it is very difficult to extract some non-polar and water-insoluble compounds owing to its intrinsic chemical properties. In terms of organic solvents, it is strongly discouraged in the food, cosmetic and pharmaceutical industries owing to its high toxicity, strong volatility, and flammability [4]. Natural deep eutectic solvents (NADES) are novel, eco-friendly solvents made by heating two or three natural components like sugars, organic acids, and polyols. Due to their low toxicity, broad polarity range, biodegradability, and low cost, NADES are widely used for extracting and separating various bioactive compounds such as phenolics, flavonoids, anthocyanins, pectin, and polysaccharides [5].

Natural deep eutectic solvent (NADES) is a novel class of green and sustainable solvent based on natural components such as carboxylic acids, choline chloride, urea, polyols and sugars. The application of NADES for the extraction of phenolic compounds has emerged as a greener approach, efficient and alternative to organic solvents [6]. NADES are formed by mixing two or more natural components that interact through hydrogen bonds, creating a eutectic mixture with a melting point much lower than that of the individual ingredients [7]. The hydrogen bond interactions take place between the hydrogen bond donor (HBD) and the hydrogen bond acceptor (HBA) [8].

NADES enable selective extraction thanks to their versatility, and when combined with modern methods like ultrasound, microwave, and high-pressure extraction, they effectively enhance the recovery of valuable compounds from plants and agricultural waste [9]

Ultrasound-assisted extraction is an emerging technology that merges ultrasonic and microwave techniques, using their combined high-energy effects to overcome drawbacks of traditional methods like long extraction times, poor temperature control, and uneven heating [10].

To the best of the authors' knowledge, no studies have explored using NADES to extract phenolic compounds from *Theobroma speciosum* ("cacauí") flowers. This study aimed to develop an efficient, eco-friendly method to extract anthocyanins from *T. speciosum* using NADES combined with ultrasound-assisted extraction. Seven different NADES formulations were tested, and antioxidant properties such as phenolic content and radical scavenging capacity were evaluated. The work focused on creating a sustainable solvent system that maximizes extraction efficiency and stabilizes compounds. This novel approach highlights the potential of acid-, sugar-, and alcohol-based NADES with ultrasound to enhance antioxidant extraction from *T. speciosum* flowers.

MATERIAL AND METHODS

Preparation of NADES

Choline chloride (ChCl), used as the hydrogen bond acceptor, was pre-dried at 40 °C for 30 minutes. It was then mixed with one or more hydrogen bond donors in specific molar ratios and heated at 80 °C with stirring for 30 minutes. The mixture was diluted with 30% distilled water to obtain a clear, homogeneous solution without crystals [10]. The NADES abbreviations and molar ratios are shown in Table 1.

Serial number	Natural deep eutectic solvents	Molar ratio
ST1	Choline chloride: Citric acid	1: 1
ST2	Choline chloride: Lactic acid	1: 1
ST3	Choline chloride: Malic acid	1: 1
ST4	Choline chloride: Glucose	1: 1
ST5	Choline chloride: Glycerol	1: 2
ST6	Choline chloride:Citric acid: Glucose	1: 1: 1
ST7	Choline chloride:Citric acid: Glycerol	1: 1: 1

Table 1: NADES (30%, v/v) abbreviations and mole ratios.

Ultrasound-assisted NADES extraction

Flowers of *T. speciosum* were collected in Manaus, Brazil (3°6′26″S, 60°1′34″W; SisGen registration A26CD5E), carefully rinsed with distilled water, freeze-dried, and ground into a fine powder for subsequent use. An aliquot of freeze-dried *T. speciosum* flowers (0.5 g) was mixed with each NADES at a solid-to-liquid ratio of 1:15 (w/v), following the protocol described by Trivisiol et al., (2020).

All NADES formulations were prepared via a combination of sonication and heating at 50 °C, using a power amplitude of 60%, which corresponds to an ultrasonic energy density of 2.7 kJ·cm^{-3}. The sonication was performed for 10 minutes in an ice bath to prevent thermal degradation, as outlined by Sayago et al., 2023. During ultrasonic homogenization, the system was treated with a 25 mm diameter probe operating at 20 kHz and 750 W nominal power (VibraCell VCX 750, Sonics, Shawnee, OK, USA), as described by Mar et al., 2023. Each prepared system was followed by filtration, and the solution was centrifuged to remove small sample particles. The resulting concentrate was collected and stored at -18 °C [10].

Antioxidant capacity assessment

Antioxidant activity was assessed using DPPH• and ABTS•$^{+}$ radical scavenging assays, ferric reducing antioxidant power (FRAP), and total phenolic content, following previously described methods with slight modifications, using an Epoch 2 microplate reader

(BioTek, USA). Additionally, antioxidant capacity was evaluated through the β-carotene/linolenic acid co-oxidation system [14].

Determination of total phenolic content of extracts (TPC)

The total phenolic content was quantified using the Folin–Ciocalteu colorimetric method. Samples were first reacted with the Folin–Ciocalteu reagent for 5 minutes, followed by the addition of 6% sodium bicarbonate solution. The mixture was then incubated for 90 minutes at room temperature. Absorbance was measured at 750 nm using a microplate reader. Gallic acid was used as the calibration standard ($y = 0.0029x + 0.2008$, $R^2 = 0.9915$), and results were expressed as milligrams of gallic acid equivalents per milliliter of sample (mg GAE/mL) [13].

Total Anthocyanin Content

The total anthocyanin content in *T. speciosum* was determined using a pH differential spectrophotometric method. This technique quantifies anthocyanins based on their structural transformation under different pH conditions, with results expressed in milligrams of anthocyanins per 100 grams of sample. An extinction coefficient of 982 (at pH 2.0) was applied, following established methodology. Absorbance measurements were performed at 535 nm using a microplate reader (Epoch 2, BioTek, Winooski, VT, USA) [15].

Carotenoid Content

The determination of carotenoid content in *T. speciosum* samples was performed using a spectrophotometric method, in which 1 mL of NADES sample was mixed with 6 mL of distilled water and 5 mL of hexane, followed by vigorous vortexing for 1 minute. The resulting supernatant (hexane phase), containing the lipid fraction, was analyzed at 452 nm using a microplate reader (Epoch 2, BioTek, Winooski, VT, USA), with hexane as the blank. Results were expressed as carotenoid content, determined by referencing a previously constructed calibration curve using a β-carotene standard [15].

Color analysis

Color measurements were performed using a DeltaVista spectrophotometer (450G, DeltaColor, Brazil), evaluating the CIELAB coordinates L*, a*, and b* under the following conditions: LED light source, D65 illumination, and a 10° standard observer, as described by de Souza Carvalho et al., 2020. The reference white sample comprised tea unaffected by any temperature influence.

Thermogravimetric Analysis (TGA) of NADES

The runs were performed on a TA Instruments SDT Q600. Samples weighing approximately 10 mg were used, with a heating rate of 10 °C/min from room temperature to a final temperature of 700 °C, under a nitrogen gas flow (N_2 5.0) of 40 mL/min. The crucible used in the tests was a 90 μL platinum pan without a lid.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) in R software (version 3.5.1). Differences among treatment means were assessed using Tukey's test at a significance level of 5% ($p \leq 0.05$).

RESULTS AND DISCUSSION

Deep ionic liquids, commonly known as NADES, typically consist of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). Organic acids, polyols, amides, and sugars are frequently used as HBDs, while quaternary ammonium bases, amino acids, and metal ions are commonly employed as HBAs [17]. Among NADES, those based on amides, acids, and alcohols demonstrate greater efficiency in extracting phenolic compounds compared to sugar-based NADES [18].

In this specific study, experiments were conducted using NADES incorporating alcohol-, acid-, and sugar-based components, containing seven HBDs (citric acid, lactic acid, malic acid, glucose, and glycerol), together with the HBA (choline chloride). The composition of NADES determines their physicochemical properties, which in turn influence their extraction efficiency. For this reason, the performance of various NADES, commonly reported in the literature and prepared using ultrasound-assisted methods, was compared in this study for the removal of bioactive compounds from *T. speciosum* flowers. In total, seven systems based on one group of HBA components (the quaternary amine choline chloride) and four types of HBDs (carboxylic acids, alcohols, and sugars) were tested. Among all these trials, only three systems resulted in satisfactory eutectic mixtures.

The variety of combinations between hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) in the context of NADES are crucial factors impacting the physicochemical properties of these ionic liquids. This explains why the compounds presented in Figure 1 exhibit different concentration values in each extraction.

Extraction is the process by which a solvent removes a desired compound from a solute. The selectivity of different solvents for target compounds arises from their distinct physical and chemical properties. ST2 has a pH in the range of 5–6 and exhibits a slightly acidic characteristic. An appropriate pH can accelerate the rate at which a solvent disrupts

the cell wall, facilitating the dissolution of compounds [19]. The four target compounds were not extracted as efficiently when glucose was used as the HBD.

NADES are increasingly being used for the extraction of anthocyanins with remarkable efficiency. Acid-based DES containing 20–30% water have proven to be the most effective in extracting anthocyanins from samples, outperforming sugar-based systems. Recently, interest in DES for the extraction of anthocyanins from cereals has significantly increased, alongside their use in vegetables, flowers, and herbs. This is due to their outstanding potential to produce plant extracts intended for direct application in the food industry [20]. The extraction of bioactive compounds most commonly utilizes low-frequency ultrasound, resulting in an overall increase in yield of 54% [21].

Conventional liquid-phase extraction using a water/glycerol mixture demonstrated high yields in extracting polyphenols such as chlorogenic acids and quercetin glycosides from *Hypericum perforatum* [22]. Conventional liquid-phase extraction using a water/glycerol mixture demonstrated high yields in extracting polyphenols such as chlorogenic acids and quercetin glycosides from *Hypericum perforatum* [23]. Flavonoids including quercetin, isoquercetin, rutin, trifolin, astragalin, hyperoside, and kaempferol were extracted from *Ribes mandshuricum* leaves using choline chloride/lactic acid [24]. Rutin extraction from flower buds of *Sophora japonica* was carried out using 11 NADES combinations as potential solvents, with choline chloride/glycerol showing the highest extraction efficiency [25]. Polyphenols are well known as antioxidants, and their health-related activities are linked to this property [26].

Carotenoids Content

Choosing a low temperature (50°C) and a short extraction time is an effective strategy to prevent oxidative degradation and isomerization of carotenoids, as well as the formation of free radicals, which can be triggered by high temperatures and cavitation. This selected condition should be easily applicable, with low-cost requirements, to other food sources or food by-products rich in carotenoids, especially β-carotene [27].

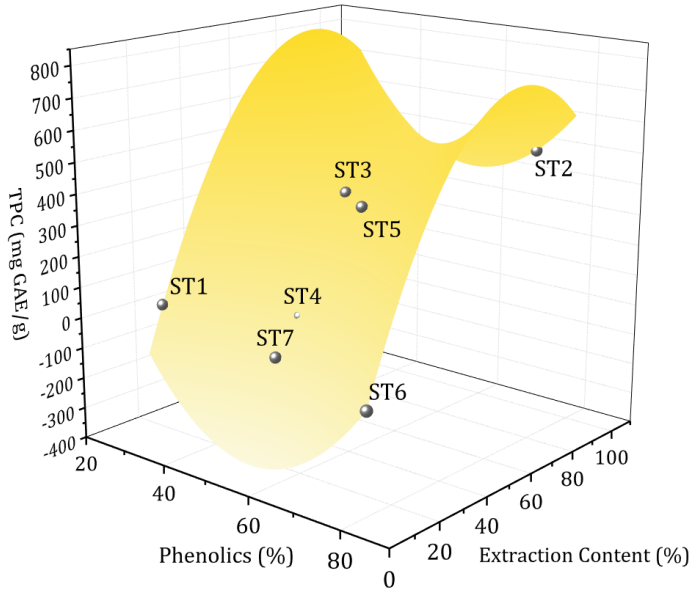


Figure 1: Phenolic compounds content in NADES systems.

The optimization of the β-carotene extraction process from pumpkin was conducted using the ANN model developed to maximize its production, ensuring the highest yield and optimal processing conditions, resulting in a high-quality and high-yield product. The optimal extraction conditions for β-carotene were determined to be a temperature of 60°C and ultrasound power of 60% (52.5 W/cm³) [28].

Based on the ANN model results, the maximum β-carotene content reached 135.39 μg/mL. The validation of the developed ANN model was performed through two independent experiments under the process conditions established to obtain the minimum and maximum β-carotene contents [29].

Based on the results, NADES ST2 stood out as the most efficient in carotenoid extraction. Extraction efficacy is influenced by various experimental parameters such as temperature, ultrasound power, and solvent-to-solid ratio. The carotenoid content in *T. speciosum* flowers may vary depending on the extraction technique used and the experimental conditions. In this research, a significant variation in carotenoid yield was observed, ranging from 15.2 to 247.5 μg/mL, depending on different experimental extraction conditions.

One of the main parameters to be optimized in an extraction process, aiming at energy efficiency and waste minimization, is the matrix-to-solvent ratio. This is because this ratio influences energy consumption and directly impacts the amount of waste produced [30]. Current trends in sustainable extraction aim to reduce solvent use, which contrasts

with our results, as larger volumes of NADES did not increase efficiency due to dilution effects.

Color and Thermal Evaluation

Quantitative color measurements, as shown in Table 2, reveal differences in parameters among the natural deep eutectic solvents. The lightness (L*) values of the samples ranged from 9 to 34, with the lowest L* values recorded for ST1 dissolved in citric acid. Positive values for the green/red parameter (a*) were obtained for all samples. All samples exhibited a* values greater than or equal to 15, and their red color intensity followed the order ST7 > ST6 > ST3 > ST5 > ST2 > ST1 > ST4. All these samples showed higher a* values than a distilled water solution tested for comparison.

Samples	L	a*	b*	ΔE
ST1	9.0 ± 0.1 ^{g}	19.4 ± 0.1 ^{e}	13.0 ± 0.1 ^{g}	44.0 ± 0.1 ^{f}
ST2	10.4 ± 0.1 ^{e}	21.7 ± 0.1 ^{d}	14.1 ± 0.1 ^{f}	47.3 ± 0.1 ^{e}
ST3	9.9 ± 0.1 ^{f}	22.4 ± 0.1 ^{c}	15.8 ± 0.1 ^{e}	48.8 ± 0.1 ^{d}
ST4	34.2 ± 0.1 ^{a}	16.6 ± 0.1 ^{f}	22.6 ± 0.1 ^{d}	35.5 ± 0.1 ^{g}
ST5	24.7 ± 0.2 ^{b}	21.6 ± 0.3 ^{d}	30.0 ± 0.1^{b}	49.2 ± 0.2 ^{c}
ST6	23.0 ± 0.1 ^{c}	31.4 ± 0.1 ^{b}	31.7 ± 0.1 ^{a}	58.3 ± 0.1 ^{b}
ST7	17.1 ± 0.1 ^{d}	38.2 ± 0.1 ^{a}	27.8 ± 0.1 ^{c}	63.6 ± 0.1 ^{a}

Table 2: Results are expressed as mean ± standard deviations (n = 3).

Regarding the blue/yellow parameter (b*), all samples exhibited positive and high b* values, indicating solutions with yellowish hues. The addition of a third HBD component to the natural deep eutectic solvent strongly influenced this color parameter, as the highest and lowest b* values were observed for ST6 and ST7, respectively. The shift toward a less intense yellow value (which could indicate an increase in blue hues) may be attributed to the presence of acid in ST1, ST2, and ST3.

The calculated maximum color intensity values (ΔE) for the samples indicate that ST6 and ST7 exhibit greater (and similar) color intensity compared to the ST4 solution. This suggests that ST1, ST2, and ST3 result in darker samples (lower L* values) with more vivid color (higher ΔE). In other words, the lower chroma (ΔE) values observed for ST6 and ST7 indicate less color vibrancy, which could suggest a faster degradation of anthocyanins in the presence of citric acid.

According to the literature, anthocyanins exhibit a variety of colors in aqueous solutions depending on pH. At very low pH (approximately pH 1), only the red flavylium cation is present. However, as pH increases, different chemical species can coexist in a

complex chemical equilibrium. In this equilibrium, the colorless hemiketal form becomes thermodynamically stable and is mainly responsible for the fading of anthocyanin color [31].

Anthocyanins are highly polar compounds with better solubility in water than in nonpolar solvents. Their chemical form and stability vary according to pH. At pH below 2, anthocyanins predominantly exist as the stable flavylium cation. However, as the pH increases, their structure changes, and degradation occurs above pH 7 [32]. Organic acid-based natural deep eutectic solvents (NADES) have the potential to fully meet the requirements for anthocyanin isolation [33]. Accordingly, seven different NADES were prepared and characterized, with the highest total anthocyanin concentrations (the sum of anthocyanins identified by HPLC) extracted using ST2 > ST3 > ST5 > ST4 > ST6 > ST7 > ST1 (Figure 2). Although polarity values [34, 35 and pH have been previously identified as crucial solvent characteristics for anthocyanin extraction, no obvious correlation was observed, since pH values ranged from 1.70 ± 0.02 to 6.80 ± 0.01.

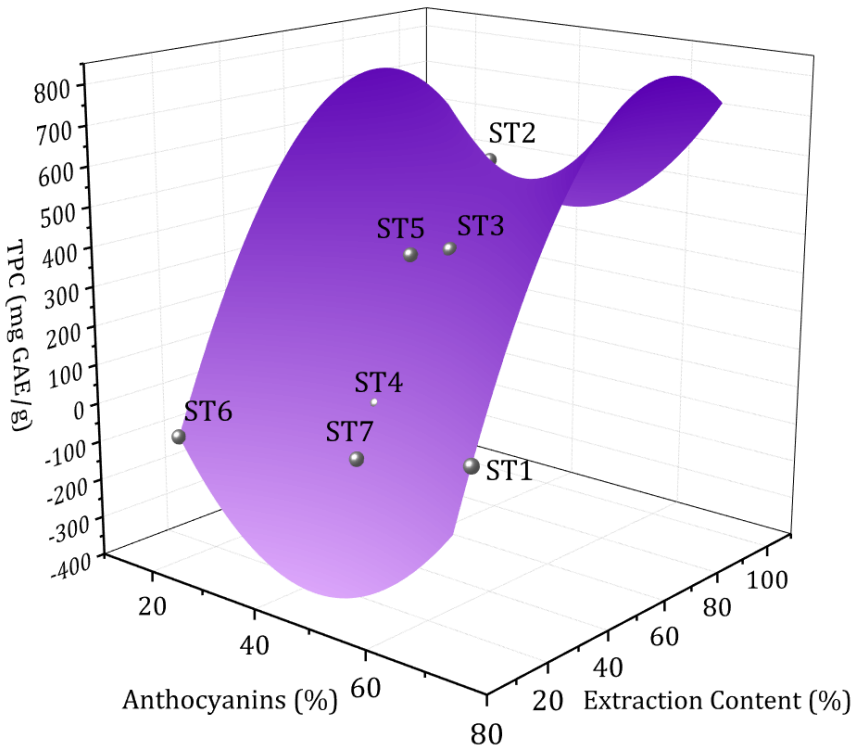


Figure 2: Anthocyanin content in NADES systems.

Similar findings have been reported, indicating that acid-based NADES exhibited lower pH values compared to alcohol- and sugar-based NADES, resulting in higher efficiency in anthocyanin extraction from blackberries [36].

This observation has been corroborated by several studies in which NADES were formed with different components [37]. NADES composed of choline chloride-sugar, choline chloride-organic acid, and choline chloride-alcohol exhibited pH ranges of 2.29 ± 0.02 to 5.05 ± 0.01, 0.76 ± 0.01 to 1.32 ± 0.01, and 5.36 ± 0.02 to 7.04 ± 0.01, respectively [38]. Acid-based solvents showed the lowest pH values, followed by alcohol- and sugar-based NADES [39].

Antioxidant Capacity

The extraction efficiencies of different types of NADES on the total phenolic content (TPC) and antioxidant capacity are illustrated in Table 3. The antioxidant activity of *T. speciosum* flower extracts prepared with NADES was assessed using the DPPH, ABTS, and FRAP methods. Our results showed that all NADES extracts exhibited DPPH and ABTS radical scavenging effects at higher concentrations than the aqueous extract (1031 μmol TE g^{-1} **and 1193 μmol TE g^{-1}, respectively) reported by** Moreira et al. (2021). The highest antioxidant power was observed with Choline chloride: Lactic acid (1401.8 μM Trolox/mL and 1811.8 μM Trolox/mL) > Choline chloride: Malic acid (776.8 μM Trolox/mL and 964.1 μM Trolox/mL) > Choline chloride: Glycerol (747.6 μM Trolox/mL and 830.8 μM Trolox/mL) for the DPPH and ABTS radicals, respectively. The greater antioxidant activity of NADES extracts compared to the aqueous extract is likely attributable to intermolecular interactions, especially hydrogen bonding that stabilizes phenolic compounds [40]. It is important to note that the investigated NADES systems, when evaluated individually, did not cause solution discoloration, suggesting that these NADES solvents cannot donate hydrogen atoms to these radicals.

Overall, a good positive correlation was observed between ABTS and DPPH, with the exception of Choline chloride: Citric acid: Glucose (1:1:1). Differences in antioxidant assay results, as determined by two distinct tests, can likely be attributed to differing mechanisms of action. ABTS assays are based on hydrogen atom transfer, which quantifies the capacity for donating hydrogen atoms [41].

One possible antioxidant mechanism is the reduction of metal ions. In the presence of antioxidant compounds, ferric ion reduction to ferrous ion occurs following a single electron transfer (SET) mechanism [42]. The FRAP method was applied in our study to evaluate the potential of NADES extracts to act as reducing agents. The highest potential to reduce Fe^{3+} to Fe^{2+} was observed with Choline chloride: Lactic acid (1186.8 μM Fe(II)/mL), followed by Choline chloride: Malic acid (726.1 μM Fe(II)/mL), while the aqueous extract showed a value of 1121 μmol Fe(II) g^{-1} **for Fe^{3+}** to Fe^{2+} reduction [14]. The Choline chloride: Citric acid extract showed the lowest electron transfer capacity (244.6 μM Fe(II)/mL). Pure NADES systems tested as controls showed no ability to reduce ferric ions. A study by Jeong et al., (2018) showed that the FRAP assay is highly correlated with total flavonoid content,

while a lower correlation was observed with the DPPH assay, indicating that flavonoids and phenolic compounds are attributed to the antioxidant activity of *T. speciosum* flowers.

Samples	DPPH (μM Trolox/mL)	ABTS (μM Trolox/mL)	TPC (mg GAE/g)	FRAP (μMFe(II)/mL)
ST1	474.3 ± 8.0 [d]	507.4 ± 12.6 [e]	39.8 ± 0.7 [e]	244.6 ± 4.0 [g]
ST2	1401.8 ± 10.4 [a]	1811.8 ± 5.1 [a]	480.1 ± 1.9 [a]	1186.8 ± 3.8 [a]
ST3	776.8 ± 8.0 [b]	964.1 ± 10.7 [b]	392.3 ± 12.1 [b]	726.1 ± 5.7 [b]
ST4	581.8 ± 11.8 [c]	601.9 ± 10.7 [d]	52.9 ± 2.3 [d]	335.4 ± 4.6 [d]
ST5	747.6 ± 12.8 [bc]	830.8 ± 6.9 [c]	369.9 ± 2.5 [c]	616.5 ± 4.2 [c]
ST6	476.8 ± 11.2 [d]	129.7 ± 8.8 [g]	32.1 ± 1.1 [e]	292.8 ± 4.2 [e]
ST7	278.5 ± 11.4 [e]	167.4 ± 13.5 [f]	33.5 ± 2.5 [e]	279.8 ± 5.4 [f]

Table 3: Results are expressed as mean ± standard deviations (n = 3).

The ABTS radical scavenging showed a correlation with the TPC values, similar to that observed in the DPPH activity assay. The results demonstrating the extraction performance of the NADES provided TPC values ranging from 32.1 ± 1.1 to 480.1 ± 1.9 mg GAE/g, while the aqueous extract showed a TPC value of 272.6 ± 0.7 mg GAE/g [14]. NADES exhibited higher TPC than water, methanol, and ethanol, except for ST1, ST4, ST6, and ST7, which showed slightly lower yields. The low extraction efficiency observed in these systems can be mainly attributed to their high viscosity. Dai et al. (2016) observed that the high viscosity of NADES hinders diffusion rates and mass transfer due to the formation of an extensive hydrogen bond network between the acidic hydrogen bond donor (HBD) and the basic hydrogen bond acceptor (HBA).

The effectiveness of the interaction between NADES and phenolic compounds is related to the availability of hydrogen bonds, such as OH and Cl groups [45]. Thus, NADES with a higher capacity to form hydrogen bonds may be more efficient in extraction, even if they exhibit higher viscosity. NADES formed with choline chloride and glycerol, lactic acid, malic acid, ethylene glycol, or acetic acid demonstrated a significant ability to establish multiple hydrogen bond networks with polar compounds, such as phenolic compounds, as reported in previous studies on phenolic compound extraction [46].

Thermogravimetric Analysis (TGA) of NADES

For the future application of natural deep eutectic solvents (NADES), it is essential to determine their thermal stability and other thermal properties. The thermal stability of NADES and their pure components was evaluated through thermogravimetric analysis (TGA), and the results are presented as weight loss versus temperature curves. The TGA curves of NADES containing different HBDs as hydrogen bond donors are summarized in the Figure 3.

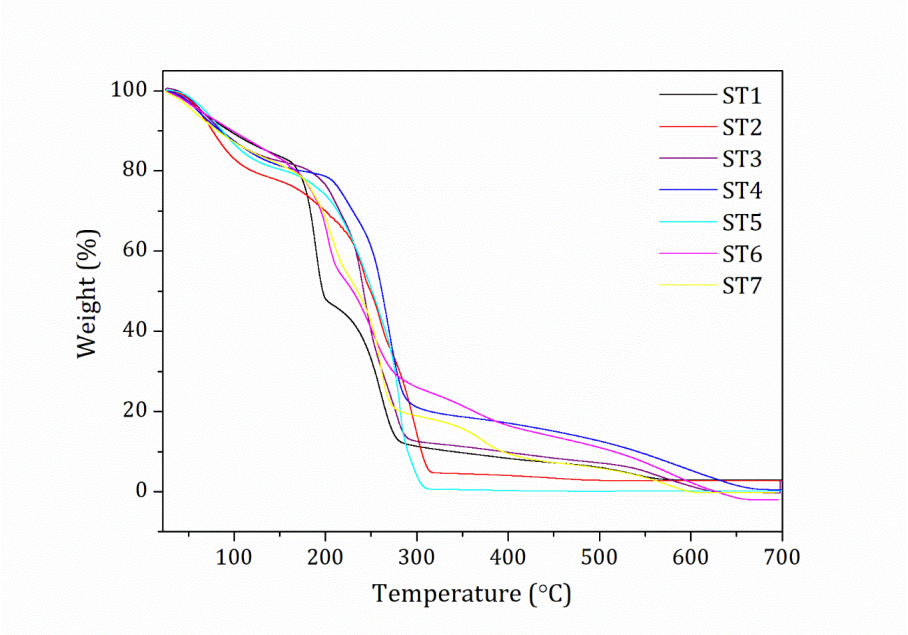


Figure 3: Thermogravimetric Analysis (TGA) Thermograms of NADES

In general, it was observed that when a 5% mass loss occurred at temperatures below 100 ºC, the TGA thermograms mainly showed mass losses associated with the removal of the aqueous component from the NADES. The analysis also indicated that the optimal operating temperatures of the NADES ranged from 190 to 110 ºC, with ST7 and ST1 showing the highest and lowest decomposition temperatures (Tdcp), respectively. The ST7 thermogram revealed a mass loss of 60% between 200 and 280 ºC, while ST1 showed a mass loss of approximately 57% between 290 and 400 ºC, likely due to the decomposition of their corresponding NADES components.

Several factors may be related to the TGA properties, including different HBD and HBA components, the ratio of these components, water content, and the synthesis method. All these elements can influence the interaction and formation of inter- and intramolecular bonds within the complex supramolecular system, thereby contributing to the thermal stability of the solvent [47].

CONCLUSION

In this study, seven methods of NADES preparation, including heating and ultrasound, were applied and compared to extract bioactive compounds from *T. speciosum* flowers. It was observed that NADES provided similar or higher yields of target compounds compared to previously studied aqueous extracts, suggesting that organic solvents can be replaced

by these systems without loss of extraction efficiency. Among the tested NADES, those containing carboxylic acids as hydrogen bond donors showed better extraction capacity for flavonoids and phenolic acids. pH also played an important role and should be considered when interpreting the results. Extracts prepared with NADES exhibited significant antioxidant capacity, as evidenced by DPPH, ABTS, and ferric reducing assays. Based on these results, NADES can be valuable for isolating bioactive compounds from plant sources and have potential for various analytical and chemical applications as sustainable and accessible solvents. Furthermore, given the nature of NADES components, these solvents may be explored in sectors such as food and pharmaceuticals, contributing to the promotion of human health.

AUTHOR CONTRIBUTIONS

J. de A. B. and E.A.S. designed the study and supervised the experiments, J.M.M. and A. de S.C. contributed in methodology, R.Z. de A.N. and S. dos S.C. made contributions in formal analysis, R.F.C. and V.F.K were responsible for data curation, and E.A.S. and P.H.C. assisted in writing – original draft preparation.

FUNDING

This research received no external funding.

ACKNOWLEDGEMENTS

The authors acknowledge the Support Program for Stricto Sensu Graduate Studies – POSGRAD (Fundação de Amparo à Pesquisa do Estado do Amazonas — FAPEAM) for the support provided through the scholarship, as well as the Analytical Centers at IFAM-CMC and UFAM for providing laboratory infrastructure support.

CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

[1] O. Zannou and I. Koca, Greener extraction of anthocyanins and antioxidant activity from blackberry (*Rubus spp*) using natural deep eutectic solvents. LWT, 2021; 8(158): 113184, 2022.

[2] S. B. Puranik, P. N. S. Pai, and G. K. Rao, Determination of Organic Volatile Impurities in Herbal Formulations and Extracts. Pharmacogn. Mag., 2009; 4(18): 139–144.

[3] J. Sousa, V. Rafael, A. Brizola, and D. Granato, High-throughput assay comparison and standardization for metal chelating capacity screening: A proposal and application. Food Chem., 2017; 214(1): 515–522, 2017.

[4] M. Zhang et al., Structure – properties relationships of deep eutectic solvents formed between choline chloride and carboxylic acids: Experimental and computational study, J. Mol. Struct., 2023; 1273(1): 1–9, 2023.

[5]Y. Tang, X. He, J. Sun, G. Liu, C. Li, and L. Li, Comprehensive evaluation on tailor-made deep eutectic solvents (DESs) in extracting tea saponins from seed pomace of *Camellia oleifera Abel*, Food Chem., 2020; 342(9): 1–10.

[6] M. C. Ali, J. Chen, H. Zhang, Z. Li, L. Zhao, and H. Qiu, Effective extraction of flavonoids from *Lycium barbarum L .* fruits by deep eutectic solvents-based ultrasound-assisted extraction, Talanta, 2019; 203(1): 16–22.

[7] M. E. Alanón, M. Ivanovic, A. M. Gómez-Caravaca, D. Arraáez-Román, and A. Segura-Carretero, Choline chloride derivative-based deep eutectic liquids as novel green alternative solvents for extraction of phenolic compounds from olive leaf, Arab. J. Chem., 2020; vol. 13(1): 1685–1701.

[8] O. Zannou, H. Pashazadeh, S. A. Ibrahim, I. Koca, and C. M. Galanakis, Green and highly extraction of phenolic compounds and antioxidant capacity from kinkeliba (*Combretum micranthum G . Don*) by natural deep eutectic solvents (NADESs) using maceration, ultrasound-assisted extraction and homogenate-assisted extraction, Arab. J. Chem., 2022; 15(5):1-13.

[9] E. A. Rebelatto, L. G. G. Rodrigues, A. R. Rudke, K. S. Andrade, and S. R. S. Ferreira, The Journal of Supercritical Fluids Sequential green-based extraction processes applied to recover antioxidant extracts from pink pepper fruits, J. Supercrit. Fluids, 2020; 166(1):22-37,

[10] S. Lin et al., Ultrasonics Sonochemistry Composition and antioxidant activity of anthocyanins from *Aronia melanocarpa* extracted using an ultrasonic-microwave-assisted natural deep eutectic solvent extraction method, Ultrason. Sonochem., 2022; 89(7): 106102.

[11] D. Trivisiol et al., Deep eutectic solvents as a biocompatible tool for the extraction of blueberry anthocyanins, J. Food Compos. Anal., 2020; 89(1): 103470.

[12]A. Sayago, J. Urbano, and E. Cort, Biomass and Bioenergy Ultrasound-assisted extraction of phenolic compounds from blueberry leaves using natural deep eutectic solvents (NADES) for the valorization of agrifood wastes, Biomass Bioenergy J., 2023; 175(6): 1-19.

[13] J. M. Mar et al., Enhancing Bioactive Compound Bioaccessibility in *Tapirira guianensis* Juices through Ultrasound-Assisted Applications, Process, 2023; 11(2718): 1–16.

[14] J. Moreira, L. Souza, W. Picanço, and A. Bezerra, Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds, Food Chem., 2021;356 (3): 1-9,

[15] A. F. A. Oliveira et al., Non-thermal combined treatments in the processing of açai (*Euterpe oleracea*) juice, Food Chem., 2018; 265(4): 57–63.

[16] L. M. de Souza Carvalho et al., Improvement of the bioaccessibility of bioactive compounds from Amazon fruits treated using high energy ultrasound, Ultrason. Sonochem., 2020; 67(4): 105148.

[17] K. C. Duru et al., An eco-friendly approach to enhance the extraction and recovery efficiency of isoflavones from kudzu roots and soy molasses wastes using ultrasound-assisted extraction with natural deep eutectic solvents (NADES), Ind. Crops Prod., 2022; 182(3)}: 114886.

[18] W. Wang, Y. Pan, J. Zhao, Y. Wang, Q. Yao, and S. Li, Development and optimization of green extraction of polyphenols in *Michelia alba* using natural deep eutectic solvents (NADES) and evaluation of bioactivity, Sustain. Chem. Pharm., 2024; 37(1): 101425.

[19] N. P. E. Hikmawanti, D. Ramadon, I. Jantan, and A. Mun'im, Natural deep eutectic solvents (Nades): Phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development, Plants, 2021; 10(10):10102091.

[20] R. Gupta, M. Meghwal, and P. K. Prabhakar, Bioactive compounds of pigmented wheat (*Triticum aestivum*): Potential benefits in human health, Trends Food Sci. Technol., 2021; 110(10): 240–252.

[21] L. Estivi, A. Brandolini, L. Condezo-Hoyos, and A. Hidalgo, Impact of low-frequency ultrasound technology on physical, chemical and technological properties of cereals and pseudocereals, Ultrason. Sonochem., 2022; 86(5): 106044.

[22] V. C. Roy, J. S. Park, A. R. Haque, M. S. Ali, H. J. Lee, and B. S. Chun, Bio-refinery of brewery spent grain utilizing natural deep eutectic solvent-induced subcritical water, J. Supercrit. Fluids, 2023; 204(9): 106108.

[23] B. Karakashov, S. Grigorakis, S. Loupassaki, and D. P. Makris, Optimisation of polyphenol extraction from *Hypericum perforatum* (St. John's Wort) using aqueous glycerol and response surface methodology, J. Appl. Res. Med. Aromat. Plants, 2015; 2(1): 1–8.

[24] H. B. Balaraman, A. Sivasubramaniyam, and S. K. Rathnasamy, High selective purification of Quercetin from Peanut hull using protic deep eutectic mixture based liquid–liquid microextraction, Microchem. J., 2019; 152(11): 104444.

[25]L. Lojková, H. Pluháčková, K. Benešová, B. Kudláčková, and R. Cerkal, The highest yield, or greener solvents? Latest trends in quercetin extraction methods, TrAC - Trends Anal. Chem., 2023; 167(7): 1-12.

[26] H. Mostafa, J. O. Airouyuwaa, F. Hamed, Y. Wang, and S. Maqsood, Structural, mechanical, antioxidant and antibacterial properties of soy protein isolate (SPI)-based edible food packaging films as influenced by nanocellulose (NC) and green extracted phenolic compounds from date palm leaves, Food Packag. Shelf Life, 2023; 38(7): 1-17.

[27] A. Stupar et al., Recovery of β-carotene from pumpkin using switchable natural deep eutectic solvents, Ultrason. Sonochem., 2021; 76(6): 1-22.

[28] P. S. Madamba, The response surface methodology: An application to optimize dehydration operations of selected agricultural crops, Lwt, 2002; 35(7): 584–592.

[29] D. Augustynska, M. Jemioła-Rzemiå ska, K. Burda, and K. Strzałka, Influence of polar and nonpolar carotenoids on structural and adhesive properties of model membranes, Chem. Biol. Interact., 2015; 239(1): 19–25.

[30] C. Vakh, A. Pochivalov, V. Andruch, L. Moskvin, and A. Bulatov, A fully automated effervescence-assisted switchable solvent-based liquid phase microextraction procedure: Liquid chromatographic determination of ofloxacin in human urine samples, Anal. Chim. Acta, 2016; 907(1): 54–59.

[31] H. K. S. Souza, N. Mateus, V. De Freitas, M. P. Gonçalves, and L. Cruz, Chemical/color stability and rheological properties of cyanidin-3-glucoside in deep eutectic solvents as a gateway to design task-specific bioactive compounds, ACS Sustain. Chem. Eng., 2020; 8(43): 16184–16196.

[32] T. Bosiljkov et al., Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins, Food Bioprod. Process., 2017; 102(1): 195–203.

[33] M. Cvjetko Bubalo, N. Ćurko, M. Tomašević, K. Kovačević Ganić, and I. Radojcic Redovnikovic, Green extraction of grape skin phenolics by using deep eutectic solvents, Food Chem., 2016; 200(1): 159–166, 2016.

[34] M. Panić, V. Gunjević, G. Cravotto, and I. Radojčić Redovniković, Enabling technologies for the extraction of grape-pomace anthocyanins using natural deep eutectic solvents in up-to-half-litre batches extraction of grape-pomace anthocyanins using NADES, Food Chem., 2019; 300 (6):1587.

[35] A. Simamora, K. H. Timotius, H. Setiawan, M. Y. Putra, and A. Munim, Natural deep eutectic solvent extraction of xanthorrhizol and curcuminoids from *Curcuma xanthorrhiza Roxb* and simultaneous determination by high-performance liquid chromatography, J. Pharm. Pharmacogn. Res., 2023; 11(6): 1056–1071.

[36] H. Huang et al., Valorization and protection of anthocyanins from strawberries (*Fragaria×ananassa Duch.*) by acidified natural deep eutectic solvent based on intermolecular interaction, Food Chem., 2023; 447(12): 138971.

[37] O. Zannou and I. Koca, Optimization and stabilization of the antioxidant properties from Alkanet (*Alkanna tinctoria*) with natural deep eutectic solvents, Arab. J. Chem., 2020; 13(8): 6437–6450.

[38] I. Adeyemi, M. R. M. Abu-Zahra, and I. M. AlNashef, Physicochemical properties of alkanolamine-choline chloride deep eutectic solvents: Measurements, group contribution and artificial intelligence prediction techniques, J. Mol. Liq., 2018; 256(1): 581–590, 2018.

[39] A. García, E. Rodríguez-Juan, G. Rodríguez-Gutiérrez, J. J. Rios, and J. Fernández-Bolaños, Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs), Food Chem., 2016; 197(1): 554–561.

[40] J. B. Barbieri, C. Goltz, F. Batistão Cavalheiro, A. Theodoro Toci, L. Igarashi-Mafra, and M. R. Mafra, Deep eutectic solvents applied in the extraction and stabilization of rosemary (*Rosmarinus officinalis L.*) phenolic compounds, Ind. Crops Prod., 2018; 144(12): 112049.

[41] M. Ivanović et al., Extraction of Bioactive Metabolites from *Achillea millefolium L.* with Choline Chloride Based Natural Deep Eutectic Solvents: A Study of the Antioxidant and Antimicrobial Activity, Antioxidants, 2022; 11(4):105879,

[42] Y. Dai, J. van Spronsen, G. J. Witkamp, R. Verpoorte, and Y. H. Choi, Natural deep eutectic solvents as new potential media for green technology, Anal. Chim. Acta, 2013; 766(1): 61–68.

[43] K. M. Jeong, Y. Jin, D. E. Yoo, S. Y. Han, E. M. Kim, and J. Lee, One-step sample preparation for convenient examination of volatile monoterpenes and phenolic compounds in peppermint leaves using deep eutectic solvents, Food Chem., 2018; 251(1): 69–76.

[44] Y. Dai, E. Rozema, R. Verpoorte, and Y. H. Choi, Application of natural deep eutectic solvents to the extraction of anthocyanins from Catharanthus roseus with high extractability and stability replacing conventional organic solvents, J. Chromatogr. A, 2016; 1434(1): 50–56.

[45] N. Alsaud, K. Shahbaz, and M. Farid, Application of deep eutectic solvents in the extraction of polyphenolic antioxidants from New Zealand Manuka leaves (*Leptospermum Scoparium*): Optimization and antioxidant activity, J. Mol. Liq., 2021; 337(1): 116385.

[46] P. V. de Almeida Pontes, I. Ayumi Shiwaku, G. J. Maximo, and E. A. Caldas Batista, Choline chloride-based deep eutectic solvents as potential solvent for extraction of phenolic compounds from olive leaves: Extraction optimization and solvent characterization, Food Chem., 2021; 352(10):1-14.

[47] M. S. C. Zain, J. X. Yeoh, S. Y. Lee, and K. Shaari, Physicochemical properties of choline chloride-based natural deep eutectic solvents (Nades) and their applicability for extracting oil palm flavonoids, Sustain., 2021; 13(23): 1-15.

CAPÍTULO 5

Theobroma spp.: Uma revisão sobre seu potencial químico e de inovação para a indústria de alimentos.

Resumo: Atualmente, o Brasil é considerado um dos países que ainda detém grande biodiversidade de fauna e flora e, dentro dessa ampla diversidade, destaca-se a família Malvaceae, que apresenta 243 gêneros e cerca de 4.300 espécies. Nessa família, o gênero *Theobroma* se sobressai pelo valor econômico e pela múltipla aplicabilidade nos setores alimentício, nutricional, medicinal e artesanal. Devido à sua relevância, o principal objetivo desta revisão foi compilar os principais artigos publicados nos últimos 10 anos sobre *Theobroma spp*., abordando suas propriedades químicas, biológicas e farmacológicas, bem como aplicações e patentes de produtos funcionais. Entre as 20 espécies do gênero *Theobroma spp*. relatadas em estudos experimentais, observou-se que sua composição química é rica em ácidos fenólicos, como os ácidos p-cumárico, cafeico e clorogênico, além de flavonóis (catequina e epicatequina), flavonas e derivados como a hipoletina. Assim, este estudo apresenta uma prospecção tecnológica com base no panorama mundial de patentes.

Palavras-chave: Antioxidante, Compostos fenólicos, Flavonoides, Cacau

Food Chemistry Advances 4 (2024) 100683

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Theobroma spp.: A review of it's chemical and innovation potential for the food industry

Josiana Moreira Mar [a,b], Edson Queiroz da Fonseca Júnior [b,c], Renilto Frota Corrêa [b], Pedro Henrique Campelo [d], Edgar Aparecido Sanches [e], Jaqueline de Araújo Bezerra [a,b,*]

[a] Postgraduate Program in Biotechnology, Federal University of Amazonas (UFAM), Manaus 69067-005, Brazil
[b] Analytical Center of the Federal Institute of Education, Science and Technology of Amazonas (IFAM), Campus Manaus Centro, Manaus 69020-120, AM, Brazil
[c] Postgraduate Program in Chemistry, Federal University of Amazonas, (UFAM), Manaus 69067-005, Brazil
[d] Department of Food Technology, Federal University of Viçosa (UFV), Viçosa 36570-900, MG, Brazil
[e] Laboratory of Nanostructured Polymers (NANOPOL), Materials Physics Department, Federal University of Amazonas (UFAM), Manaus 69067-005, AM, Brazil

ARTICLE INFO

Keywords:
Antioxidant
Phenolic compounds
Flavonoids
Cocoa

ABSTRACT

Nowadays, Brazil is considered one of the countries that still has a great biodiversity of fauna and flora, and within this wide diversity, the Malvaceae family stands out, presenting 243 genera and 4,300 species. In this family, the genus *Theobroma.* Is highlighted for the economic value of multiple applicability in food, nutritional, medicinal and craft sectors. Due to its importance, the major objective of this review was to compile the main articles of the last 10 years on the *Theobroma* spp. based on chemical, biological and pharmacological properties, as well as applications and patents of functional products. Of the 20 species of the genus *Theobroma* spp. reported in experimental studies, it was observed that its chemical composition is rich in phenolic acids, such as p-coumaric acids, caffeic and chlorogenic, as well as flavonols (catechin and epicatechin), flavones and derivatives such as hypoletin. Thus, this study shows the technological prospection based on the worldwide patent landscape.

1. Introduction

The Amazon rainforest has one of the highest rates of biodiversity in the world. It is a gene pool for many animal and plant species and an endless source of variability for endemic species, including *Theobroma* spp., a prominent genus of the Malvaceae family, formerly called Sterculiaceae. *Theobroma* spp. is distributed in the rainforests of the southern hemisphere, from Mexico to the southern Amazon rainforest (Giustina et al., 2014).

The Malvaceae family, presents species that are part of the list of medicinal plants and, therefore, with relevance for chemical and pharmacological studies. This family contains 246 genera and more than 4225 species distributed worldwide, specifically in tropical and temperate regions, and in Brazil there are approximately 80 genera and 400 species found (Giustina et al., 2014; Mar et al., 2021; Pérez-Mora et al., 2018).

The *Theobroma* genus has 20 species, which 9 are native to the Amazon region. These species are a vital resource for indigenous communities in Latin America and other communities in countries with a tropical climate distributed across different continents such as Asia and Africa. It is part of the human and animal diet in the Amazon, with potential interest in the agro-industrial sector (Motamayor et al., 2013).

Theobroma spp. fruits have a pleasant smell and taste, it also has multiple uses in the manufacture of juices, jellies, ice cream, liqueurs, marmalades and other products. Although they are cultivated only at a regional level, some species such as *T. bicolor* Bonpl. (macambo or bacao) and *T. grandiflorum* (Willd. ex Spreng.) K. Schum (cupuaçu or cupu) are in the process of domestication and technification (Pérez-Mora et al., 2018).

The antioxidant activity and phenolic composition of the pulp juice of *T. cacao L., T. bicolor* and *T. grandiflorum* were described by Pérez-Mora et al. (2018), studies of cupuaçu pulp and seeds were reported by Pugliese et al. (2013) and according to Oliveira and Genovese (2013) the same activity has been discussed in composition of cocoa liquors.

The seeds of *T. cacao, T. bicolor* e *T. grandiflorum* are used in all of the world in the production of chocolate (roasted seeds), applied in sweets and cosmetics. It is important to emphasize that the close relatives of

* Corresponding author.
E-mail address: jaqueline.araujo@ifam.edu.br (J.A. Bezerra).

https://doi.org/10.1016/j.focha.2024.100683
Received 8 February 2024; Received in revised form 19 March 2024; Accepted 22 March 2024
Available online 27 March 2024
2772-753X/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

cocoa, from other *Theobroma* species, can be used in genetic improvement programs, as a source of genes related to the desired phenotypes, highlighting their disease resistance, drought tolerance, high protein content and other agronomic characteristics along with its economic interest (Motamayor et al., 2013).

An interesting and promising example of *T. speciosum* Willd. ex Spreng, a species native to the Amazon region, is popularly known as cocoaí, still little widespread in Brazil, despite its traditional areas of occurrence. It has agronomic importance for presenting a possible source of resistance genes for other species of the genus, in genetic improvement and great potential to produce derived foods for human consumption, it can also be found throughout the humid tropics and among the species of the genus (Colli-Silva & Pirani, 2020).

The efficient use of these species requires a detailed knowledge of their biology, especially from a genetic and molecular point of view; however, till this date, only a few studies have been published about this topic. A substantial equivalence analysis between commercial cacao and its wild relatives is needed as a preliminary step before validating, producing and commercializing Amazonian *Theobroma* species as a food alternative. This result will improve consumer confidence and acceptance, opening new markets interested in exotic fruits, avoiding problems of illicit crops (non-standard), encouraging good practices with applied technologies and increasing the income of family farmers or indigenous producers (Pérez-Mora et al., 2018).

In the southern hemisphere of Latin America, studies involving the fruits of *Theobroma* species are limited, mainly regarding their chemical composition and biological activities. The *T. cacao* widely used in the food industry, are rich in polyphenols and methylxanthines, which are related to the blockade of adenosine receptors, central nervous system stimulants, and beneficial effects on the cardiovascular system (Franco et al., 2013).

Tauchen et al. (2016) demonstrated the antiproliferative activity of *T. bicolor* seed in relation to cancer cells Hep-G2 (hepatocellular carcinoma) and HT-29 (colon carcinoma). Chemical and biological data of *T. angustifolium* DC, *T. mammosum* Cuatrec. & J. León, *Herrania mariae* (Mart.) Goudot, *T. microcarpum* Mart., *T. simiarum* Donn. Sm., *T. speciosum* Willd. Ex Spreng. and *T. guianense* (Aubl.) J.F. Gmel. are still scarce. The development of foods with functional properties is a growing field aiming to attend the new demands of the consumer market. Therefore, the insertion of new ingredients plays an important role in the composition of foods, as it exerts a great influence on the characteristics, adding value to the final product (for example, color, flavor, rheological properties, water and oil retention capacity), stimulating the productive chain of the species, the cultivation and generating employment and income for the human communities of rural producers (Silva et al., 2014).

Several studies have shown that peels, bagasse and seeds of *Theobroma* fruits are good sources of food nutrients and phytochemicals bioactives, including phenolic compounds, carotenoids and others. These co-products are promising sources of alternative food ingredients in the various branches of the food, pharmaceutical and other industries (Kababie-Ameo et al., 2022).

This review represents an updated and comprehensive compilation of articles published in the last 10 years on the genus *Theobroma* spp. The main objective was to show the chemical composition, as well as the chemical, biological and pharmacological properties, including the applications in patents of the species, belonging to this genus, to arouse the interest of researchers and entrepreneurs for the development of new functional products and processes of industrial production.

2. Research strategy and eligibility criteria

This review was searched in Scopus, Capes Periodics, Google Database and PubMed. The search was performed using the terms "*Theobroma* AND caffeine OR theobromine OR theophylline OR products OR nutritional OR chemical OR antibacterial OR activity", considering title, abstract and keywords. The search for data on chemical composition, constituents and biological activities of species of the genus *Theobroma* were realized in Scopus and PubMed. The keywords consisted of "*Theobroma* AND alkaloids OR organic acids OR flavonoids OR phenolic compounds OR biological OR activity", evaluating titles and abstracts. All of the articles resulting from the researched selection were read in their entirety, based on the adopted criteria. Following the steps: the elaboration of a tool with the following information: author, date, species, main constituents, quantification and identification of constituents and also biological activities. 30 articles were gathered for this selection of chemical composition. Published articles were considered from 2012 to May 2022. The selected articles reported the chemical composition and properties of *Theobroma* fruits, totalizing 56 articles.

Have been performed an analysis of 500 patents contained in the patent databases: GOOGLE PATENTS, USTPO and ESPACENET, using the Booleans: "Fruits of *Theobroma*" AND "Food Technology" AND "Functional Food" AND "Biotechnological application" AND "Medicinal Application". Patents (30) were selected using as an evaluative criterion fruits of *Theobroma* and their applications in the fields of Food Technology, Functional Foods, Biotechnological Application and Medicinal Application. The search was performed on November 28, 2022.

3. Chemical composition of *Theobroma* (malvaceae) species

The Malvaceae family comprises several classes of chemical compounds. In the case of phytochemical studies of this family, the present compounds, especially of the genus *Theobroma*, are alkaloids, organic acids, flavonoids and their derivations. Besides the classes of compounds mentioned, the family also has many studies with substances from the class of phenylpropanoids and derivatives, as well as many phenolic compounds, a class of considerable value since they have been associated with various health-promoting effects, such as prevention of different types of cancer and coronary heart disease (Perez-Vizcaino & Fraga, 2018).

The chemical composition of the genus *Theobroma* has high levels of fat consisting of stearic, oleic, palmitic and linoleic fatty acids, in varying amounts. Sterols, fibers, minerals and methylxanthine compounds such as theobromine, theophylline and caffeine were relevant in its composition, being also known for their rich polyphenol contents (Jean-Marie et al., 2021).

The main compounds quantified were alkaloids from the flour of the *T. cacao* species, with theobromine, caffeine and the two main catechins being found. The fruit peel had the highest theobromine content (10.21 μg /g), while the seed had the highest content of caffeine, epicatechin and catechin (1.11 μg/g, 3.40 μg/g e 3.09 mg/g), respectively.

Signs of organic acids were attributed to citric and malic acids, the phenolic compounds to protocatechuic acid, quercetin, quercetin pentoside and quercetin-3-*O*-glucoside (Mar et al., 2021), quercetin being reported in *T. cacao* (Sánchez-Rabaneda et al., 2003).

Caffeine, theobromine and theophylline occur naturally in a wide variety of foods and beverages such as coffee, cocoa, tea, yerba mate as well as energy drinks and are widely consumed throughout the world (Martínez-lópez et al., 2014).

Caffeine and theobromine are considered biomarkers for caffeinated beverages (Dozier et al., 2020). Although considerable amounts of caffeine and theobromine can be consumed from dietary sources (coffee, tea, cola drinks and chocolate), it is believed that only relatively small amounts of theophylline are ingested from the same sources. Theophylline occurs naturally in tea and in trace amounts in cocoa and coffee beans. Theobromine levels have been reported to represent only 0.15 %−0.46 % in different types of chocolate (Monteiro et al., 2016).

3.1. Alkaloids (methylxanthines)

Alkaloids, derived from the special (secondary) metabolism of plants, have as their primary function the protection of the species

against herbivores and pathogenic organisms (Huang et al., 2021). Some alkaloids (Fig. 1) were isolated from species of the genus *Theobroma* (Table 1). Of the different types of alkaloids, the *Theobroma* genus presents in its composition common purine alkaloids such as caffeine, theobromine and theocrine, found especially in seeds and leaves of different species of the genus. Purine alkaloids are secondary metabolites derived from purine nucleotides. Present in about 100 species in 13 orders of the plant kingdom (Ashihara & Crozier, 1999), including Malvales. Hammerstone et al. (1994) described the content of purine alkaloids by high performance liquid chromatography in seeds and leaves of eleven species of *Theobroma*, and showed that 1,3,7,9-tetramethyluric acid (theacrine) was the main purine alkaloid present in the samples. The authors have not detected the presence of theophylline in the studied samples.

3.2. Phenylpropanoids

Phenylpropanoids, obtained from the deamination of L-phenylalanine, are non-essential phytochemicals for the survival of plants, thus being classified as secondary compounds. In recent years, the attention given to the class is due to the substances present that carry out medicinal action as an antioxidant (Vladimir-Knežević et al., 2012), UV filter (Takshak & Agrawal, 2019), anti-inflammatory agents (Cunha et al., 2019), healing (Ambrosone et al., 2014), antibacterial and anticancer (Gao et al., 2019).

The genus *Theobroma* (Malvaceae) has two major classes of phenyl propanoids, such as cinnamic acid and to coopherol derivatives (Table 1). Two common cinnamic acid derivatives, p-coumaric acid and caffeic acid, and two cinnamic acid derivatives containing quinic acid, p-coumaroylquinic acid and chlorogenic acid, were isolated from *T. cacao* leaf by Jalal and Collin (1977). The study from Tauchen et al. (2016) characterized the samples by UHPLC-MS/MS detecting the same constituents in leaves, seeds and pericarp of *T. bicolor, T. cacao* and *T. grandiflorum*. In another study, Bruni et al. (2002) isolated four types of tocopherol, α, β, γ and tocopherol δ, from the seed of *T. subincanum*. Febrianto and Zhu (2022) detected the derivative of the phenolic acid Clovamide in samples of *T. grandiflorum, T. bicolor* and *T. subincanum*.

3.3. Flavonoids

Flavonoids are an important class of natural products found in fruits, vegetables and some beverages. They have a polyphenolic structure and are known to be synthesized in specific locations in the plant and are mainly responsible for the color and aroma of the flowers, pollination and dispersal of seeds and spores, besides the development of some seedlings (Griesbach, 2010). Flavonoids from the Malvaceae family include flavones, flavonols and their derivatives (Table 1). Simple flavonols such as catechin and epicatechin have been found in *T. cacao* grains by Quiroz-Reyes and Aguilar-Méndez (2022) and seeds of *T. grandiflorum* by De Oliveira et al. (2015). Ramos-Escudero et al. (2021) isolated procytian (proanthocyanidin) in *T. cacao* beans and Pugliese et al. (2013) flavone vitexin obtained from *T. grandiflorum* seeds. Flavone glycosides, such as hypoletin, quercetin and isoscutelarein are distributed in seeds and flowers of species of the *Theobroma* genus (Febrianto & Zhu, 2022a; Mar et al., 2021; Pugliese et al., 2013).

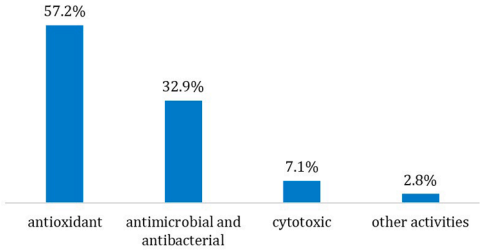


Fig. 1. Properties reported in studies of *Theobroma* spp.

4. Antioxidant activity of the genus *Theobroma*

The FRAP from the grain flour exhibited a weak reducing power relative to the pod bark, but the total phenolic content (111.05 mg GAE/g flour) was higher than that reported for cocoa pod bark flour (5.4 a 16.6 mg GAE/g flour). Furthermore, the DPPH content reported for wheat flours varied between 4 and 5 μmol equivalents of Trolox/g, lower value than cocoa residue flours (46 and 87 μmol equivalents of Trolox/g) and cocoa bark flours, respectively (Vargas-arana et al., 2022).

Lee et al. (2003) investigated the total phenolic and flavonoid concentration of cocoa, black tea, green tea, and red wine. The phenolic and flavonoid content of the cocoa per serving was 611 mg of gallic acid equivalents (GAE) and 564 mg of epicatechin equivalents (ECE), respectively. As a result, the highest amount of phenolic and flavonoid content was detected in cocoa as compared to black tea (124 mg GAE and 34 mg ECE), green tea (165 mg GAE and 47 mg ECE), and red wine (340 mg GAE and 163 mg ECE). Moreover, ABTS and DPPH radical scavenging activity assays indicated the highest antioxidant activity in cocoa.

Comparing the antioxidant potential of *T. cacao* species from French Guiana and Brazil, French-Guyanese seeds showed DPPH activity equivalent to two mixtures of Brazilian cacao hybrids capable of 324.06 ± 51.7 and 363.3 ± 120.1 μmol TE/g of dry matter, respectively. For the ORAC essay, Guyana showed a higher capacity when compared to seeds of the *T. cacao* species from Africa Forastero (303 ± 5 μmol TE/g dry matter). In the analysis of total polyphenols, for cupuaçu pulp, the values varied from 27.9 ± 1 mg EAG/100 g to 32.5 ± 1 mg EAG/100 g (Jean-Marie et al., 2021).

In the literature, *Theobroma* species have represented a great pharmacological potential (Fig. 1), having as main activities described the antioxidant potential (57.2 %), antimicrobial activity (18.1 %), antibacterial activity (14.8 %), cytotoxic activity (7.1 %) and hematic, antiobesity and cytoprotective activity (2.8 %). This demonstrates the importance of gender-related studies for the development of future products.

5. Chemical, biological and pharmacological properties

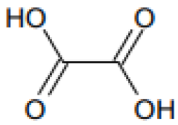
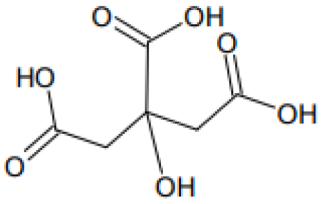
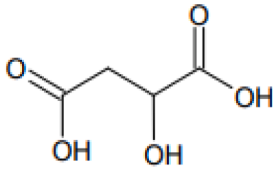
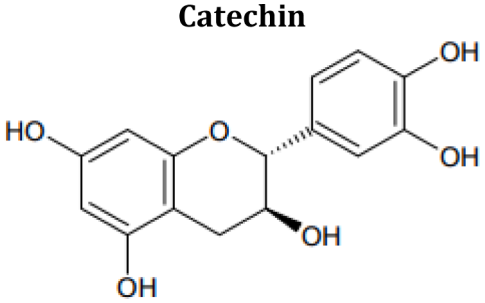
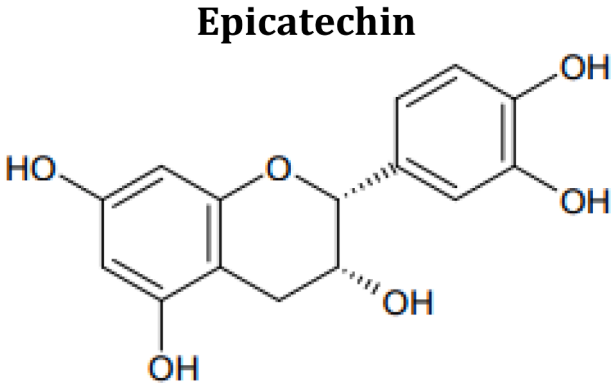
5.1. Food applications

Grains from *T. grandiflorum, T. bicolor*, and *T. subincanum* species showed high potential as alternatives for functional food ingredients, in addition to their flavoring potential. *T. bicolor* samples with their white color may be suitable for the development of products resembling chocolate with a shiny appearance (Febrianto & Zhu, 2022b). Substantial equivalence studies were conducted on three *Theobroma* spp., *cacao, bicolor*, and *grandiflorum* through analysis of chemical composition and protein profile of fruits (pulp juice and seeds). Principal component analysis of sugar content, organic acid, and phenol in pulp juice revealed equivalence among the three species, with differences in some compounds leading to distinct organoleptic properties (Pérez-mora et al., 2018).

To preserve the compounds and their activity, cupuaçu pulp was subjected to spray drying using blends of inulin/maltodextrin as wall materials, under simulated intestinal fluid conditions, resulting in the release of 76.7 % of the ascorbic acid present in the species. These findings indicate the great potential of cupuaçu microencapsulates as a strategy to enhance the value of Amazonian fruits (Acosta-Vega et al., 2023). Research on the addition of cupuassu pulp and flour has contributed to improving the physicochemical and sensory characteristics of fermented dairy beverages, potentially increasing their nutritional value (Gutiérrez-Álzate et al., 2023).

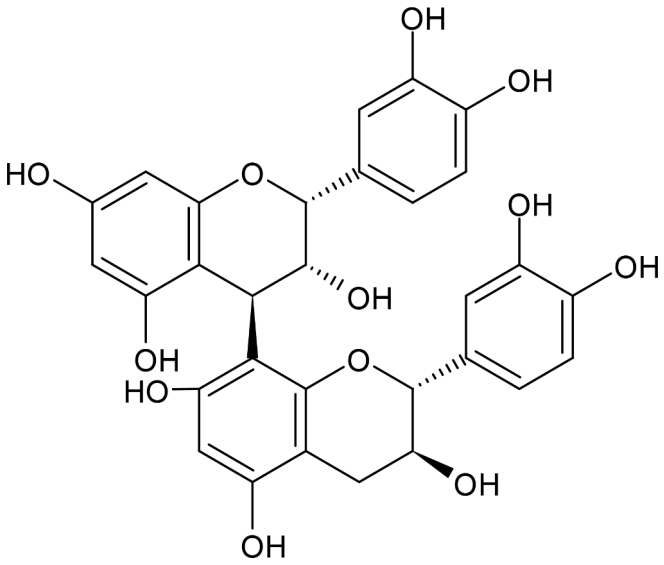
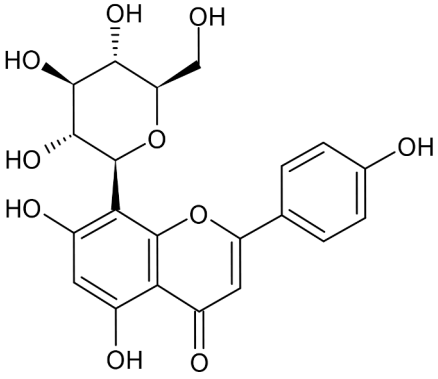
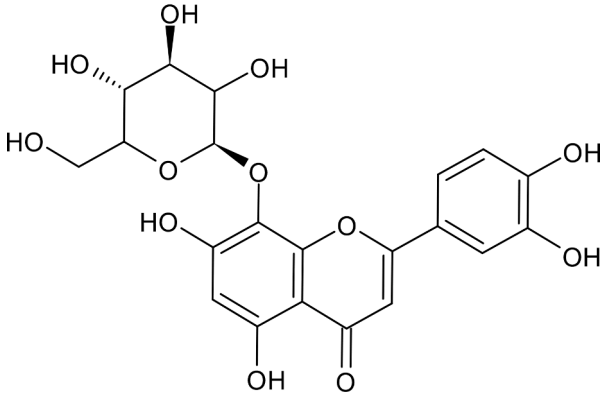
A fermentation process of cupuaçu juice (*T. grandiflorum*) using the

Table 1
. Summary of common bioactive compounds identified in *Theobroma* spp.

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
Organic acids	**Oxalic acid** 	*T. cacao* (pericarp)	HPLC-PDA	(Pérez-Mora, Jorrin-Novo & Marina, 2018)
	Citric acid 	*T. bicolor* (pericarp) *T. cacao* (pericarp) *T. grandiflorum* (seeds; bark) *T. speciosum* (flowers)	HPLC-PDA ^{1}H NMR	(Pérez-Mora, Jorrin-Novo & Marina, 2018); (Mar et al., 2021); (Andrade et al., 2022)
	Malic acid 	*T. bicolor* (pericarp) *T. cacao* (pericarp) *T. grandiflorum* (bark) *T. speciosum* (flowers)	HPLC-PDA ^{1}H NMR	(Mar et al., 2021); (Pérez-Mora, Jorrin-Novo & Marina, 2018); (Andrade et al., 2022)
Flavonoids	**Catechin** 	*T. cacao* (beans) *T. grandiflorum* (seeds)	HPLC-DAD UFLC-DAD	(Oliveira & Genovese, 2013); (Quiroz-Reyes & Aguilar-Méndez, 2022); (Andrade et al., 2022)
	Epicatechin 	*T. cacao* (beans) *T. grandiflorum* (seeds)	HPLC-DAD; UFLC-DAD	(Quiroz-Reyes & Aguilar-Méndez, 2022); (Andrade et al., 2022)

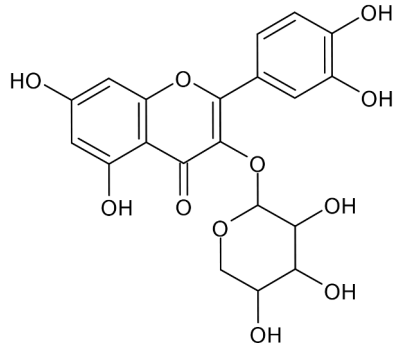
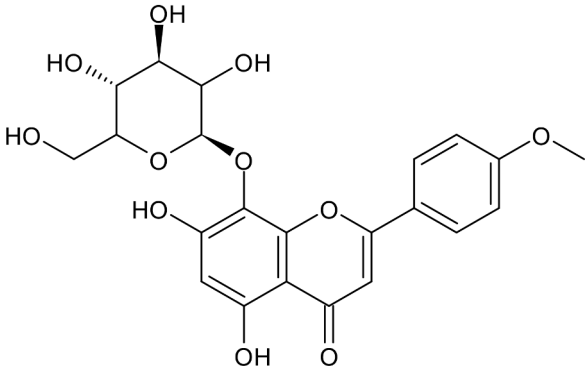
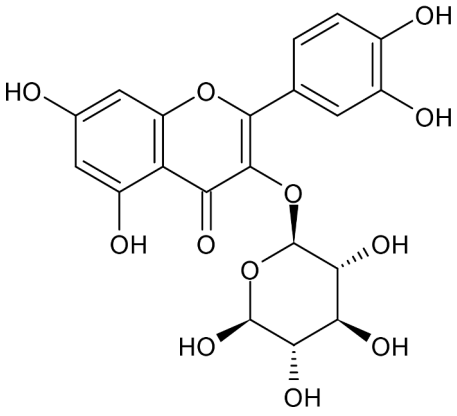
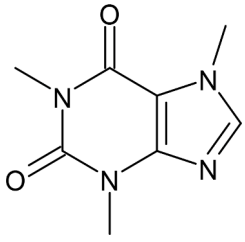
(*continued on next page*)

Table 1 (*continued*)

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
	Procyanidin 	*T. cacao* (beans)	HPLC-DAD	(Ramos-Escudero et al., 2021)
	Vitexin (apigenin-8-C-glucoside) 	*T. grandiflorum* (seeds)	HPLC-DAD/MS	(Pugliese et al., 2013)
	Hypolaetin 8-*O*-β-D glucuronide 	*T. grandiflorum* (seeds) *T. subincanum* (seeds)	HPLC-DAD/MS	(Pugliese et al., 2013); (Febrianto & Zhu, 2022)

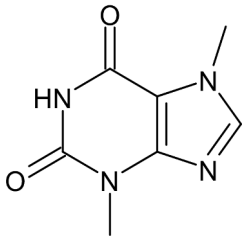
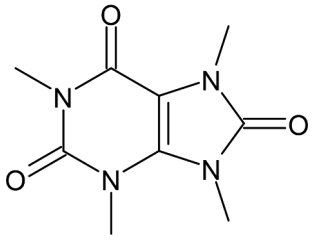
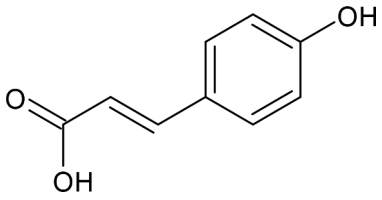
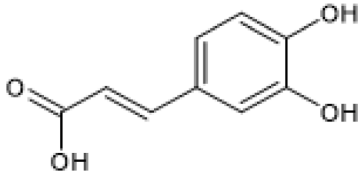
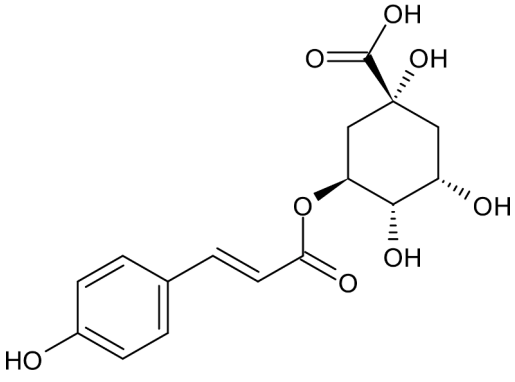
(*continued on next page*)

Table 1 (*continued*)

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
	Quercetin 3-*O*-pentoside 	*T. grandiflorum* (seeds)	HPLC-DAD/MS	(Pugliese et al., 2013)
	Isoscutelarein 8-*O*-β-D-glucuronide 	*T. grandiflorum* (seeds)	HPLC-DAD/MS	(Pugliese et al., 2013)
	Quercetin 3-*O*-glucoside 	*T. speciosum* (flowers)	^{1}H NMR	(Mar et al., 2021)
Alkaloids	**Caffeine** 	*T. angustifolium* (seeds) *T. bicolor* (seeds; leaves) *T. cacao* (seeds; leaves) *T. grandiflorum* (leaves) *T. mammosum* (leaves) *T. simiarum* (leaves) *T. speciosum* (leaves)	GC–MS; HPLC; HPLC-MS	(Hammerstone, Romanczyk & Aitkent, 1994); (Pereira-caro et al. 2013); (Marx & Maia, 1991); (Dozier, Kim & Russell, 2020)

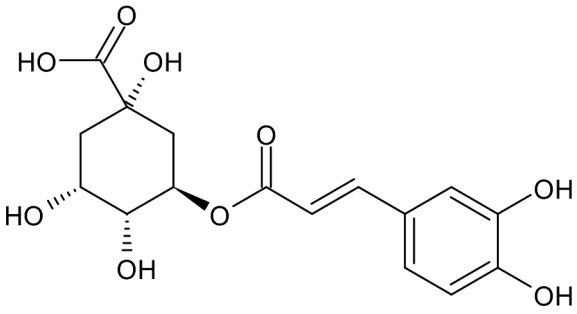
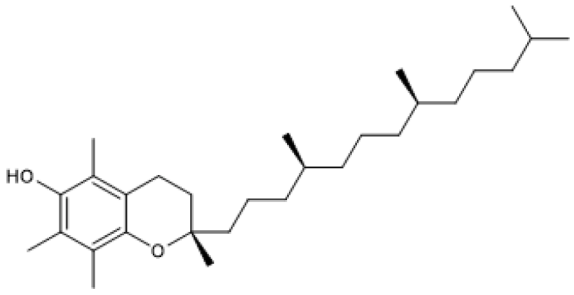
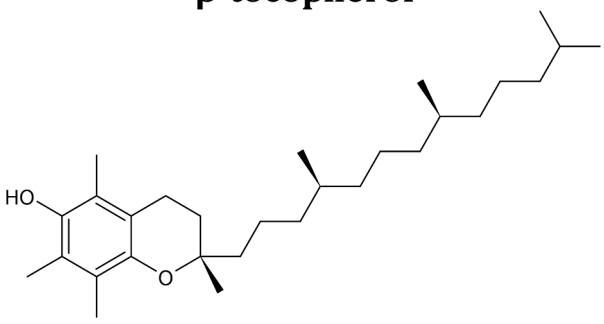
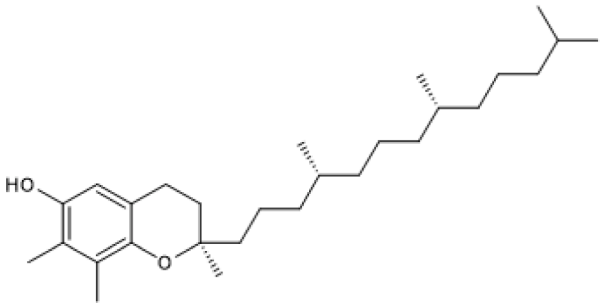
(*continued on next page*)

Table 1 (*continued*)

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
	Theobromine 	*T. angustifolium* (seeds) *T. bicolor* (leaves) *T. cacao* (seeds; leaves) *T. grandiflorum* (seeds) *T. mammosum* (seeds) *T. mariae* (seeds) *T. speciosum* (seeds)	HPLC-MS	(Hammerstone, Romanczyk & Aitkent, 1994); (Marx & Maia, 1991); (Dozier, Kim & Russell, 2020)
	Theacrine 	*T. angustifolium* (seeds) *T. bicolor* (seeds) *T. grandiflorum* (seeds) *T. mammosum* (seeds) *T. microcarpum* (seeds) *T. speciosum* (seeds) *T. subincanum* (seeds)	HPLC-MS	(Hammerstone, Romanczyk & Aitkent, 1994); (Marx & Maia, 1991); (Dozier, Kim & Russell, 2020)
Phenolic compounds (Phenylpropanoids)	***p*-coumaric acid** 	*T. bicolor* (seeds; pericarp) *T. cacao* (seeds; leaves; pericarp) *T. grandiflorum* (seeds; leaves; pericarp)	MS/MS	(Tauchen et al., 2016)
	Caffeic acid 	*T. bicolor* (seeds; pericarp) *T. cacao* (seeds; leaves; pericarp) *T. grandiflorum* (leaves)	MS/MS	(Tauchen et al., 2016); (Jala & Collin, 1977)
	***p*-coumaroylquinic acid** 	*T. cacao* (leaves)	MS/MS	(Jala & Collin, 1977)

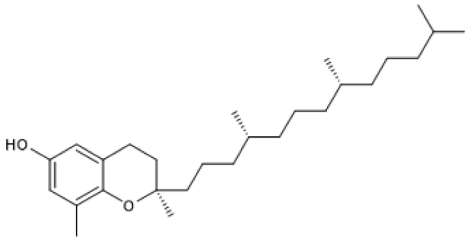
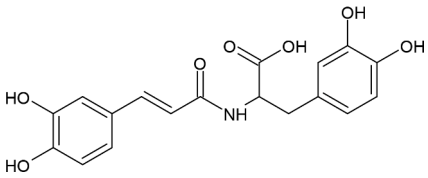
(*continued on next page*)

Table 1 (*continued*)

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
	Chlorogenic acid 	*T. bicolor* (pericarp) *T. cacao* (leaves) *T. grandiflorum* (seeds; leaves; pericarp)	MS/MS	(Jala & Collin, 1977); (Tauchen et al., 2016)
	α-tocopherol 	*T. subincanum* (seeds)	HPLC-UV/ vis	(Bruni et al., 2002)
	β-tocopherol 	*T. subincanum* (seeds)	HPLC-UV/ vis	(Bruni et al., 2002)
	γ-tocopherol 	*T. subincanum* (seeds)	HPLC-UV/ vis	(Bruni et al., 2002)

(*continued on next page*)

Table 1 (*continued*)

Chemical class	Compound name	Source (Plant Part)	Analytical method	Reference
	δ-tocopherol 	*T. subincanum* (seeds)	HPLC-UV/vis	(Bruni et al., 2002)
	Clovamide 	*T. bicolor* (beans) *T. grandiflorum* (beans) *T. subincanum* (beans)	HPLC-DAD/MS	(Febrianto & Zhu, 2022)

probiotic bacterium *Lactiplantibacillus plantarum* Lp62 resulted in increased phenolic content, flavonoids, and antioxidant potential. The fermentation enhanced the functional properties of cupuaçu juice, making it an effective vehicle for delivering the probiotic bacterium L. plantarum Lp62 (Lúcia et al., 2017). The study investigated the impact of ultrasound-assisted homogenization (US) and arabic gum (GA) on the colloidal stability of cupuaçu juice. The results showed that increasing energy density and GA concentration resulted in higher juice viscosity, reducing sedimentation. This increase in energy density was attributed to greater vitamin C bioavailability in the cupuaçu juice serum. It is concluded that the use of US and GA may be a promising strategy for the food industry to maintain the physicochemical properties of cupuaçu juice during storage (Silva et al., 2019).

An investigation into the analysis of the complex composition of cocoa beans during fermentation stages provided crucial insights into assessing their quality and nutritional attributes. Besides contributing to the development of flavor precursors, the results suggest that fermenting cocoa beans to the optimal point reduces the risk of heavy metal contamination and positively enhances the bioavailability of essential minerals for human nutrition (Millena et al., 2023). As alterations in protein composition during cocoa seed ripening and maturation, along with potential modifications during post-harvest processing (fermentation, drying, and roasting), have been investigated. These studies have revealed a significant reservoir of potential bioactive compounds derived from the protein components, which could be utilized in food functionalization (Rawel et al., 2019).

A study was conducted to determine the profile of bioactive compounds in cocoa residues, including mucilage and bean husks, and to assess their antioxidant activity in two varieties. The results indicated the presence of bioactive compounds in the external parts of the fruits, such as bean husks, pod husks, mucilage, and placenta, which are considered byproducts. These cocoa residues showed potential to be used as functional ingredients in various applications in the food industry (Llerena et al., 2023). The iced tea derived from cocoa almond husks offers a cost-effective and environmentally friendly way to boost antioxidant consumption. The powder is rich in dietary fiber (37.5 %), lipids (19.7 %), proteins (14.6 %), and contains 25.7 mg of gallic acid equivalents per gram. Retaining 85.3 % of the phenolics, the preferred formulation comprises 30 g of powder mixed with 1 liter of water (Lopes et al., 2021).

Enhancing local diets with nutritionally rich ingredients can have a significantly positive impact on the health status of local populations. By introducing foods that are sources of essential nutrients such as vitamins, minerals, and antioxidants, people can improve their overall health, strengthen their immune systems, and reduce the risk of nutritional deficiencies and diet-related diseases. This not only promotes individual well-being but also contributes to the sustainable development of communities by increasing productivity, reducing healthcare costs, and enhancing overall quality of life.

5.2. Applications medicinal

The use of the *Theobroma* genus as food has been widely proposed for a long time, as this is common for byproducts of food processing. The *T. cacao* seed bark, for example, has considerable amounts of proteins, minerals and vitamins that makes it an interesting and inexpensive material for human and animal food. (Table 2) (Rojo-Poveda et al., 2020).

Despite the presence of theobromine, studies have revealed positive effects when using diets fortified with the right amount of bark from the seed of *T. cacao* for poultry, rabbits, ruminants or pigs.

It is widely accepted that oxidative stress is involved in the development and/or secondary pathology of several human diseases. Several studies show evidence that the regular consumption of plant-based foods is associated with a lower risk of incidence of these (Ponce-Sánchez et al., 2021). It is believed that the beneficial health effect of plant-based foods can be mainly credited to the number of phenolic compounds and their ability to promote the antioxidant effect. Currently, antioxidant activity is mostly examined in common food plants such as fruits and vegetables. However, recent studies indicate that other categories of plants, such as medicinal plants, also have significant antioxidant efficacy (Tauchen et al., 2016).

An investigation of *T. cacao* by-products for the extraction of bioactive compounds using subcritical water revealed that the extracts contained levels of total proteins, total phenols, and total flavonoids. The antiradical activity against DPPH radicals (IC_{50} ~ 11–13 μg/mL) and ABTS radicals (IC_{50} ~ 7–9 μg/mL), as well as the total antioxidant activity (~14–20 mg EAK/100 g DE), indicated suitability for formulating a functional cosmetic product (Švarc-Gajić et al., 2023).

The antidiabetic capacity was assessed, revealing that among sixteen Brazilian native fruits and six commercial frozen pulps, cupuassu fruit exhibited the most robust inhibition of α-amylase activity (De Souza Schmidt Gonçalves et al., 2010). Recently, the impact of the daily consumption of cupuassu liquor, derived from fermented seeds, on oxidative stress and lipid profile in rats with streptozotocin-induced diabetes was examined. The findings indicated that supplementing with cupuassu

Table 2
Form of consumption of parts of *Theobroma* species.

BOTANICAL NAME	PART(S) TESTED	WAY OF CONSUMPTION	REFERENCE
T. bicolor	Pericarp, Aril, seed	Pulp is eaten fresh, seeds are consumed roasted	(Tauchen et al., 2016)
T. cacao	Leaf, pericarp, Aril, seed	Pulp is eaten fresh, seeds are consumed roasted; leaves used as infusion/decoction	(Tauchen et al., 2016)
	Bark, pulp and seed	Nanoparticles (Ag nps) using silver nitrate and extracts of different parts of *T. cacao*: the husk (h-Ag nps), pulp (p-Ag nps), and seed (s-Ag nps)	(Thatikayala et al. 2019)
	Bark e seed	Assessment of the antibiofilm potential of hydroalcoholic extracts from *T. cacao* (cacao) waste	(Binartha, Kardinal, and Widyarman 2021)
	Cocoa bean	Proposing the elaboration of chocolate cake with substitution of wheat flour by cocoa bean shell powder (csp).	(Nogueira et al. 2022)
	Bean	Cocoa tea made from the husk of the cocoa bean	(Rojo-poveda et al., 2020)
	Leaf, pericarp, Aril, seed	The use of cocoa on diabetic neuropathy.	(Kababie-Ameo et al., 2022)
	Fruit	Cacau liqueurs	(Nascimento et al. 2014)
	Fruit	Cacau liqueurs	(Melo Neto et al. 2020)
T. grandiflorum	Leaf, pericarp, aril	Pulp is eaten fresh, seeds are consumed roasted; leaves used as Infusion/decoction	(Tauchen et al., 2016)
	Fruit juice pulps	Cytotoxicity and antimicrobial potential of methanolic extracts of *T. grandiflorum* (cupuaçu) against clinical isolates of uropathogenic *E. coli*.	(Chen et al. 2015)
	Pulp	Fruit pulp flours as complementary antioxidants, prebiotics, immunomodulators and dietary anti-inflammatories	(Curimbaba et al., 2020)
	Seed	Microencapsulated extract of cupuaçu seed by-product	(Nascimento et al. 2014)
	Fruit	Formulation of phytosomes containing cocoa pod extract, in the preparation of facial serum	(Priani et al. 2019)
	Bark	Microencapsulated cocoa bark phenolic extracts for bakery products	(Grassia et al. 2021)
	Fruit	Cupuaçu fermented drinks	(Rosa et al. 2020)
	Pulp	Goat's milk yogurt made with cupuaçu pulp	(Costa et al. 2017)
	Fruit	Cocoa honey obtained from the fruit	(Nascimento et al. 2014)
	Fruit	Cupuaçu liqueurs	(Nascimento et al. 2014)

liquor led to decreased plasma triacylglycerol levels, elevated HDL cholesterol levels, and enhanced plasma antioxidant capacity (T. B. De Oliveira & Genovese, 2013). Persistent consumption of a phenolic-rich extract derived from cupuassu liquor, in the context of a high-fat diet, resulted in diminished lipid peroxidation, heightened plasma antioxidant capacity, and enhanced glucose tolerance (K. de De Oliveira et al., 2015).

Several clinical studies have also demonstrated the cardioprotective effects of cocoa oral supplementation, preventing chronic cardiovascular events, reducing blood pressure, improving endothelial dysfunction, suppressing the formation of atherosclerotic lesions, and inhibiting atherosclerosis (Sesso et al., 2022).

The genus *Theobroma* features species abundant in secondary metabolites, with a notable presence of phenolic compounds, among which theobromine stands out as an example (Meng et al., 2024). The theobromine alkaloid showed the ability to stimulate the formation of apatite, which increases the crystallinity of tooth enamel, making it more resistant to acid dissociation. Due to its promising results when added to other types of materials, the addition of theobromine to glass ionomer cements (GIC) could enhance its anti-cariogenic effects without altering the mechanical properties of the material (Badrie et al., 2015). Moreover, there is growing evidence indicating that theobromine might have the ability to promote bone growth. In vitro studies have shown that theobromine can enhance the osteogenic capacity of human mesenchymal stem cells (hMSCs) and accelerate skeletal development in rats (Clough et al., 2017).

Theobromine has also exhibited neuroprotective properties, demonstrating its capacity to prevent neuronal damage and improve motor memory and cognitive regulatory functions. Due to its low incidence of side effects and minimal harm to the human body at suitable doses, theobromine and its derivatives hold potential as effective agents for preventing brain-related disorders, offering significant prospects in the medical field (Meng et al., 2024). Theobromine has shown the ability to enhance memory in the hippocampus, influencing the activity of hippocampal neurons and protecting them from harm by regulating adenosine receptors (Valada et al., 2022). In a study conducted by Wen et al. (2021), the impact of theobromine on a non-alcoholic fatty liver disease (NAFLD) mouse model was investigated. The findings showed that theobromine resulted in decreases in body weight and liver weight, along with improvements in liver morphology.

However, it is important to note that further research is needed to fully understand the effects of theobromine on human health and to determine the optimal doses to achieve benefits without adverse effects.

5.3. Antibacterial activity and anticariogenic effects

The silver nanoparticles (AgN) Pssynthesized from extracts of leaves and pods of cocoa plants showed smaller particle sizes and higher purity in the range of 10–100 nm. This indicates that the cocoa leaf extract has richer phenolic compounds than the pod extract, resulting in a different rate of nucleation and growth of the AgNPs, resulting in antibacterial activity against *E. coli,* with zone of inhibition of 21 mm in a concentration of 0.15 g/mL of the aqueous extract (Efavi et al., 2022).

Biological and antioxidant activities of aqueous extracts of cocoa pod barks revealed that ethyl acetate extracts were active against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*, which had the concentration of 0,01 mg/mL, the inhibition diameters were (6.62 ± 0.10), (6.52 ± 0.02) and (11.72 ± 0.36) mm, respectively (Wheni et al., 2021).

The cocoa bean husk is one of the main by-products of chocolate manufacturing and has several compounds with biofunctionality. It can function as an antibacterial agent, and its action is mainly reported against *Streptococcus mutans* (Campos-Vega et al., 2018). *T. cacao* pod shell extract of both variants (yellow and purple) showed good antioxidant activity and antidiabetic activity with IC_{50} as of 41.3 and 44.5 μg/mL for the DPPH assay and 41.6 and 27.7 μg/mL for the inhibitory assay of α-glucosidase, respectively. Meanwhile, for the antibacterial test, the pod shell of *T. cacao* of both variants also had the most active extracts with MIC (minimum inhibitory concentration) from 0.62 mg/mL against *S. aureus* (Wheni et al., 2021).

Active compounds derived from plants could be used as substitutes for pharmaceuticals to control diseases caused by microorganisms. The antibacterial potential of the hydroalcoholic extract of the leaves of the fruits from *T. cacao* species was evaluated on gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa.* The results obtained for gram-negative bacteria, antibacterial potential was observed only in *Pseudomonas aeruginosa* in all its concentrations (Luiz et al., 2016).

The 30 % aqueous extract of *T. cacao* was highly efficient against the broad spectrum of clinical isolates of gram-negative and gram-positive bacteria (*Klebsiella pneumoniae, Proteus meirabilis, Proteus vulgaris, Salmonella typhimurium, S. typhi, Staphylococcus aureus, S. epidermidis, S. saprophytics* and *Streptococcus pyogenes*), with more effective behavior than the commercially available antibiotic ciprofloxacin, in addition to a high inhibition against adherents and biofilm formation (Wheni et al., 2021).

The cocoa shell (*T. cacao* L.) has antioxidant and antimicrobial activity with potential as a natural food preservative, but in the use of cocoa husk extract it presented a disadvantage due to its short shelf life and limited application in food, so one of the efforts that can be made is to encapsulate the extract, this study concluded that encapsulated extract of cocoa husk using 20 % maltodextrin showed the highest antioxidant and antimicrobial activity compared to other treatments, having a concentration of 30 %; 40 %; 50 % and 60 %, but for the treatment of 20 % and 30 % there is no difference. Ethanol extracts from fruit peels can be made into encapsulated forms which are very likely to be used as natural preservatives (Hasanuddin et al., 2019). In addition to that, the plant extract of *T. cacao* alone or combined with conventional antibiotics showed benefits in the treatment of infections involving multidrug-resistant bacteria (Silva et al., 2014).

5.4. Centesimal composition

Theobroma bicolor is widely distributed in the neotropical region with great potential for economic use. The seeds, as well as the flowers of *T. bicolor*, have a high nutritional value. Seed nutrients are high in protein, fat, carbohydrates and fiber, as well as copper, magnesium, manganese, potassium and zinc (Ponce-Sánchez et al., 2021). When reviewing the use of the species, the pulp and grains of the species *T. bicolor* are mainly used for the preparation of sweets (marzipan, nougat and marshmallow), chocolate (mixture of patashte and cocoa seeds), beverages such as atole, pozol, powder and popo, and in a smaller extent, they are consumed as fresh fruits (Parada-Berríos et al., 2022).

Theobroma cacao is considered a super food due to its high nutrient content and proven health benefits, its beans are high in carbohydrates (31 %), protein (11 %), fat (54 %), fiber (16 %) and minerals (Shahanas et al., 2019). The knowledge of bioactive compounds in cocoa and cocoa products suggests that they can be considered as part of healthy nutritional foods and health promoters. It provides a wide variety of valuable food products and has potential health benefits (Campos-Vega et al., 2018).

The cocoa beans contain over 300 volatile compounds, most of which are responsible for their distinctive aroma and flavor. The cocoa flavor is characterized by aliphatic esters, polyphenols, aromatic carbonyls and theobromine, which are primarily responsible for preventing rancidity in cocoa butter. A review of the composition of 1 g of powdered cocoa bean showed that it contains 3 % mineral elements (i.e. Copper [0.04 mg], Iron [0.14 mg], Manganese [0.04 mg], Magnesium [4.99 mg], Phosphorus [7.34 mg], Zinc [0.07 mg], Potassium [15.24 mg], Calcium [1.28 mg] and Selenium [0.14 μg], respectively), 5 % amino acids (i.e. Valine [11.77 mg], Isoleucine [7.60 mg], Tryptophan [2.93 mg], Threonine [7.76 mg], Leucine [11.90 mg], Lysine [9.83 mg] and Histidine [3.40 mg], respectively) (i.e. Carbohydrate [0.28 g], Total dietary fiber [0.37 g], Protein [0.20 g], and Total lipid fat [0.14 g], respectively), and < 1 % vitamins (i.e. Vitamin B2 [0.002 mg] and Niacin [0.02 mg], respectively) (Etaware, 2021).

The contents of the metals K, Mg, P and Ca found in the aqueous extracts of the species of *T. speciosum* constituted the following values, 115 ± 2 μg mL^{-1}, 18.4 ± 0.2 μg mL^{-1}, 7.0 ± 0.0 μg mL^{-1}, 3.1 ± 0.1 μg mL^{-1}. The flowers aqueous extract represents a new promising food source rich in antioxidant compounds (Mar et al., 2021).

The cupuaçu pulp is considered the most important part, it is consumed for its flavor, color and pleasant aroma, offering potential for the manufacture of derivative products, both industrially and handcrafted (Etaware, 2021). Both the pulp and the aroma extracted from it can be used as an input for the preparation of various products, such as alcoholic and non-alcoholic beverages, among others, which has attracted scientific interest over the last few years due to its bioactive compounds (Vriesmann & Oliveira, 2009). From the study realized with the pulp of the *T. grandiflorum* species, were obtained values of calcium content (13 mg/100 g), phosphor (21 mg/100 g), iron (0.5 mg/100 g), potassium (331 mg/100 g), sodium (3 mg/100 g), manganese (18 mg/100 g) and zinc (0.3 mg/100 g) (Ramos et al., 2022).

6. *Theobroma* spp. products

Among the by-products resulting from the processing of the *Theobroma* spp. are the seeds and bark, while in the cosmetics industry the residues are used empirically as fertilizer or are discarded. The residues generated in processing are not properly treated, with no use of seeds or peels, with seeds equivalent to 25 % of the weight of the fruit (Alba et al., 2021).

Cocoa pulp has a physical-chemical composition that enables the development of products such as nectars, liqueurs, jellies, vinegars, among others (Puerari et al., 2012). Cocoa honey is a regional name for the transparent liquid extracted from the pulp that surrounds the seed before the cocoa fermentation process, composed of water, fermentable sugars (10–18 %), non-volatile acids (0,77–1.52 % of citric acid) and pectin (0.9–2.5 %) (Alba et al., 2021). When we pay attention to the losses of cocoa byproducts and market demands, there is a need to develop products that can be included in human food (Freitas, 2017). Cupuaçu nectar was microbiologically safe, as well as with potential for commercialization, since they obtained similar results in sensory acceptance. This nectar formulation has high antioxidant capacity and high vitamin C contente (Freitas, 2017).

Obtaining flour of good physical quality in relation to its physical characteristics with good pH values, water content, titratable acidity, total sugar content and with high yield through the residues of the frozen cupuaçu fruit pulp, led to the use of the waste generated by the industrialization of the fruit, which could be an alternative for minimizing waste and controlling environmental wear (Puerari et al., 2012). The flours produced from co-products of the frozen cupuassu fruit pulp industry have considerable phenolic contents and expressive constituents of antioxidant capacity, have technological functional potential, and can be considered as possible raw materials for obtaining extracts rich in antioxidants or for extracting natural antioxidants in the food and pharmaceutical industries (Curimbaba et al., 2020).

The alcoholic formulation of 40 % of *T. cacao*, in which pH analysis data, titratable and fixed acidity, vitamin C, total and reducing sugars and alcohol content were obtained, remained stable during the commercial stability analysis period, with reductions being observed significant, but small, in the physical chemical parameters analyzed at time zero (0) to 90 days, proving to be a good alternative in the food industry (Alba et al., 2021). Regarding the physicochemical analyses, for the sparkling product of *T. grandiflorium*, it generated a product with more attractive sensory characteristics and with a higher nutritional quality (higher protein and carbohydrate content) (Curimbaba et al., 2020).

7. Patents of *Theobroma* spp .

The evaluation of patent deposits directly related to the *Theobroma* genus, proved the interest in research and development - R&D of products and processes correlated with their applications by countries, which sought individual protection, by country or Wold Intellectual Property Organization - WIPO through the Patent Cooperation Treaty – PCT described below: **Brazil** with 2 patents, **WIPO (PCT)** with 7 patents, **United States** with 7 patents, **Japan** with 2 patents, **Australia** with 1 patent, **South Korea** with 3 patents, **Canada** with 6 patents, **France** with 2 patents, **Philippines** with 1 patent, **European Patent Office** with

1 patent, **Romania** with 1 patent and **Russia** with 1 patent, however, there were deposits with double protection, for the same patent as an example between WIPO (PCT) and the country of origin or protection in two countries simultaneously, as described below: **WIPO (PCT)** and **Australia** with 1 patent, **Canada** and **France** with 1 patent in common, and **WIPO (PCT)** and **Canada** with 2 separate patents.

Of the evaluated application areas of the 30 patents involving the genus *Theobroma*; 26/30 patents were in the area of Food Technology, BR112013001959B1, US7521238B2, BR102019021218A2, WO2007062206A2, US20220338519A1, JP2003088372A, US11077162B2, (WO2019/153046Al, AU2019216877A1), US10543242B2, (WO2021074316A1, CA3154121A1), (WO2020225056A1, CA3135952A1), CA3056180A1, PH22018000886U1, WO2014018822A2, CA3056038A1, KR100923538B1, EP2934561B1, JP2008195652A, KR101139376B1, US20060134230A1, US20210046135A1, US20210236572A1, RO128712A2, CA2600301A1, WO2008134648A2 and RU2479313C2, which correspond approximately (86.66 %); 22/30 patents went to Functional Foods, BR112013001959B1, BR102019021218A2, WO2007062206A2, US20220338519A1, JP2003088372A, US11077162B2, (WO2019/153046Al, AU2019216877A1), KR101361186B1, PH22018000886U1, WO2014018822A2, CA3056038A1, KR100923538B1, EP2934561B1, JP2008195652A, KR101139376B1, US20060134230A1, US20210046135A1, US20210236572A1, RO128712A2, CA2600301A1, WO2008134648A2 and RU2479313C2, roughly corresponding (73.33 %); 10/30 patents apply to the area of Biotechnology, BR112013001959B1, US7521238B2, JP2003088372A, KR100923538B1, EP2934561B1, KR101139376B1, FR2904542A1, US20210236572A1, WO2008134648A2 and RU2479313C2, that correspond approximately (33.33 %) and 20/30 patents have Medicinal Application, BR112013001959B1, WO2007062206A2, US20220338519A1, JP2003088372A, US11077162B2, (WO2019/153046Al, AU2019216877A1), KR101361186B1, US10543242B2, (CA3098361A1, FR3080767A1), WO2016046375A1, KR100923538B1, EP2934561B1, JP2008195652A, KR101139376B1, FR2904542A1, US20210046135A1, US20210236572A1, RO128712A2, WO2008134648A2 and RU2479313C2, roughly corresponding (66.66 %). However, of the 30 patents analyzed and their respective applications, patents with multiple applications stand out, in ascending order: 5/30 patents have only 1 following field of application: (CA3098361A1, FR3080767A1), WO2016046375A1, (WO2021074316A1, CA3154121A1), (WO2020225056A1, CA3135952A1) and CA3056180A1, which correspond to 16.66 %, 10/30 patents that have 2 fields of application such as: US7521238B2, BR102019021218A2, KR101361186B1, US10543242B2, PH22018000886U1, WO2014018822A2, CA3056038A1, US20060134230A1, FR2904542A1 and CA2600301A1, that express 33,33 %, 7/30 patents present 3 fields of application in the sequence: WO2007062206A2, US20220338519A1, US11077162B2, (WO2019/153046Al, AU2019216877A1), JP2008195652A, US20210046135A1 and RO128712A2, that provided 23.33 % and 8/30 patents demonstrated 4 fields of application such as: BR112013001959B1, JP2003088372A, KR100923538B1, EP2934561B1, KR101139376B1, US20210236572A1, WO2008134648A2 and RU2479313C2, corresponding to 26.66 % of the total number of patents selected.

The analysis of the thirty selected patents related to the application of *Theobroma* fruits allowed us to observe that industrial interest in the area of food technology represented 86.66 % of the patents, followed by functional foods with 73.33 %, followed by medicinal application with 66.66 % and in the area of biotechnology with 33.33 %. Demonstrating the power and diversity of markets, involving these products or processes applied to industries with multiple applications, always looking for innovative products to improve the quality of life of human beings (Fig. 2; Table 3).

For the evaluation of the technological complexity required to obtain the results proposed in the patents, these were classified as: Low, Medium and High Technology. The results obtained showed relative classification equivalence. With 10/30 patents classified as Low

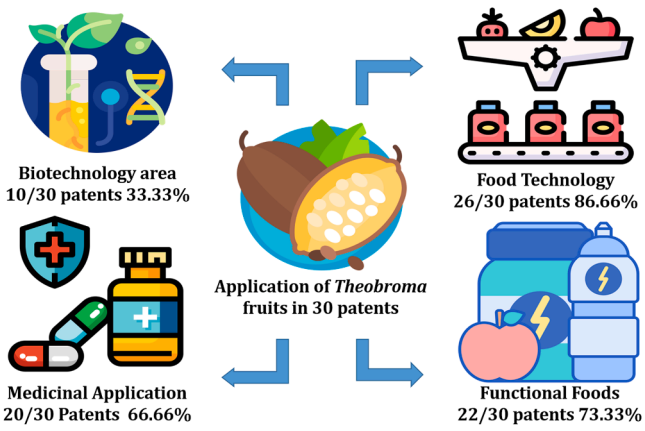


Fig. 2. Areas with potential industrial application for the fruits of *Theobroma*, of the thirty patents evaluated.

Technology, (WO2019/153046Al, AU2019216877A1), KR101361186B1, (CA3098361A1, FR3080767A1), (WO2021074316A1, CA3154121A1), (WO2020225056A1, CA3135952A1), CA3056180A1, PH22018000886U1, CA3056038A1, US20060134230A1 and CA2600301A1, corresponding to 30 %, 9/30 patents were classified as Medium Technology, US7521238B2, BR102019021218A2, WO2007062206A2, US20220338519A1, US11077162B2, WO2014018822A2, JP2008195652A, US20210046135A1 and RO128712A2, representing 33.33 % and 11/30 patents, by distinction, classified as High Technology, BR112013001959B1, JP2003088372A, US10543242B2, WO2016046375A1, KR100923538B1, EP2934561B1, KR101139376B1, FR2904542A1, US20210236572A1, WO2008134648A2 and RU2479313C2, providing 36.66 % of the total number of carefully analyzed patents.

The correlation between the added value in a patented product is directly related to its technological complexity, the greater or higher complexity is expected to be more concerned about protecting that invention, however it was observed that of the patents evaluated, the holders of these deposits preferred to protect the patents of *Theobroma* fruits with high technology aggregated in collective protections (Offices or treaties involving several countries eg: PCT with 152 countries) or individual by country, inventions with medium complexity for technologies derived from *Theobroma* fruits, accompanied the line of protection of high complexity, thus opting for collective and individual protection by country. However, for patents on inventions using the fruits of *Theobroma*, with low technological complexity, applicants opted for double protection (between countries or between the country of origin and a patent office), or for simple protection by country. The only country that overvalued the research of products and processes that evolved into patents derived from the fruits of *Theobroma*, regardless of the technological complexity, was the United States of America, with 2 high patents, 4 medium and 1 low, totaling 7 patents out of the 30 evaluated, this indicates how much the American market values the products and processes derived from the fruits of *Theobroma,* stimulating the heating of the market, in the areas of food technology, functional foods, biotechnology and medicinal application, with important technological innovations for the human societies (Fig. 3).

8. Conclusion

The fruits of the *Theobroma* genus have great potential in the food, pharmaceutical and cosmetic industries, as they have a high antioxidant and antimicrobial potential. The fruits have a high nutritional content and have antibacterial and anticariogenic properties. In the last 10 years, only two (*T. cacao* and *T. grandiflorum*) species were more explored in terms of chemistry and their chemical and biological properties. As a perspective, there is potential to study other promising

Table 3
Evaluation of patents related to the fruits of *Theobroma* and its applications.

Patent title	Application	Patent number	Country	Reference
Use of succinate dehydrogenase inhibitors and/or respiratory chain complex iii inhibitors to improve the ratio of harmful microorganisms to beneficial	The present invention refers to the use of succinate dehydrogenase inhibitors and/or complex iii inhibitors to control undesirable pathogenic fungi	BR112013001959B1	Brazil	(Fought et al., 2011)
Tissue culture medium for Macadamia and *T. cacao*	The present invention provides a method and a culture medium for the production of food biomass by directly culturing seed kernel tissue, or seed cotyledonary differentiated tissue.	US7521238B2	United States	(Atehortua et al., 2011)
Medium chain triglyceride vegetable butter	The invention is situated in the field of human needs, more specifically of food compositions and presents in its composition medium chain triglycerides	BR102019021218A2	Brazil	(Sabará; Dean, 2019)
Chocolate formulations with functional properties	This invention is directed to bioactive substances from cacao and methods for concentrating extractions. The invention is also directed to formulations, pharmaceuticals preparations, nutritional preparations, and dietary supplements extracted from food materials.	WO2007062206A2	WIPO (PCT)	(Martin, 2006)
Oral supplements containing enriched phenolic compound extracts combined with organic acid sodium salts, creatines and/or creatine salts	When taken together in an oral supplement, the particular phenolic compound(s) found in such extracts and in other genera containing significant quantities of total phenols are believed to enhance the activity of the phosphocreatine salts in a synergistic manner and result in enhancement of physical activity as well as provide recovery from inflammation.	US20220338519A1	United States	(Daugherty; Tempesta, 2022)
Theobromine synthase and gene for coding the synthase	The present invention refers to the theobromine synthase, DNA or RNA encoding the enzyme, and D. A method of producing caffeine and theobromine or a precursor thereof, using the microorganism, plant or a cell or tissue cultivated thereof. Refers to a method of variation of the amount of precursor.	JP2003088372A	Japan	(Mizuno et al., 2003)
Preparation of protective plants of the heart to prevent and improve coronary heart disease	The present invention provides a new natural plant preparation that can prevent and control various risk factors for coronary heart disease, including vascular inflammatory processes that cause cardiovascular events	US11077162B2	United States	(Shandong University of Technology, 2021)
Formulation and method of use	This invention relates to formulations for the promotion of gastrointestinal health and methods of treatment and/or use thereof. The formulations are useful for a variety of applications, such as modulating a subject's gut microbiome and/or the treatment of diseases, disorders or conditions characterised, at least in part by poor gastrointestinal health, including inflammatory bowel disease (IBD)	WO2019/153046Al AU2019216877A1	WIPO (PCT) Australia	(Legge, 2019)
Novel cosmetic and/or pharmaceutical compositions and applications thereof	The present invention comprises the compositions of extracts of Polynia species and extracts of *Theobroma* species, or extracts of Salix species and zinc sulfate as active ingredients, methods to prepare these compositions, and cosmetic and/ or pharmaceutical uses of these compositions.	KR101361186B1	South Korea	(Ulmann et al., 2007)
Composition for the treatment of joint conditions	The present invention is related to a composition for improving the health status of various joints in the human body. The composition may be used to treat osteoarthritis, rheumatic joint disease, joint swelling, pain, hallux valgus, traumatic conditions of the joints including sprains, strains, post-surgical recovery, bone edema, and speed up the recovery of athletes post-injury.	US10543242B2	United States	(Stancioiu; Catanas, 2014)
Extract of shells of *T. cacao* beans for controlling rosacea and skin redness	The present invention relates in particular to an extract of cocoa bean shells or a cosmetic or dermatological composition comprising said extract for preventing and / or treating rosacea.	CA3098361A1 FR3080767A1	Canada France	(Áries et al., 2019)
T. cacao extract for use in the treatment or prevention of receptor tyrosine kinases related disorders	The present invention relates to the treatment or prevention of receptor tyrosine kinases-related disorders. More particularly, the present invention concerns a *T. cacao* extract for use in the treatment of receptor tyrosine kinases related disorders and a pharmaceutical composition comprising such extract.	WO2016046375A1	WIPO (PCT)	(Legeai-Mallet; Carretero, 2016)
Chocolate products, ingredients, processes and uses	The present invention provides a new composition obtainable from a plant in the *Theobroma* genus and the use of that composition as an ingredient in a chocolate product.	WO2021074316A1 CA3154121A1	WIPO (PCT) Canada	(Abu-Hardan, 2019)
Foodstuff and beverage products, ingredients, processes and uses	The present invention provides the use of a composition obtainable from the pulp of a plant in the *Theobroma* genus or an extract of said pulp, as an ingredient in a foodstuff product.	WO2020225056A1 CA3135952A1	WIPO (PCT) Canada	(Dupas-Langlet, 2019)

(*continued on next page*)

Table 3 (*continued*)

Patent title	Application	Patent number	Country	Reference
Chocolate products, ingredients, processes and uses	The present invention refers to the field of new chocolate products, new ingredients for use in such products and uses of the new ingredients. In addition, it refers to the compositions of tropicalizing agents based on fat, their methods of preparation, chocolate tropicalization methods.	CA3056180A1	Canada	(Festring et al., 2019)
Composition of making cacao (*Theobroma*) butter	The utility model disseminates cocoa butter (*T. cacao*) and its production method, which employs cocoa bean as the main component of the product and honey, vegetable oil and fresh milk with low fat content as ingredients.	PH22018000886U1	Philippines	(Lobaton; Pabelona, 2018)
Methods for increasing the production of phenolic compounds from *T. cacao*	Described here are methods to increase the production of phenolic compounds from *T. cacao*. For example, the devices and methods described increase the production of phenolic compounds from cocoa plants with industrial and economic value.	WO2014018822A2	WIPO (PCT)	(Cuero Rengifo, 2014)
Foodstuff products, ingredients, processes and uses	The present invention provides the use of a composition obtained from pulp of a plant of the genus *Theobroma*, preferably cocoa pulp, or a pulp extract of a plant of the genus *Theobroma*, preferably cocoa pulp, as an ingredient in a food, preferably a confectionery product.	CA3056038A1	Canada	(Vieira et al., 2018)
Angiotensin-I converting enzyme inhibitor, Manufacturing process thereof, and Functional foods containing the same	The object of the present invention is to provide angiotensin converting enzyme (ACE) inhibitor derived from natural products having high safety and high activity as a food, a preparation method thereof, and a food and drink containing the same.	KR100923538B1	South Korea	(Maeda et al., 2009)
Composition comprising raphanus, *Theobroma* and *Passiflora* for treating opioid and alcohol abuse	A pharmaceutical composition for use in the treatment of opioid abuse, opioid dependence, alcohol abuse and / or alcohol dependence, and / or for use in the treatment of symptoms of opioid and / or alcohol withdrawal, wherein the pharmaceutical composition.	EP2934561B1	European Patent Office	(Qadan, 2012)
Colitis inhibitor comprising *T. cacao* ingredient	The present invention relates to an inhibitor and method for suppressing colitis. A colitis inhibitor and food comprise a ingredient *T. cacao* or a cocoa polyphenol derived from *T. cacao*.	JP2008195652A	Japan	(Matsuyama et al., 2008)
Anti-influenza viral drug and, viral-infection suppression products and foods comprising the same	The present invention provides a high inhibiting effect against flu virus infection and provides an anti-influenza virus agent with no side effects and contained in functional foods. Influenza anti-virus agents and infection inhibitors present in the mixture of foods containing orange (*Citrus spp*.), cat's nail (*Uncaria tomentosa*), cocoa (*T. cacao*) and others.	KR101139376B1	South Korea	(Shimizu et al., 2011)
Weight loss composition and formulation	This invention discloses a new and unique combination of *Dahurian angelica* Root extract, *Polygonum multiflorum* extract, *T. cacao* extract, *Yerba mate* extract, Oolong tea extract, Kohki Tea extract, *Horse chesnut* extract, and *Ligustrum lucidum* extract useful as a dietary supplement for increasing weight loss in humans.	US20060134230A1	United States	(Abraham, 2006)
Use of herbal extract obtained e.g. from i.e. *T. cacao* and/or at least another active agent e.g. to enhance augmentation of melanin synthesis in melanocytes of epidermis and to improve natural skin pigmentation	The invention refers to the use in a cosmetic composition or for the preparation of a pharmaceutical composition, of an effective amount of cocoa extract (*T. cacao* L.); and contains the extract or composition intended to promote increased melanin synthesis in the melanocytes of the epidermis or capillary bulb, with a view to giving it a tanned appearance, or to prepare the skin for exposure to the sun, or for therapeutic purposes, to repigmentthe depigmented skin, for example in the case of vitiligo, or to pigment the hair.	FR2904542A1	France	(Claude Dal, 2011)
Synergistic herbal compositions for the treatment of obesity and overweight	The invention relates to synergistic herbal composition comprising combination of first ingredient selected from the extracts, fractions, phytochemicals and mixtures thereof derived from *T. cacao* and a second ingredient selected from the extracts, fractions, phytochemicals and mixtures thereof derived from *Citrus aurantifolia* and optionally containing at least one ingredient selected from pharmaceutically.	US20210046135A1	United States	(Gokaraju et al., 2021)
Synergistic herbal compositions for testosterone boosting	The invention relates to synergistic herbal compositions comprising at least one ingredient selected from the extracts and fractions derived from *Punica granatum* and at least one ingredient selected	US20210236572A1	United States	(Gokaraju G.R.; et al.; 2018)

(*continued on next page*)

Table 3 (*continued*)

Patent title	Application	Patent number	Country	Reference
	from the extracts and fractions derived from *T. cacao* for improving testosterone levels in humans.			
Method for treating rheumatism	The invention refers to a method of treatment of rheumatism. According to the invention, the method consists of administering 4 to 5 g of cocoa powder dissolved in cold water, associated with 250 ml of cowberry tea, administered concomitantly vitamin C to support the immune system, and can apply anti-infection treatment and possibly vitamin and mineral supplements, as appropriate.	RO128712A2	Romania	(Scurtu S.I.; 2010)
Compositions and methods for increasing metabolism, thermogenesis and/or muscular definition	Compositions and Methods for Increasing Metabolism, Thermogenesis and/or Muscular Definition Field of the Invention The present invention relates to compositions and methods for increasing a person's natural metabolic rate, increasing thermogenesis or decreasing water retention.	CA2600301A1	Canada	(Heuer M. A.; 2005)
Cocoa ingredients having enhanced levels of stilbene compounds and methods of producing them	The invention provides methods of making food products and ingredients having enhanced levels of natural stilbene compounds, such as resveratrol. Chocolate liquor, cocoa powders, and cocoa extracts having enhanced levels of resveratrol prepared from cacao beans are disclosed.	WO2008134648A2	WIPO (PCT)	(Hurst W. J.; et al.; 2008)
Method of treating and preventing respiratory diseases involving administration of cholecalciferol	The present invention provides treatment, prevention and relief of respiratory symptoms ensured by the combined application of cholecalciferol and said excipients. The excipient is specified in a group consisting of tea extract, vitamin A, probiotics, vitamin C, Andrographis extract, 1-tryptophan, *Allium sativum, T. grandiflorum*, coenzyme Q10, acai berry, grapes, guarana and others	RU2479313C2	Russia	(Clymer J. W. & Ranney P. J.; 2006)

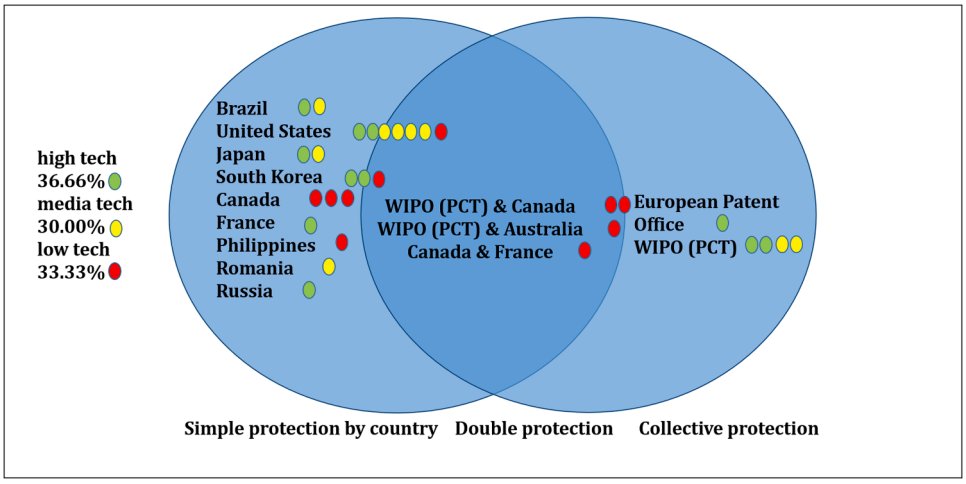


Fig. 3. Relationship technological complexity and patent protection of products derived from the fruits of the genus *Theobroma* in the thirty patentes evaluated.

species of bioactive compounds in their different plant parts for further industrial application. This review intends to stimulate new studies to evaluate the biological and nutritional potential of different plant parts of the genus *Theobroma*, aiming the promotion of human health.

CRediT authorship contribution statement

Josiana Moreira Mar: Writing – original draft, Investigation, Conceptualization. **Edson Queiroz da Fonseca Júnior:** Writing – original draft, Investigation, Conceptualization. **Renilto Frota Corrêa:** Writing – original draft, Investigation. **Pedro Henrique Campelo:** Writing – review & editing. **Edgar Aparecido Sanches:** Writing – review & editing. **Jaqueline de Araújo Bezerra:** Writing – review & editing, Conceptualization, Project administration, Supervision.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

Acknowledgments

The authors wish to acknowledge to Fundação de Amparo à Pesquisa do Estado do Amazonas – FAPEAM (Process number 01.02.016301.03335/2021–56, Edital n. 010/2021- CT&I áreas

prioritárias, Edital n. 013/2022–Produtividade-CT&I), CAPES Programa de Desenvolvimento da Pós-Graduação (PDPG) Pós-Doutorado Estratégico - PPGBIOTEC – UFAM, Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPq (Process number 311522/2020–3) for financial support, fellowships and Analytical Center at IFAM-CMC for the infrastructure.

References

Švarc-Gajić, J., Brezo-Borjan, T., Dzedik, V., Rodrigues, F., Morais, S., & Delerue-Matos, C. (2023). ESG approach in the valorization of cocoa (*Theobroma cacao*) by-products by subcritical water: Application in the cosmetic industry. *Sustainable Chemistry and Pharmacy, 31*(11), Article 100908.

Acosta-Vega, L., Martínez-Suárez, J. F., Sánchez-Garzón, F. S., Hernández-Carrión, M., & Nerio, L. S. (2023). Optimization of the encapsulation process of Cupuassu (*Theobroma grandiflorum*) pulp by spray drying as an alternative for the valorization of Amazonian fruits. *Lwt, 184*(2).

Alba, K., Nguyen, P. T. M., & Kontogiorgos, V. (2021). Sustainable polysaccharides from *Malvaceae* family: Structure and functionality. *Food Hydrocolloids, 118*(1), Article 106749.

Ambrosone, L., Guerra, G., Cinelli, M., Filippelli, M., Mosca, M., Vizzarri, F., Giorgio, D., & Costagliola, C. (2014). Corneal epithelial wound healing promoted by verbascoside-based liposomal eyedrops. *BioMed Research International, 4*(8), 1–8.

Andrade, J.K.S., Barros, R.G.C., Pereira, U.C., Gualberto, N.C., de Oliveira, C.S., Shanmugam, S., & Narain, N. (2022). α-Amylase inhibition, cytotoxicity and influence of the in vitro gastrointestinal digestion on the bioaccessibility of phenolic compounds in the peel and seed of Theobroma grandiflorum. Food Chemistry, 373 (October 2021).

Ashihara, H., & Crozier, A. (1999). Biosynthesis and metabolism of caffeine and related purine alkaloids in plants. *Advances in Botanical Research, 30*(1), 117–205.

Badrie, N., Bekele, F., Sikora, E., & Sikora, M. (2015). Cocoa agronomy, quality, nutritional, and health aspects. *Critical Reviews in Food Science and Nutrition, 55*(5), 620–659.

Bruni, R., Medici, A., Guerrini, A., Scalia, S., Poli, F., Romagnoli, C., Muzzoli, M., & Sacchetti, G. (2002). Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (*Sterculiaceae*) seeds. *Food Chemistry, 77*(3), 337–341.

Campos-Vega, R., Nieto-Figueroa, K. H., & Oomah, B. D. (2018). Cocoa (*Theobroma cacao* L.) pod husk: Renewable source of bioactive compounds. *Trends in Food Science & Technology, 81*(1), 172–184.

Clough, B. H., Ylostalo, J., Browder, E., McNeill, E. P., Bartosh, T. J., Rawls, H. R., Nakamoto, T., & Gregory, C. A. (2017). Theobromine upregulates osteogenesis by human mesenchymal stem cells in vitro and accelerates bone development in rats. *Calcified Tissue International, 100*(3), 298–310.

Colli-Silva, M., & Pirani, J. R. (2020). Estimating bioregions and undercollected areas in South America by revisiting Byttnerioideae, Helicteroideae and Sterculioideae (*Malvaceae*) occurrence data. *Flora (Ankara, Turkey), 271*(1), Article 151688.

Cunha, F. V. M., Coelho, A. G., Azevedo, P. S., da, S., da Silva, A. A., Oliveira, F., de, A., & Nunes, L. C. C. (2019). Systematic review and technological prospection: Ethyl ferulate, a phenylpropanoid with antioxidant and neuroprotective actions. *Expert Opinion on Therapeutic Patents, 29*(2), 73–83.

Curimbaba, T. F. S., Almeida-Junior, L. D., Chagas, A. S., Quaglio, A. E. V., Herculano, A. M., & Di Stasi, L. C. (2020). Prebiotic, antioxidant and anti-inflammatory properties of edible Amazon fruits. *Food Bioscience, 36*(10), Article 100599.

de Oliveira, T. B., & Genovese, M. I. (2013). Chemical composition of cupuassu (*Theobroma grandiflorum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats. *Food Research International, 51*(2), 929–935.

De Souza Schmidt Gonçalves, A. E., Lajolo, F. M., & Genovese, M. I. (2010). Chemical composition and antioxidant/antidiabetic potential of brazilian native fruits and commercial frozen pulps. *Journal of Agricultural and Food Chemistry, 58*(8), 4666–4674.

Dozier, C. A., Kim, D., & Russell, D. H. (2020). Chemical residue evidence in Leon Plain pottery from the Toyah phase (1300–1650 CE) in the American Southern Plains. *Journal of Archaeological Science, 32*(1), Article 102450.

Efavi, J. K., Nyankson, E., Kyeremeh, K., Manu, G. P., Asare, K., & Yeboah, N. (2022). Monodispersed AgNPs synthesized from the nanofactories of *Theobroma cacao* (Cocoa) leaves and pod husk and their antimicrobial activity. *International Journal of Biomaterials, 1*(9), Article 4106558.

Etaware, P. M. (2021). Molecular sciences the effects of the phytochemistry of cocoa on the food chemistry of chocolate (s) and how disease resistance in cocoa can be improved using CRISPR/Cas9 technology. *Food Chemistry: Molecular Sciences, 3*(1), Article 100043.

Febrianto, N. A., & Zhu, F. (2022). Comparison of bioactive components and flavor volatiles of diverse cocoa genotypes of *Theobroma grandiflorum, Theobroma bicolor, Theobroma subincanum* and *Theobroma cacao*. *Food Research International, 161*(3), Article 111764.

Franco, R., Oñatibia-Astibia, A., & Martínez-Pinilla, E. (2013). Health benefits of methylxanthines in cacao and chocolate. *Nutrients, 5*(10), 4159–4173.

Freitas, E. C. (2017). Phenolic compounds and screening of the antioxidant capacity of *Theobroma grandiflorum* dehydrated coproducts. *Revista Virtual de Química, 9*(6), 2193–2203.

Gao, X. N., Gao, E. J., & Zhu, M. C. (2019). Synthesis, crystal structure, DNA binding, and cytotoxicity of a Zn(II) complex constructed from phenylacetic acid. *Journal of Structural Chemistry, 60*(7), 1180–1188.

Giustina, L. D., Luz, L. N., Vieira, F. S., Rossi, F. S., Soares-Lopes, C. R. A., Pereira, T. N. S., & Rossi, A. A. B. (2014). Population structure and genetic diversity in natural populations of *Theobroma speciosum* Willd. Ex Spreng (*Malvaceae*). *Genetics and Molecular Research, 13*(2), 3510–3519.

Griesbach, R. J. (2010). Biochemistry and genetics of flower color. In *Plant Breeding Reviews, 25* pp. 89–114). John Wiley & Sons, Inc.

Gutiérrez-Álzate, K., Rosario, I. L. S., de Jesus, R. L. C., Maciel, L. F., Santos, S. A., de Souza, C. O., Vieira, C. P., Cavalheiro, C. P., & Costa, M. P.da (2023). Physicochemical, rheological, and nutritional quality of artisanal fermented milk beverages with cupuassu (*Theobroma grandiflorum*) pulp and flour. *Foods (Basel, Switzerland), 12*(11), Article 12112217.

Hammerstone, J. F., Romanczyk, L. J., & Martin Aitken, W. (1994). Purine alkaloid distribution with *Herrania* and *Theobroma*. *Phytochemistry, 35*(5), 1237–1240.

Hasanuddin, A., Anwar, K., Mappatoba, M., & Hafsah. (2019). Antibacterial and antioxidant activities of ethanol extracts of cocoa husk (*Theobroma cacao* L.) with maltodextrine in various concentration. *IOP Conference Series: Earth and Environmental Science, 255*, Article 012017.

Huang, J. P., Wang, Y. J., Tian, T., Wang, L., Yan, Y., & Huang, S. X. (2021). Tropane alkaloid biosynthesis: A centennial review. *Royal Society of Chemistry, 38*(9), 1634–1658.

Jalal, M. A. F., & Collin, H. A. (1977). Polyphenols of mature plant, seedling and tissue cultures of *Theobroma cacao*. *Phytochemistry, 16*(9), 1377–1380.

Jean-Marie, E., Bereau, D., Robinson, J.-C., Poucheret, P., Guzman, C., Boudard, F., & Robinson, J.-C. (2021). Antioxidative and immunomodulatory potential of the endemic French Guiana wild cocoa "Guiana. *Foods (Basel, Switzerland), 10*(3), 522.

Kababie-Ameo, R., Rabadán-Chávez, G. M., Vázquez-Manjarrez, N., & Gutiérrez-Salmeán, G. (2022). Potential applications of cocoa (*Theobroma cacao*) on diabetic neuropathy: Mini-review. *Frontiers in Bioscience-Landmark, 27*(2), 057.

Lúcia, A., Pereira, F., Corrêa, S., Kelly, V., Abreu, G., De Oliveira, T., Faria, W., Narain, N., & Rodrigues, S (2017). Impact of fermentation conditions on the quality and sensory properties of a probiotic cupuassu (*Theobroma grandi* florum) beverage. *Food Research International Journal, 100*(5), 603–611.

Lee, K. W., Kim, Y. J., Lee, H. J., & Lee, A. C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *Journal of Agricultural and Food Chemistry, 51*(1), 7292–7295.

Llerena, W., Samaniego, I., Vallejo, C., Arreaga, A., Zhunio, B., Coronel, Z., Quiroz, J., Angós, I., & Carrillo, W. (2023). Profile of bioactive components of cocoa (*Theobroma cacao* L.) by-products from ecuador and evaluation of their antioxidant activity. *Foods (Basel, Switzerland), 12*(13), 1–18.

Lopes, S. M. D. A., Martins, M. V., Souza, V. B.de, & Tulini, F. L (2021). Evaluation of the nutritional composition of cocoa bean shell waste (*Theobroma cacao*) and application in the production of a phenolic-rich iced tea. *Journal of Culinary Science & Technology, 21*(5), 818–828.

Luiz, S., Rochelle, D. A., Cássia, J. De, Sardi, O., Almeida, I., Câmara, L., Galvão, D. C., Goldoni, J., Matias, S., Alencar, D., & Luiz, P (2016). The anti-biofilm potential of commonly discarded agro-industrial residues against opportunistic pathogens. *Industrial Crops & Products, 87*(1), 150–160.

Mar, J. M., da Silva, L. S., Moreira, W. P., Biondo, M. M., Pontes, F. L. D., Campos, F. R., Kinupp, V. F., Campelo, P. H., Sanches, E. A., Bezerra, J., & de, A. (2021). Edible flowers from *Theobroma speciosum*: Aqueous extract rich in antioxidant compounds. *Food Chemistry, 356*(5), Article 129723.

Martínez-lópez, S., Sarriá, B., Gómez-juaristi, M., Goya, L., Mateos, R., & Bravo-clemente, L. (2014). Theobromine, caffeine and theophylline metabolites in human plasma and urine after consumption of soluble cocoa products with different methylxanthine contents. *FRIN, 63*(1), 446–455.

Marx, F., & Maia, J. G. S. (1991). Purine alkaloids in seeds of Theobroma species from the Amazon. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung, 193*(5), 460–461.

Meng, X., Zhou, J., Zhao, C., & Gan, R. (2024). Health benefits and molecular mechanisms. *Journal of Functional Foods, 115*(3), 1–27.

Millena, C. G., Balonzo, A. R. R., Rentoy, J. R., Ruivivar, S. S., & Bobiles, S. C. (2023). Effect of fermentation stages on the nutritional and mineral bioavailability of cacao beans (*Theobroma cacao* L.). *Journal of Food Composition and Analysis, 115*(9), Article 104886.

Monteiro, J. P., Alves, M. G., Oliveira, P. F., & Silva, B. M. (2016). Structure-bioactivity relationships of methylxanthines: Trying to make sense of all the promises and the drawbacks. *Molecules (Basel, Switzerland), 21*(8), 974.

Motamayor, J. C., Mockaitis, K., Schmutz, J., Haiminen, N., III, D. L., Cornejo, O., Findley, S. D., Zheng, P., Utro, F., Royaert, S., Saski, C., Jenkins, J., Podicheti, R., Zhao, M., Scheffler, B. E., Stack, J. C., Feltus, F. A., Mustiga, G. M., Amores, F., … Kuhn, D. N. (2013). The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. *Genome Biology, 14*(6), r53.

Oliveira, T. B. De, & Genovese, M. I. (2013). Chemical composition of cupuassu (*Theobroma grandi florum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats. *FRIN, 51*(2), 929–935.

Oliveira, K. De, North, S., Beck, B., & Hopp, J. (2015a). Promoting collaboration and cultural competence for physician assistant and physical therapist students: A cross-cultural decentralized interprofessional education model. *Journal of Educational Evaluation for Health Professions, 12*(1), 1–20.

de Oliveira, T. B., Rogero, M. M., & Genovese, M. I (2015b). Poliphenolic-rich extracts from cocoa (*Theobroma cacao* L.) and cupuassu (*Theobroma grandiflorum* Willd. Ex Spreng. K. Shum) liquors: A comparison of metabolic effects in high-fat fed rats. *PharmaNutrition, 3*(2), 20–28.

Pérez-Mora, W., Jorrin-Novo, J. V., & Melgarejo, L. M. (2018). Substantial equivalence analysis in fruits from three *Theobroma* species through chemical composition and protein profiling. *Food Chemistry, 240*(1), 496–504.
Parada-Berríos, F. A., Vásquez-Osegueda, E. A., Lovo-Lara, L. M., Arias-de-Linares, A. Y., & Molina-Escalante, M. O. (2022). Rescate de patashte (*Theobroma bicolor* Humb & Bonpl.) y cushta (*Theobroma angustifolium*) dos especies promisorias en peligro de extinción en El Salvador. *Revista Minerva, 2*(1), 109–121.
Perez-Vizcaino, F., & Fraga, C. G. (2018). Research trends in flavonoids and health. *Archives of Biochemistry and Biophysics, 646*(1), 107–112.
Ponce-Sánchez, J., Zurita-Benavides, M. G., & Peñuela, M. C. (2021). Reproductive ecology of white cacao (*Theobroma bicolor* Humb. & Bonpl.) in Ecuador, western Amazonia: Floral visitors and the impact of fungus and mistletoe on fruit production. *Brazilian Journal of Botany, 44*(1), 479–489.
Puerari, C., Magalhães, K. T., & Schwan, R. F. (2012). New cocoa pulp-based kefir beverages: Microbiological, chemical composition and sensory analysis. *Food Research International, 48*(2), 634–640.
Pugliese, A. G., Tomas-Barberan, F. A., Truchado, P., & Genovese, M. I. (2013). Flavonoids, proanthocyanidins, vitamin C, and antioxidant activity of *Theobroma grandiflorum* (Cupuassu) pulp and seeds. *Journal of Agricultural and Food Chemistry, 61*(11), 2720–2728.
Quiroz-Reyes, C. N., & Aguilar-Méndez, M.Á. (2022). Continuous ultrasound and pulsed ultrasound: Selective extraction tools to obtain enriched antioxidants extracts from cocoa beans (*Theobroma cacao* L.). *Innovative Food Science & Emerging Technologies, 80*(1), Article 103095.
Ramos, S. N. M., Glezer, J. W., Garcia, A.de O., Behrens, J. H., & Efraim, P (2022). Cupuassu from bean to bar: Sensory and hedonic characterization of a chocolate-like product. *Food Research International, 155*(1), Article 111039.
Ramos-Escudero, F., Casimiro-Gonzales, S., Fernández-Prior, Á., Cancino Chávez, K., Gómez-Mendoza, J., Fuente-Carmelino, L.de.la, & Muñoz, A. M. (2021). Colour, fatty acids, bioactive compounds, and total antioxidant capacity in commercial cocoa beans (*Theobroma cacao* L.). *LWT, 147*(1), Article 111629.
Rawel, H. M., Huschek, G., Sagu, S. T., & Homann, T. (2019). Cocoa bean proteins-Characterization, changes and modifications due to ripening and post-harvest processing. *Nutrients, 11*(2), 428.
Rojo-Poveda, O., Barbosa-Pereira, L., Zeppa, G., & Stévigny, C. (2020). Cocoa bean shell—A by-product with nutritional properties and biofunctional potential. *Nutrients, 12*(4), 1123.
Sánchez-Rabaneda, F., Jáuregui, O., Casals, I., Andrés-Lacueva, C., Izquierdo-Pulido, M., & Lamuela-Raventós, R. M. (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry, 38*(1), 35–42.
Sesso, H. D., Manson, J. E., Aragaki, A. K., Rist, P. M., Johnson, L. G., Friedenberg, G., Copeland, T., Clar, A., Mora, S., Moorthy, M. V., Sarkissian, A., Carrick, W. R., Anderson, G. L., Manson, J. E., Sesso, H. D., Rist, P. M., Lagerstrom, S. R., Bassuk, S. S., Wang, L., … Stern, L. (2022). Effect of cocoa flavanol supplementation for the prevention of cardiovascular disease events: The cocoa supplement and multivitamin outcomes study (cosmos) randomized clinical trial. *American Journal of Clinical Nutrition, 115*(6), 1490–1500.
Shahanas, E., Panjikkaran, S. T., Aneena, E. R., Sharon, C. L., & Remya, P. R. (2019). Health benefits of bioactive compounds from cocoa (*Theobroma cacao*). *Agricultural Reviews, 40*(2), 143–149.
Silva, E. N.da, Ramos, D.da C., Menezes, L. M., Souza, A. O.de, Lannes, S. C.da S., & da Silva, M.v. (2014). Nutritional value and antioxidant capacity of "cocoa honey" (*Theobroma cacao* L.). *Food Science and Technology, 34*(4), 755–759.
Silva, L. F. R.da, Gomes, A.da. S., Castro, D. R. G., Souza, F.das. C.do. A., Mar, J. M., Laiane Souza da Silva, E. A. S., Bezerra, J.de. A., Bakry, A. M., & Campelo, P. H (2019). Ultrasound-assisted homogenization and gum Arabic combined to physicochemical quality of cupuaçu juice. *Journal Food Processing Preservation, 43*(9), e14072.
Takshak, S., & Agrawal, S. B. (2019). Defense potential of secondary metabolites in medicinal plants under UV-B stress. *Journal of Photochemistry and Photobiology B: Biology, 193*(1), 51–88.
Tauchen, J., Bortl, L., Huml, L., Miksatkova, P., Doskocil, I., Marsik, P., Villegas, P. P. P., Flores, Y. B., Damme, P. Van, Lojka, B., Havlik, J., Lapcik, O., & Kokoska, L (2016). Phenolic composition, antioxidant and anti-proliferative activities of edible and medicinal plants from the Peruvian Amazon. *Revista Brasileira de Farmacognosia, 26*(6), 728–737.
Valada, P., Alçada-Morais, S., Cunha, R. A., & Lopes, J. P. (2022). Theobromine targets adenosine receptors to control hippocampal neuronal function and damage. *International Journal of Molecular Sciences, 23*(18), 10510.
Vargas-arana, G., Merino-zegarra, C., Tang, M., Pertino, M. W., & Simirgiotis, M. J. (2022). UHPLC – MS characterization, and antioxidant and nutritional analysis of cocoa waste flours from the Peruvian Amazon. *Antioxidants (Basel), 21*(11), 1–14.
Vladimir-Knežević, S., Blaženković, B., Štefan, M. B., & Babac, M. (2012). Plant polyphenols as antioxidants influencing the human health. *Phytochemicals as Nutraceuticals, 23*(1), 155–180.
Vriesmann, L. C., & de Oliveira Petkowicz, C. L. (2009). Polysaccharides from the pulp of cupuassu (*Theobroma grandiflorum*): Structural characterization of a pectic fraction. *Carbohydrate Polymers, 77*(1), 72–79.
Wen, H., Li, L., Zhan, L., Zuo, Y., Li, K., Qiu, M., Li, H., Sun, W., & Xu, E. (2021). Hypoxic postconditioning promotes mitophagy against transient global cerebral ischemia via PINK1/Parkin-induced mitochondrial ubiquitination in adult rats. *Cell Death and Disease, 12*(7), 630.
Wheni, A., Purnami, M., Windarsih, A., Kumar, D., Suzuki, T., & Katayama, T. (2021). Biocatalysis and Agricultural Biotechnology In vitro studies of antioxidant, antidiabetic, and antibacterial activities of *Theobroma cacao, Anonna muricata* and *Clitoria ternatea*. *Biocatalysis and Agricultural Biotechnology, 33*(4), Article 101995.

CAPÍTULO 6

Aplicação de plasma atmosférico frio em folhas de *Hibiscus acetosella*: Impactos nos fenóis totais, na atividade antioxidante e na morfologia da superfície.

Resumo: Este estudo avaliou o efeito do plasma atmosférico frio sobre o perfil fenólico, a atividade antioxidante, os parâmetros de cor e a microestrutura das folhas de *Hibiscus acetosella*. As amostras foram submetidas a diferentes níveis de tensão (18, 20 e 22 kV) e tempos de tratamento (2, 6 e 8 minutos). Os compostos fenólicos foram caracterizados por UPLC-QToF-MS/MS, e a capacidade antioxidante foi determinada pelos ensaios DPPH, ABTS e FRAP. Os parâmetros de cor (L*, a*, b* e ΔE) e as alterações microestruturais também foram avaliados. Os resultados demonstraram que o tratamento com plasma modulou significativamente a composição bioativa de forma dependente da tensão aplicada. A condição de 18 kV, especialmente por 6 minutos, promoveu a maior atividade antioxidante e os maiores teores de fenóis totais e antocianinas, sugerindo maior liberação ou transformação estrutural dos compostos fenólicos. Em contraste, tratamentos mais intensos levaram à redução da capacidade antioxidante, provavelmente devido à degradação oxidativa induzida por espécies reativas de oxigênio e nitrogênio. A análise cromatográfica revelou a predominância de ácido 2-O-cafeoilhidroxicítrico e ácido cafeico, cuja modulação esteve associada às mudanças na atividade antioxidante.
A análise microestrutural indicou uma ruptura progressiva da parede celular com o aumento da intensidade do tratamento, o que pode facilitar a liberação de compostos, mas também promover sua degradação em condições mais severas. O plasma atmosférico frio representa uma tecnologia verde promissora para a modulação controlada de compostos bioativos em matrizes vegetais, com potenciais aplicações no desenvolvimento de ingredientes funcionais e produtos alimentícios de maior valor agregado.

Palavras-chave: Plasma atmosférico frio; Compostos fenólicos; Atividade antioxidante; *Hibiscus acetosella*.

Application of Cold Atmospheric Plasma on *Hibiscus acetosella* Leaves: Impacts on Total Phenolics, Antioxidant Activity, and Surface Morphology

***Josiana Moreira Mar*[a,d], *Andreza Cavalcante de Oliveira*[d], *Daniel de Queiroz Rocha*[a,d], *Adriano de Souza Carolino*[b], *Erickson Oliveira dos Santos*[e], *Cleideane Cunha Costa*[e], *Kidney de Oliveira Gomes Neves*[h], *Marcos Batista Machado*[h], *Renilto Frota Correa*[d], *Edgar Aparecido Sanches*[b], *Pedro Henrique Campelo*[f], *Jaqueline de Araújo Bezerra*[a,g*]**

[a]*Posgraduate Program in Biotechnology, Federal University of Amazonas, Manaus 69067-005, Brazil;*

[b]*Laboratory of Nanostructured Polymers, Materials Physics Department, Federal University of Amazonas, Manaus 69067-005, Brazil;*

[c]*LPMAT, Laboratório de processamento de materiais tecnológicos, Federal University of Amazonas, Manaus 69067-005, Brazil;*

[d]*NECTAM, Analytical Center, Federal Institute of Education, Science and Technology of Amazonas, Campus Manaus Centro, Manaus 69020-120, Brazil;*

[e]*Research Laboratory for Water Reuse, Samsung Research & Development Institute Brazil, Samsung Eletrônica da Amazônia, Manaus 69075-842, Brazil;*

[f]*Department of Food Technology, Federal University of Viçosa, Viçosa 36570-900, Brazil;*

[g]*Coordination of Technology and Innovation (COTEI), National Institute for Amazonian Research (INPA), Manaus, Amazonas, Brazil;*

[h]*Nuclear Magnetic Resonance Laboratory, Analytical Center, Universidade Federal do Amazonas, Manaus, 69067-005, Brazil.*

Corresponding Authors:

*Jaqueline de A. Bezerra (jaqueline.bezerra@inpa.gov.br)

ABSTRACT

This study evaluated the effect of atmospheric cold plasma on the phenolic profile, antioxidant activity, color parameters, and microstructure of *Hibiscus acetosella* leaves. The samples were subjected to different voltage levels (18, 20, and 22 kV) and treatment times (2, 6, and 8 min). Phenolic compounds were characterized by UPLC-QToF-MS/MS, and antioxidant capacity was determined using DPPH, ABTS, and FRAP assays.

Color parameters (L*, a*, b*, and ΔE) and microstructural changes were also assessed. The results demonstrated that plasma treatment significantly modulated the bioactive composition in a voltage-dependent manner. The condition of 18 kV, particularly for 6 min, promoted the highest antioxidant activity and the highest total phenolic and anthocyanin contents, suggesting enhanced release or structural transformation of phenolic compounds. In contrast, more intense treatments led to reductions in antioxidant capacity, likely due to oxidative degradation induced by reactive oxygen and nitrogen species. Chromatographic analysis revealed the predominance of 2-*O*-caffeoylhydroxycitric acid and caffeic acid, whose modulation was associated with changes in antioxidant activity. Microstructural analysis indicated progressive cell wall disruption with increasing treatment intensity, which may facilitate compound release but also promote degradation under severe conditions. Atmospheric cold plasma represents a promising green technology for the controlled modulation of bioactive compounds in plant matrices, with potential applications in the development of functional ingredients and value-added food products.

Keywords: Atmospheric cold plasma; Phenolic compounds; Antioxidant activity; *Hibiscus acetosella*

1. Introduction

In recent years, driven by the growing demand for safe foods with superior nutritional quality, novel low-temperature technologies have been developed to preserve food while reducing processing impacts on its sensory and nutritional properties (Fernández & Thompson, 2012). Recognized as the fourth state of matter, plasma is composed of a complex mixture of excited species (atoms, molecules, ions, and radicals), reactive components (electrons, cations, anions, and free radicals), ground-state molecules, as well as ultraviolet (UV) photons and visible light (Nwabor et al., 2022). Cold plasma is typically generated by applying electrical or microwave energy to gases such as air, oxygen, hydrogen, nitrogen, helium, argon, or their mixtures, under either low or atmospheric pressure conditions (Mir et al., 2020).

Its ability to generate reactive species at mild temperatures makes it ideal for interacting with heat-sensitive molecules, such as anthocyanins and carotenoids, natural pigments widely recognized for their antioxidant properties and benefits to human health

(Ramezan et al., 2024). The application of cold plasma can enhance the antioxidant potential of foods and plant extracts through various mechanisms. One of the main mechanisms is the generation of reactive oxygen and nitrogen species (Moszczyńska et al., 2023). When present at controlled concentrations, these reactive species can interact with bioactive compounds in the materials, such as polyphenols, flavonoids, and vitamins, inducing structural modifications that result in increased antioxidant activity (Zargarchi et al., 2024).

A thorough understanding of the mechanisms by which cold plasma influences these bioactive compounds, as well as the microstructural changes occurring in the treated materials, is essential for advancing its applications (Heydari et al., 2023). In this context, scanning electron microscopy (SEM) plays a crucial role, enabling direct visualization of the modifications at the cellular and tissue levels induced by cold plasma (Birania et al., 2022). According to Bezerra et al. (2023), cold plasma promotes surface rupture, pore formation, and increased roughness due to the action of reactive species on plant tissues, which enhances mass transfer and extraction processes. In this context, scanning electron microscopy (SEM) is essential for directly visualizing these structural alterations at the cellular level (Bezerra et al., 2023).

Hibiscus acetosella, commonly known as cranberry hibiscus or roselle, has emerged as a promising source of bioactive compounds with remarkable antioxidant properties. Its flowers and leaves are rich in phenolic compounds, including anthocyanins (responsible for their red-purple coloration), flavonoids such as myricetin, quercetin, and kaempferol, as well as organic acids like caffeic acid (dos Santos Silva et al., 2023). The presence of these phytochemicals endows *H. acetosella* with significant free radical scavenging ability and reducing power, as demonstrated in various antioxidant activity assays (Lyu et al., 2020). This abundance of antioxidants suggests its potential for

applications in the food, pharmaceutical, and cosmetic industries, contributing to human health and the prevention of oxidative stress-related diseases (Marques Mandaji et al., 2022).

The effects of cold plasma on bioactive compounds are highly complex, requiring a multifaceted approach. To address this, the present study integrates cold plasma treatment with NMR-based chemical characterization, assessment of antioxidant potential, quantification of anthocyanins and carotenoids, and microstructural analysis using Scanning Electron Microscopy (SEM). The main objective was to elucidate how cold plasma modulates the content and bioactivity of anthocyanins and carotenoids by linking plasma-induced structural changes to compound functionality. The findings underscore the potential of cold plasma to enhance extractive yield and functional properties, with promising implications for the food industry.

2. Materials and methods

2.1 Atmospheric cold plasma treatment (ACP)

One gram of *Hibiscus acetosella* tea powder was infused in 30 mL of water for 10 min. The infusion was transferred to sterile Petri dishes (90 mm diameter) and treated using a cold atmospheric pressure plasma (CAP) system (ALT2030, Inergiae Conversores Estáticos, Brazil). Plasma treatments were conducted at atmospheric pressure and a constant frequency of 6 kHz, using voltages of 18, 20, and 22 kV and exposure times of 2, 6, and 8 min (Hemmati, et al., 2021). Ambient relative humidity and temperature were 61% and 20 °C, respectively.

2.2 Color analysis

Color measurements were performed using a DeltaVista spectrophotometer (450G, DeltaColor, Brazil), which evaluated the CIELAB L*, a*, and b* coordinates. Measurement conditions included an LED light source, D65 illuminant, and a 10° standard observer, as described by Souza Carvalho et al., 2020. A tea sample unaffected by any temperature influence served as the white reference.

2.3 Antioxidant capacity assessment

The antioxidant capacity was determined by the DPPH• and $ABTS^{\bullet+}$ radical scavenging assays, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC). DPPH corresponds to the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, expressed as μM Trolox equivalents per mL (μM Trolox/mL). ABTS refers to the 2,2′-azino-*bis*-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, also expressed as μM Trolox equivalents per mL (μM Trolox/mL). FRAP (Ferric Reducing Antioxidant Power) was expressed as μM Fe^{2+} per mL (μM Fe^{2+}/mL). The analyses were performed according to previously reported methodologies with slight modifications, using an Epoch 2 microplate reader (BioTek Instruments, USA) (Moreira et al., 2021).

2.4 Determination of total phenolic content of extracts (TPC)

The quantification of total phenolic content was performed using the Folin–Ciocalteu colorimetric method. First, samples were brought into contact with the Folin–Ciocalteu reagent for 5 minutes. Subsequently, 6% sodium bicarbonate solution was added, and the resulting mixture was incubated for 90 minutes at room temperature. Absorbance was determined at 750 nm using a microplate reader, with results presented as mg of gallic acid equivalents per mL of sample (mg GAE/mL) (Mar et al., 2023).

2.5 Total Anthocyanin Content

The total anthocyanin content in *H. acetosella* was determined by the pH differential spectrophotometric method. This technique quantifies anthocyanins based on their structural transformation under different pH conditions. Results were expressed in milligrams of anthocyanins per 100 grams of sample, using an extinction coefficient of 982 (at pH 2.0), according to established methodology. Absorbance measurements were performed at 535 nm using a microplate reader (Epoch 2, BioTek, Winooski, VT, USA) (Oliveira et al., 2018).

2.6 MEV

The microstructure of *H. acetosella* teas was analyzed using a NA3 microscope (TECSCAN, Czech Republic) operating at 20 keV and 25 °C. The samples were mounted on carbon tape and coated with a thin layer of gold prior to analysis.

2.7 UPLC-QToF-MS/MS analysis

A 2.0 mg portion of the freeze-dried hydromethanolic extract samples was weighed into 2 mL eppendorff tubes and diluted with 2 mL of 10% HPLC-grade methanol solution. The analytical samples were filtered using a 0.22 µm PVDF syringe filter directly into 2 mL amber vials and injected into the UPLC-QToF-MS/MS system.

The chromatographic analysis was carried out using an ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) equipped with a Sample Delivery System (SDS), a Flow-Through Needle (FTN) autosampler, and a column oven. The system was interfaced to a Xevo G2-XS qTOF mass spectrometer (Waters Corporation) equipped

with an electrospray ionization source (ESI). Instrument control, data acquisition, and processing were performed using MassLynx™ 4.2 software (Waters).

Separation was achieved on an ACQUITY UPLC® BEH C_{18} column (100 × 2.1 mm, 1.7 µm particle size) maintained at 40 °C. The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). The elution was performed under a gradient program as follows: 0–1 min, 5% B; 1–15 min, 20% B; 15–20 min, 40% B; 20–25 min, 80% B; 25–27 min, 95% B; 27.1–30 min, return to 5% B for column re-equilibration. The flow rate was 0.25 mL/min, and the injection volume was 10 µL. The column and sample tray temperatures were maintained at 40 °C and 15 °C, respectively, to ensure stability and reproducibility.

The qTOF mass spectrometer was operated in negative electrospray ionization mode (ESI^-). Mass spectra were acquired in enhanced resolution over an m/z range of 100–1200. The instrumental parameters were set as follows: capillary voltage at 2.5 kV, cone voltage at 30 V, source temperature at 120 °C, and desolvation temperature at 350 °C. Nitrogen was used as the desolvation and cone gas at specific flow rates. Leucine enkephalin (m/z 554.2615, negative mode) was continuously infused as a reference lock mass for real-time mass correction, ensuring mass accuracy within ±2 ppm.

All analyses were performed in full scan TOF mode and processed using MassLynx™ and UNIFI™ software to obtain accurate mass measurements, isotopic patterns, and fragmentation spectra for compound identification. Tentative identification of metabolites was based on exact mass, elemental composition, and MS/MS fragmentation patterns, compared with authentic standards or literature data.

2.8 Isolation of caffeic acid derivative by HPLC Preparatory

Following analysis by UPLC-QToF-MS/MS, a major compound derived from caffeic acid was isolated by preparative HPLC for complete structural elucidation by 1D and 2D NMR. Preparative chromatographic separation for compound isolation from the extract was performed using a semi-preparative high-performance liquid chromatography (HPLC) system from Shimadzu, equipped with two high-pressure pumps (LC-6AD and LC-8A), a diode array detector (SPD-M20A), a system controller module (CBM-20A), an in-line five-channel degasser (DGU-20A5R), a Rheodyne 7725i manual injection valve, and an automatic fraction collector (FRC-10A). Separations were carried out on an ODS column (Shim-Pack PREP-ODS (H), 20 × 250 mm, 5 μm; Shimadzu).

The mobile phase consisted of water (solvent A) and methanol (solvent B), delivered at a constant flow rate of 16 mL/min. The chromatographic separation was performed using a linear gradient elution program as follows: 10% solvent B from 0 to 5 min; increased to 60% solvent B from 5 to 35 min; increased to 80% solvent B from 35 to 42 min; increased to 100% solvent B from 42 to 46 min; followed by re-equilibration at 5% solvent B from 46 to 50 min. Eluted compounds were isolated and collected using the automatic fraction collector (FRC-10A).

2.9 High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) analysis

The samples analyzed by UPLC-QToF-MS/MS were also injected into HPLC-DAD. Chromatographic analyses were performed using a high-performance liquid chromatography system (Prominence series, Shimadzu Corporation, Kyoto, Japan), equipped with a quaternary pump (LC-20AD), an online degasser (DGU-20A5), an autosampler (SIL-20A HT), a column oven (CTO-20A), a diode array detector (SPD-

M20A), and a system controller (CBM-20A). System control, data acquisition, and processing were carried out using LabSolutions software (Shimadzu).

The separation of analytes was achieved on a CORTECS T3 column (4.6 × 100 mm, 2.7 µm particle size; Waters Corporation, Milford, MA, USA), maintained at 40 °C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (methanol containing 0.1% formic acid). The elution was performed using a linear gradient program optimized for phenolic compound profiling, as follows: 0–5 min, isocratic at 5% B; 5–15 min, 5–60% B; 15–20 min, 60–80% B; 20–22 min, 80–100% B, followed by a re-equilibration period of 2 min under the initial conditions. The flow rate was 1.0 mL min^{-1}, and the injection volume was 10 µL. Detection was carried out using a diode array detector (DAD), scanning from 200 to 600 nm.

The calibration curve for caffeic acid was prepared from a gravimetrically standardized stock solution (1000.00 mg/L), yielding six concentration levels: 0.67, 3.4, 7.1, 15.3, 30.3, and 49.7 mg/L. Each level was analyzed in triplicate, and the resulting chromatographic peak areas were used to calculate the mean, standard deviation (SD), and relative standard deviation (RSD%). RSD values ranged from 0.14% to 1.78%, confirming high repeatability across the entire working range.

The analytical response exhibited a robust linear behavior, with simple linear regression applied using the mean peak area as the dependent variable. The determination coefficient (R^2 = 0.9958) demonstrated excellent linearity within the studied interval. Residual analysis confirmed homoscedasticity and the absence of systematic trends. These results indicate that the constructed calibration curve is suitable for the quantification of caffeic acid and its derivatives in the samples.

Statistical analysis was conducted to evaluate the effect of treatment factors (Voltage and Time) on the response variables: Antioxidant Activity (AA) and Caffeic

Acid Concentration (CafAc). Initially, Pearson's correlation was used as an exploratory analysis to quantify the linear relationship between AA and the concentrations of the quantified phenolic compounds.

To determine the existence of statistically significant differences between the treatment groups, a one-way Analysis of Variance (ANOVA) was applied, considering Voltage (18 V, 20 V, 22 V) and Time (2 min, 6 min, 8 min) as independent factors. In cases where the ANOVA indicated significance ($p < 0.05$), Tukey's post-hoc test was used to identify which pairs of treatment groups were significantly different from each other. All analyses were performed excluding the control sample (VP).

2.10 Qualitative NMR Analysis

The NMR spectra of the extracts were acquired at the Nuclear Magnetic Resonance Laboratory (NMRLab) of the Federal University of Amazonas (UFAM), using a Bruker Avance IIIHD NMR spectrometer operating at 11.7 T and equipped with a 5 mm BBI probe with a z-axis gradient.

2.11 Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) in R software (version 3.5.1). Differences among treatment means were evaluated by Tukey's test, with a significance level of 5% ($p \leq 0.05$).

3. Results and discussion

3.1 Effect of CAP pretreatment on antioxidant activity

The antioxidant activity of *Hibiscus acetosella* leaves is directly associated with the presence of bioactive compounds, particularly phenolic compounds (Mar et al., 2020).

In the extract of the species, phenolic acids such as caffeic acid, rosmarinic acid, and caffeoyl-hydroxycitric acid derivatives were identified, as well as flavonoids derived from quercetin and kaempferol, as described by Santos Silva et al. (2023). These metabolites exhibit well-recognized antioxidant potential due to the presence of conjugated aromatic systems and multiple hydroxyl groups in their chemical structures, features that favor electron or hydrogen atom donation and, consequently, the neutralization of free radicals (Mathew et al., 2015).

However, these compounds may react with reactive oxygen species generated by atmospheric cold plasma, such as atomic oxygen, singlet oxygen, hydroxyl radicals, and peroxyl radicals (Brandenburg et al., 2007). Oxidation induced by these reactive species may compromise the structural integrity of phenolic compounds, reducing their ability to interact with ferric ions and DPPH radicals and, consequently, decreasing the observed antioxidant activity

In this context, plasma treatment duration and applied voltage significantly influenced the antioxidant activity of the samples, as evidenced by the DPPH, ABTS, and FRAP assays (Table 1). In the group treated at 18 kV, increasing the exposure time from 2 to 6 minutes promoted a significant increase *($p < 0.05$)* in antioxidant activity, with DPPH values rising from 1382.6 to 1476.8 μM Trolox/mL, ABTS from 1457.4 to 1586.3 μM Trolox/mL, and FRAP from 1577.2 to 1666.9 μMFe(II)/mL. However, extending the treatment to 8 minutes resulted in a reduction of these values, suggesting that prolonged exposure may favor oxidative processes and degradation of bioactive compounds.

At 20 kV, a progressive and significant decrease *($p < 0.05$)* in antioxidant activity was observed with increasing exposure time, with sample V20KV8 presenting the lowest values across all three assays. A similar trend was observed at 22 kV, although the values remained higher than those recorded for the 20 kV group. Overall, comparison among

voltage levels indicates that increasing plasma intensity, particularly when combined with longer exposure times, may promote a decline in antioxidant activity.

The intermediate condition (18 kV for 6 min – V18KV6) stood out by presenting the highest values in all three antioxidant assays, even surpassing the control (VP). In contrast, more severe conditions, particularly 20 kV for 8 min, resulted in significant reductions in antioxidant activity. These findings are consistent with the total phenolic content (TPC) values, as samples with lower levels of these compounds exhibited reduced antioxidant capacity.

Table 1: Results are expressed as mean ± standard deviations (n = 3).

Amostras	DPPH (µMTrolox/mL)	ABTS (µMTrolox/mL)	TPC (mgGAE/mL)	FRAP (µMFe(II)/mL)
V18KV2	1382.6 ± 5.2^{d}	1457.4 ± 5.0^{d}	502.2 ± 0.6^{d}	1577.2 ± 1.7^{d}
V18KV6	1476.8 ± 6.3^{a}	1586.3 ± 6.7^{a}	576.3 ± 0.6^{a}	1666.9 ± 1.9^{a}
V18KV8	1427.6 ± 6.3^{b}	1526.3 ± 8.9^{b}	524.0 ± 1.2^{b}	1605.4 ± 2.3^{b}
V20KV2	1157.6 ± 3.8^{g}	1171.9 ± 7.7^{h}	426.5 ± 0.9^{h}	1485.0 ± 2.7^{h}
V20KV6	1129.3 ± 6.3^{h}	1126.3 ± 8.8^{i}	403.3 ± 0.9^{i}	1468.7 ± 2.3^{i}
V20KV8	1091.8 ± 3.8^{i}	1069.7 ± 5.8^{j}	344.1 ± 0.9^{j}	1308.7 ± 3.9^{j}
V22KV2	1361.8 ± 5.2^{e}	1369.7 ± 6.7^{e}	481.2 ± 0.5^{e}	1564.3 ± 2.3^{e}
V22KV6	1348.5 ± 5.0^{e}	1296.3 ± 8.8^{f}	473.3 ± 0.9^{f}	1543.2 ± 1.7^{f}
V22KV8	1296.0 ± 6.6^{f}	1221.9 ± 3.8^{g}	457.8 ± 0.5^{g}	1519.5 ± 1.7^{g}
VP	1399.3 ± 3.8^{c}	1504.1 ± 6.9^{c}	509.4 ± 1.3^{c}	1595.0 ± 2.8^{c}

(a–j) Means with the same letter in the same column are not significantly different. VP = untreated sample (control). V18KV, V20KV, and V22KV indicate plasma treatments at 18, 20, and 22 kV, respectively, and the numbers 2, 6, and 8 correspond to exposure time (min).

The obtained results corroborate previous studies demonstrating that antioxidant activity depends on plasma treatment conditions. Hemmati, et al. (2021) observed that increasing voltage and exposure time in green tea powder resulted in a reduction in phenolic compound content and antioxidant activity. Similarly, Castro et al. (2020) reported a non-linear behavior of antioxidant activity in camu-camu juice treated with

dielectric barrier discharge (DBD) plasma, with an increase at low frequency (200 Hz), a reduction at intermediate frequencies (420–628 Hz), and a new significant increase at a higher frequency (960 Hz), accompanied by variations in antioxidant compound concentrations.

These findings indicate that the effect of atmospheric cold plasma depends on the interaction between voltage, exposure time, and other operational parameters, which influence the generation and reactivity of the active species formed during the process (Ji et al., 2020). Thus, moderate conditions may favor the release or structural modification of phenolic compounds, enhancing antioxidant activity, whereas more intense or prolonged treatments tend to promote their degradation.

Overall, plasma treatment influenced the antioxidant activity of the samples, as certain conditions, such as V18KV6, resulted in increased antioxidant activity compared to the control (VP), while more severe conditions, represented by the V20 group, led to reduced antioxidant capacity. These findings are consistent with the results reported by Hemmati et al. (2021) for atmospheric cold plasma treatments in green tea powder, in which increasing voltage and exposure time decreased phenolic content and antioxidant activity.

3.2 Colour analysis

Table 2 shows that cold plasma treatment promoted significant changes in the color parameters of the tea samples ($p < 0.05$), highlighting the strong influence of voltage and exposure time.

A reduction in the L* parameter (lightness) was observed, particularly under more intense treatments, such as 20 kV/6 min (38.8 ± 0.2) and 22 kV/8 min (39.6 ± 0.1), indicating darkening of the tea at higher plasma intensities. Previous studies have reported

chromatic alterations in foods and plant extracts subjected to cold plasma, which are attributed to pigment oxidation and degradation of phenolic compounds (Amiri et al., 2025).

Regarding the a* parameter, an increase was observed under intermediate treatments, with the highest value at 20 kV/6 min (13.5 ± 0.1), suggesting intensification of the reddish hue, possibly associated with structural modification and greater exposure of phenolic compounds, including anthocyanins. This behavior has been previously described in plant extracts where plasma-induced microstructural modifications enhanced the exposure of pigments such as anthocyanins and other flavonoids, resulting in changes in chromaticity (Xi et al., 2024).

The b* parameter showed a progressive increase with rising voltage and exposure time, reaching 24.8 ± 0.1 at 22 kV/8 min, indicating a tendency toward yellowing of the beverage. Consequently, ΔE increased according to treatment severity, ranging from 23.8 ± 0.1 (18 kV/2 min) to 31.0 ± 0.1 (22 kV/8 min), confirming pronounced and visually perceptible color changes (ΔE > 3).

Table 2: Color parameters (L, a, b*) and ΔE of wine samples subjected to different cold plasma conditions. Values are expressed as mean ± standard deviation (n = 3). Different lowercase letters in the same column indicate significant differences among samples ($p < 0.05$) according to Tukey's test.

Samples	L*	a*	b*	ΔE
V18KV2	42.1± 0.1^{a}	11.7± 0.1^{f}	17.3± 0.1^{i}	23.8± 0.1^{i}
V18KV6	40.9 ± 0.1^{d}	12.1± 0.1^{d}	17.5± 0.1^{h}	24.6± 0.1^{h}
V18KV8	41.5± 0.1^{b}	12.3± 0.1^{c}	18.2± 0.1^{g}	25.2± 0.1^{g}
V20KV2	40.4± 0.1^{g}	12.9± 0.1^{b}	18.6± 0.1^{f}	26.5± 0.1^{e}
V20KV6	38.8± 0.2^{i}	13.5± 0.1^{a}	19.5± 0.1^{e}	28.1± 0.1^{c}
V20KV8	41.0± 0.1^{c}	10.9± 0.1^{g}	20.0± 0.1^{d}	26.3± 0.1^{f}
V22KV2	40.7± 0.1^{e}	11.9± 0.1^{e}	20.9± 0.1^{c}	27.8± 0.1^{d}
V22KV6	40.5± 0.1^{f}	10.8± 0.1^{h}	23.1± 0.1^{b}	29.7± 0.1^{b}
V22KV8	39.6± 0.1^{h}	9.7± 0.1^{i}	24.8± 0.1^{a}	31.0± 0.1^{a}

VP	41.9± 0.1^{j}	11.6± 0.1^{f}	17.0± 0.1^{j}	23.4± 0.1^{j}

(a–j) Means with the same letter in the same column are not significantly different. VP = untreated sample (control). V18KV, V20KV, and V22KV indicate plasma treatments at 18, 20, and 22 kV, respectively, and the numbers 2, 6, and 8 correspond to exposure time (min).

These results indicate that more severe cold plasma conditions may induce oxidative reactions and structural transformations in tea pigments and bioactive compounds, directly affecting their stability and visual quality.

Studies on foods and plant extracts subjected to cold plasma have also reported high ΔE values as a consequence of changes in phenolic compounds, anthocyanins, and natural pigments (Dantas et al., 2021), reinforcing that plasma can trigger both degradation reactions and chromatic reorganization depending on the applied conditions (Amorim et al., 2024).

3.3 Total anthocyanins determination (TAC)

The total anthocyanin results (Figure 1) demonstrate that atmospheric cold plasma treatment exerted a voltage- and time-dependent effect. The V18KV6 condition showed the highest anthocyanin content (9.8 mg/mL), differing significantly from the other samples ($p < 0.05$), whereas V20KV8 presented the lowest value (4.0 mg/mL). Overall,

moderate treatments favored the preservation or even enhancement of anthocyanin content, while more severe conditions promoted marked reductions.

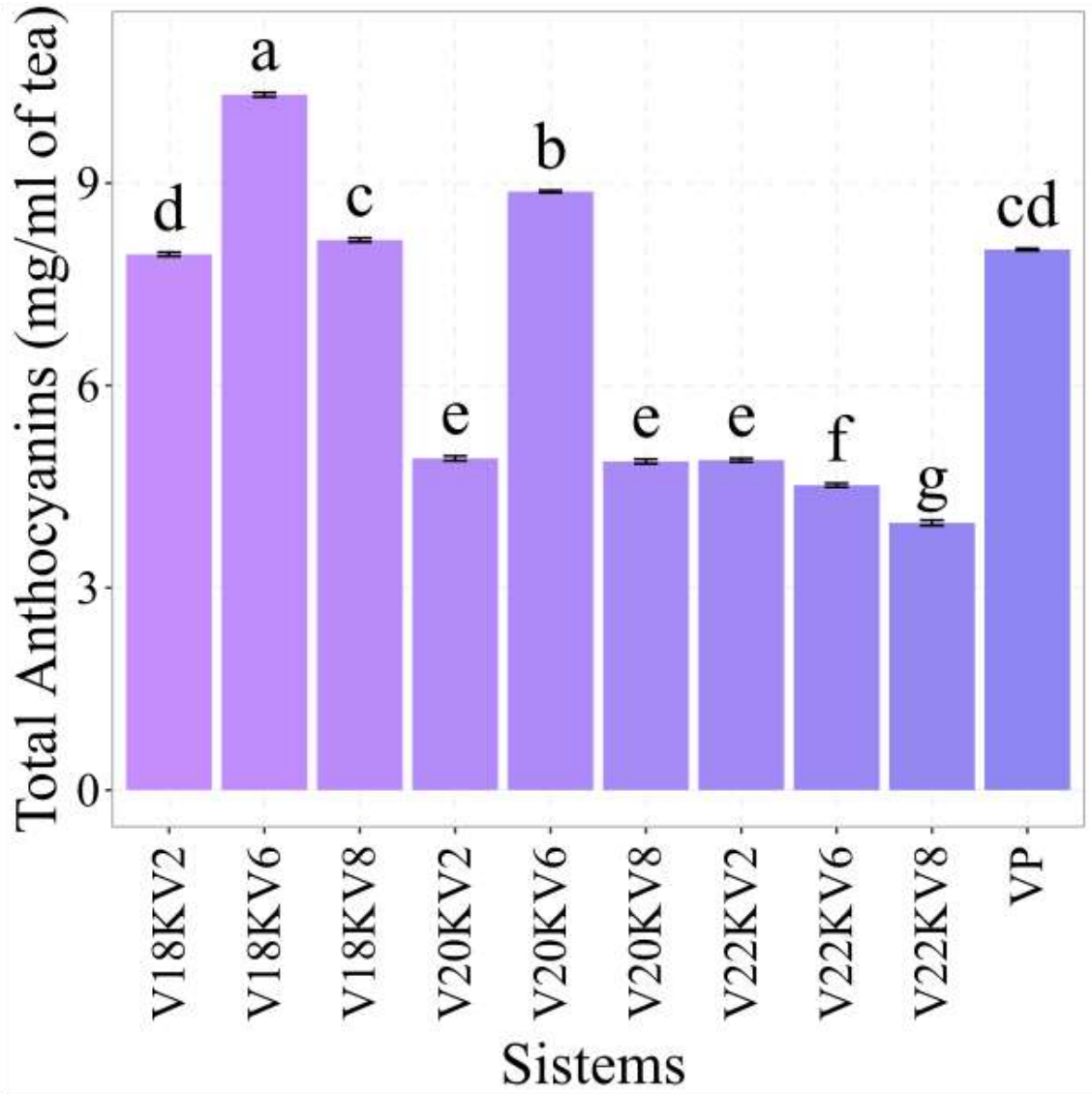


Figure 1: Total anthocyanin content in *Hibiscus acetosella* leaves subjected to atmospheric cold plasma treatment under different voltage levels and exposure times.

This behavior largely follows the results observed for antioxidant activity (DPPH, ABTS, and FRAP) and total phenolic content (TPC). The V18KV6 sample (9.8 mg mL^{-1}), which showed the highest anthocyanin content, also exhibited the highest DPPH (1476.8 µM Trolox/mL), ABTS (1586.3 µM Trolox/mL), TPC (576.3 mg GAE/mL), and

FRAP (1666.9 μM Fe(II)/mL) values, even surpassing the control VP (8.1 mg/mL). This result suggests that, under intermediate processing conditions, plasma may promote structural modifications in the plant matrix that facilitate the release of phenolic compounds and anthocyanins, thereby enhancing antioxidant potential.

In contrast, samples treated at 20 kV showed a progressive reduction in anthocyanins, TPC, and antioxidant activity with increasing exposure time. The V20KV2 condition presented an anthocyanin content of approximately 5.0 mg/mL, decreasing in V20KV6 (4.9 mg/mL) and reaching the lowest value in V20KV8 (4.0 mg/mL). This trend was accompanied by reductions in DPPH (1091.8 μM Trolox/mL), ABTS (1069.7 μM Trolox/mL), TPC (344.1 mg GAE/mL), and FRAP (1308.7 μM Fe(II)/mL) values in V20KV8, indicating possible degradation of bioactive compounds under higher treatment intensity.

At 22 kV, a similar gradual decrease was observed with increasing treatment time, with values of 5.0 mg/mL (V22KV2), 4.6 mg/mL (V22KV6), and 4.3 mg/mL (V22KV8). Despite this reduction, the values remained higher than those observed at 20 kV under the longest exposure time. Samples V22KV2 and V22KV6 presented antioxidant activity relatively close to the control, suggesting that although structural alterations occurred, the level of chemical degradation was less pronounced than at 20 kV.

The positive correlation between TPC, anthocyanins, and antioxidant assays confirms that these compounds play a central role in the extract's antioxidant capacity. As anthocyanins belong to the flavonoid class and significantly contribute to total phenolic content, variations in their concentration directly impact DPPH, ABTS, and FRAP results.

Overall, the data indicate that the plasma effect is non-linear and dependent on the combination of voltage and time. Moderate conditions, such as 18 kV for 6 min

(V18KV6), favored greater availability of antioxidant compounds, whereas more intense treatments, particularly 20 kV for 8 min (V20KV8), resulted in structural degradation and reduced antioxidant potential. Comparison of total anthocyanin content relative to the control sample (VP 8.0 mg/mL) further confirmed that the effect of cold plasma was operational-condition dependent. Treatment at 18 kV for 6 min promoted the greatest increase, with an approximate 25% rise in anthocyanin content (Figure 2).

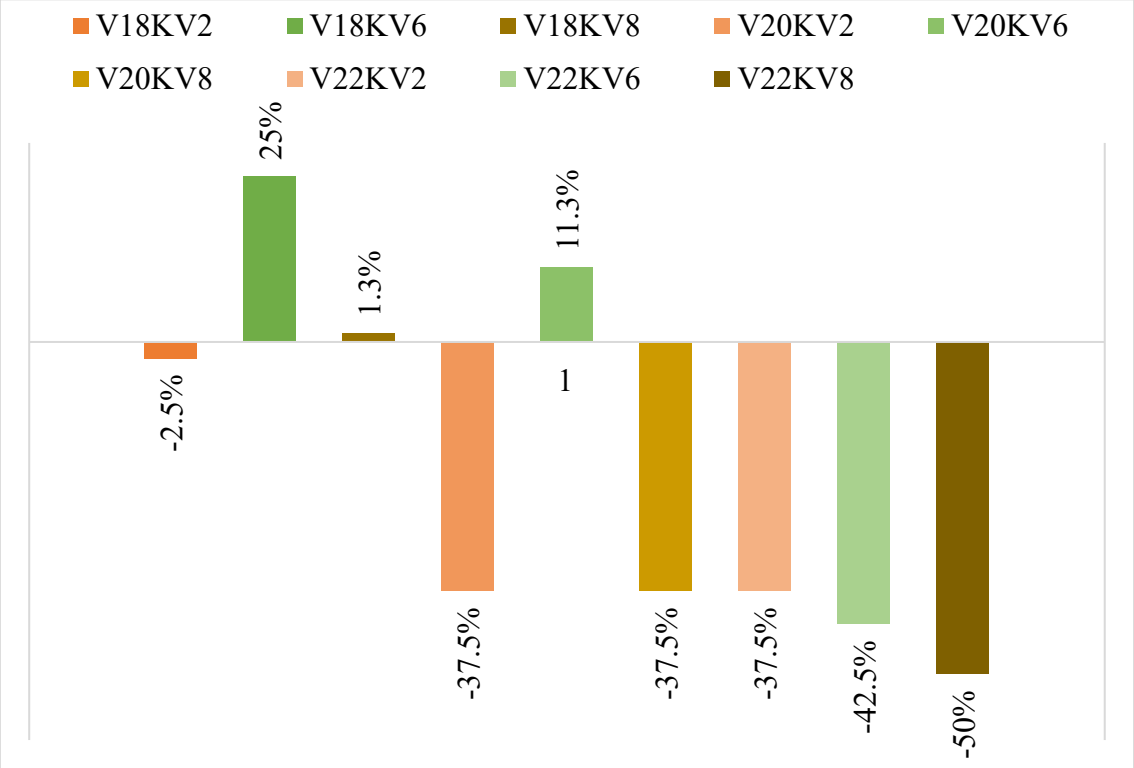


Figure 2: Percentage variation of total anthocyanins relative to VP.

On the other hand, more intense treatments, particularly at 20 and 22 kV with longer exposure times, promoted marked reductions (up to −50% in V22KV8), indicating possible oxidative degradation of these pigments due to the action of reactive species generated by the plasma. This pattern corroborates the findings of Fernandes et al. (2014) who reported that anthocyanin content may either increase (34–77%) or be completely degraded after cold plasma treatment, depending on operational parameters such as time

and voltage. Similarly, studies on pomegranate juice demonstrated increases of 21–35% in anthocyanin content under suitable atmospheric cold plasma conditions, suggesting a positive effect on the preservation or extraction of these compounds (Bursać Kovačević et al., 2016). In addition, research on blueberry juice indicates that changes in anthocyanin content and antioxidant activity vary significantly according to oxygen concentration and plasma exposure time, reinforcing that parameter optimization is essential to maximize the stability of these sensitive pigments (Hou et al., 2019). Taken together, these findings confirm that cold plasma can act either as a promoter of anthocyanin extraction or as a degradation factor, depending on the intensity of the applied treatment.

3.4 Effect of CAP pretreatment on surface microstructure

SEM images (Figure 1) of roselle leaves showed that atmospheric cold plasma promoted morphological changes dependent on voltage and exposure time. The control samples, untreated system (ST) and pure roselle (VP), exhibited relatively intact surfaces, with preserved cell walls and well-defined structural organization.

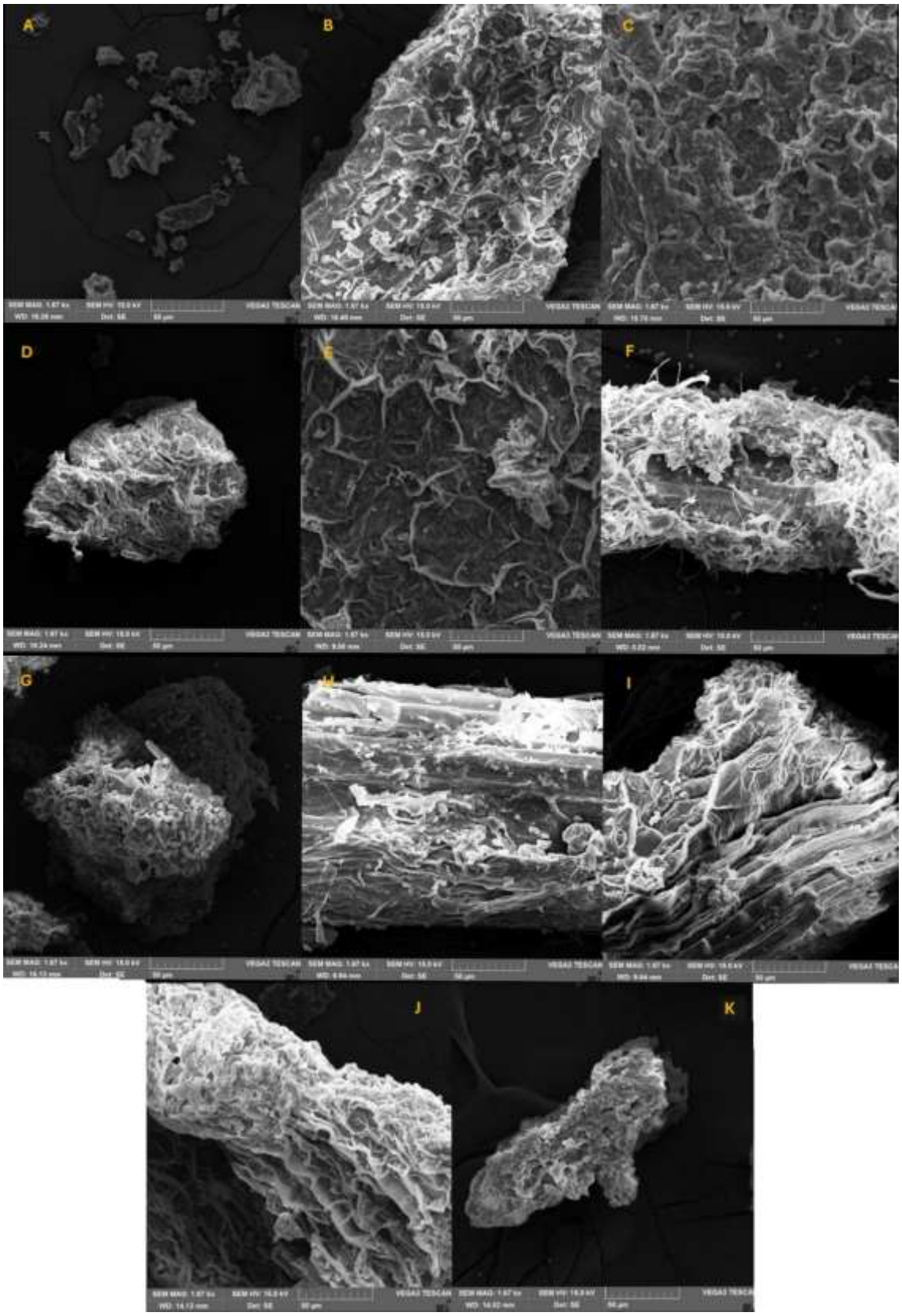


Figure 3: SEM (Scanning Electron Microscopy) images of *H. acetosella* tea powder: untreated sample (A); fresh roselle in plant material form (B); samples treated with cold plasma at V18kV, at 50× magnification, for 2, 6, and 8 minutes (C, D, and E, respectively); samples treated at V20kV for 2, 6, and 8 minutes (F, G, and H,

respectively); and samples treated at V22kV for 2, 6, and 8 minutes (I, J, and K, respectively).

For treatments at 18 kV, the 2 min condition (V18KV2) did not promote marked structural alterations, maintaining morphology similar to the control. However, increasing the exposure time to 6 min (V18KV6) resulted in visible surface wrinkling and the onset of cell wall disorganization. At 8 min (V18KV8), these modifications became more evident, with the presence of fissures and increased roughness, indicating that prolonged exposure intensifies plasma effects even at intermediate voltage.

For treatments at 20 kV, structural damage was more pronounced. At 2 min (V20KV2), clear signs of cellular disorganization and surface fragmentation were already observed. After 6 min (V20KV6), the structure showed greater collapse and increased surface irregularity. The most severe treatment, 20 kV for 8 min (V20KV8), resulted in intense cell wall rupture and significant loss of structural integrity, characterizing strong degradation of the plant matrix.

Similarly, treatments at 22 kV promoted evident structural alterations at all evaluated times. At 2 min (V22KV2), fiber deformation and compaction were already noticeable. These effects intensified at 6 min (V22KV6) and became even more pronounced at 8 min (V22KV8), with visibly shrunken and deformed cell walls and marked surface roughness.

Overall, increasing voltage and plasma exposure time intensified structural rupture and disorganization. These findings suggest that the observed changes in antioxidant activity and phenolic content may be associated with the initial release of bioactive metabolites under moderate conditions, followed by their possible degradation under more severe treatments due to plasma-induced cellular damage. Similar results, including cellular ablation of green tea leaves, surface rupture, and increased roughness,

have been reported for different plant matrices subjected to cold plasma (Keshavarzi et al., 2020), confirming that treatment intensity is a determining factor in the degree of microstructural modification. Comparable findings were also described for turmeric treated with cold plasma (Hemmati, Garavand, Goudarzi, et al., 2021).

3.5 Chemical composition by Qualitative NMR analysis and UPLC-QToF-MS/MS

The analysis of the aqueous extract of *Hibiscus acetosella* leaves by NMR spectroscopy enabled the identification of four compounds, including three organic acids, citric acid (1), hydroxycitric acid (2), and caffeic acid (4) and hydroxycitric acid lactone (3) (Figure 1). Citric acid (1) was assigned based on the identification of the diastereotopic protons H-2 and H-4, observed as a pair of doublets at δ_H 2.99 (d, J = 16.4 Hz) and δ_H 3.10 (d, J = 16.4 Hz), as well as their HSQC correlations with δ_C 43.6 (C-2 and C-4) and HMBC correlations with the carboxylic carbon signal at δ_C 177.0 (COOH) (Köstereli & Severin, 2015). Hydroxycitric acid (2), derived from compound 1, was assigned by the presence of a singlet at δ_H 4.45, attributed to proton H-2, and by a carbon signal at δ_C 77.7 (C-2). Hydroxycitric acid lactone (3) was identified by the characteristic downfield shift of proton H-2 to δ_H 5.38 (s) and by the carbon signals at δ_C 87.7 (C-2), δ_C 81.7 (C-3), and δ_C 179.6 (lactone C=O) (Seethapathy et al., 2018). Finally, caffeic acid (4) was assigned based on the aromatic proton signals at δ_H 7.21 (H-2, d, J = 1.4 Hz), δ_H 6.94 (H-5, d, J = 8.3 Hz), and δ_H 7.14 (H-6, dd, J = 1.4 and 8.3 Hz), along with the vinylic protons at δ_H 7.73 (H-7) and δ_H 6.48 (H-8) (Jeong et al., 2011). The magnitude of the coupling constant (J = 16.0 Hz) between H-7 and H-8 confirmed the E stereochemistry of the double bond, while the carboxylic carbon was observed at δ_C 171.2.

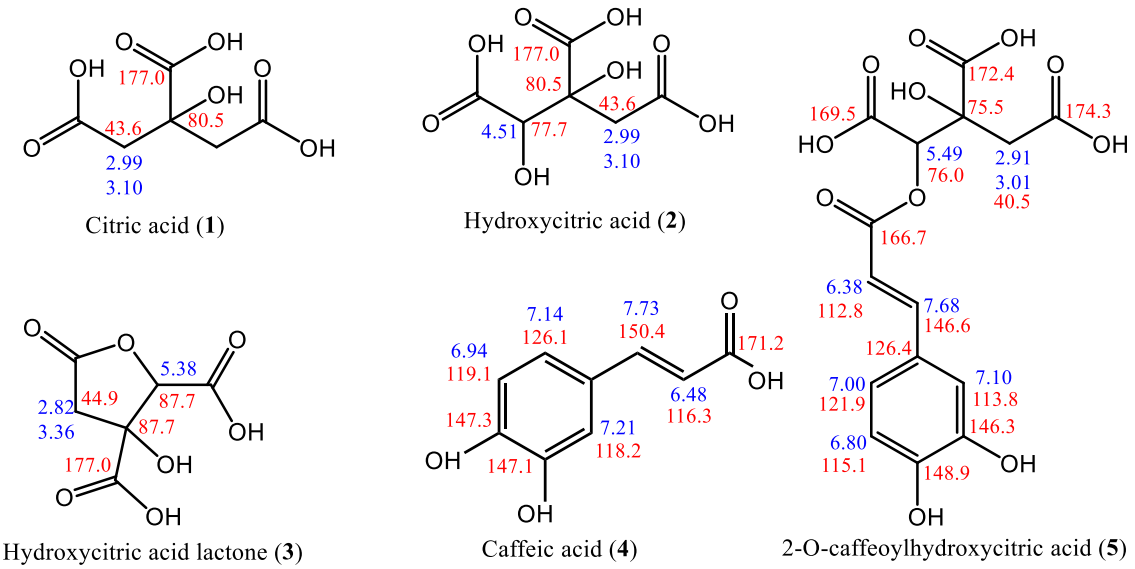


Figure 4: Chemical structures with ^{1}H and ^{13}C NMR data of the main compounds identified by NMR in the aqueous extract of *Hibiscus acetosella* leaves.

In order to isolate caffeic acid and its derivatives, the aqueous extract of *Hibiscus acetosella* leaves was subjected to HPLC fractionation, which enabled the isolation of 2-*O*-caffeoylhydroxycitric acid (5) (Figure 4). Its identification was accomplished by 1D and 2D NMR experiments, in which characteristic signals were assigned at δ 5.49 (s), δ 2.91 (H_A, d, J = 16.2 Hz) and δ 3.01 (H_B, d, J = 16.2 Hz), δ 6.38 (d, J = 16.1 Hz), δ 7.68 (d, J = 16.1 Hz), δ 7.10 (d, J = 2.0 Hz), δ 6.80 (d, J = 8.1 Hz), and δ 7.00 (dd, J = 2.0, 8.1 Hz) in the ^{1}H NMR spectrum. Additionally, the key HMBC correlation between the signal at δ 5.49 and the carbon signal at δ 166.5 confirmed the esterification of caffeic acid by hydroxycitric acid at the C-2 position (Parveen et al., 2008).

The hydroethanolic extract of *Hibiscus acetosella* was characterized by UPLC-QToF-MS/MS operating in negative ion mode. Nine phenolic compounds were identified based on accurate mass measurements with low ppm error, diagnostic MS/MS fragmentation patterns, and comparison with literature data (Table 3).

Table 3: Chemical composition by UPLC-QToF-MS/MS.

N	TR (min)	Compound (Molecular formule)	Exact molecular mass	$[M-H]^-$ (erro ppm)	m/z fragment (Intensity)	Reference
1	2.31	Hydroxycitric acid ($C_6H_8O_8$)	208.0219	207,0160 (9.2)	189.0046 (90) 127.0050 (100)	(Parveen et al., 2008)
2	3.46	Hydroxycitric acid lactone ($C_6H_6O_7$)	190.0114	189.0046 (6,34)	127.0024 (100)	(Ikeda et al., 2023)
3	10.18	Rosmarinic Acid ($C_{18}H_{16}O_8$)	360.0845	359.0781 (3.89)	179.0361 (100) 135.0466 (55)	(Grzegorczyk-Karolak et al., 2020)
4	13.66	Caffeic acid ($C_9H_8O_4$)	180.0423	179.0331 (-7,26)	135.0466 (100) 134.0387 (65)	(Ikeda et al., 2023)
5	14.28	2-*O*-caffeoylhydroxycitric acid ($C_{15}H_{13}O_{12}$)	370.0536	369.0424 (-9.21)	189.0046 (100) 127.0024 (72) 163.0396 (5) 135.0439 (10)	(Ikeda et al., 2023)
6	20.84	Quercetin 3-*O*-sambubioside ($C_{26}H_{28}O_{16}$)	596.1377	595.1324 (4.20)	300.0288 (100) 301.0344 (25) 271.0240 (10)	(Deng et al., 2009)
7	21.92	Quercetin 7-glucoside ($C_{21}H_{20}O_{12}$)	464.0955 463.0877	463.0887 (2.16)	301.0344 (55) 300.0288 (100) 271.0240 (20)	(Xiao et al., 2021)
8	23.01	Kaempferol-*O*-glycoside ($C_{21}H_{20}O_{11}$)	448.1006	447.0913 (-3.13)	284.0316 (70) 285.0368 (100) 297.1518 (20) 255.0308 (5)	(Wu et al., 2024)
9	23.32	Kaempferol-3-*O*-rutinoside ($C_{27}H_{30}O_{15}$)	594.1585	593.1531 (4.21)	285.0406 (100)	(Wu et al., 2024)

The chemical profile was predominantly composed of hydroxycitric acid derivatives, hydroxycinnamic acids, and flavonol glycosides, indicating a phenolic composition consistent with the bioactivity previously reported for species of the genus *Hibiscus*.

Compounds 1, 2, and 5 were assigned as hydroxycitric acid derivatives. Their MS/MS spectra showed characteristic sequential losses of H_2O and CO_2, generating the diagnostic fragment at *m/z* 127, typical of the hydroxycitrate skeleton. Compound 5 ($[M–H]^-$ *m/z* 369.0424) exhibited a base peak at *m/z* 189, corresponding to ester bond cleavage and formation of the hydroxycitric acid core, along with fragments at *m/z* 135 and 127, confirming the presence of caffeoyl and hydroxycitrate moieties.

Compounds 3 and 4 were identified as rosmarinic acid and caffeic acid, respectively, showing $[M–H]^-$ ions at *m/z* 359 and 179. Their fragmentation patterns were consistent with ester bond cleavage and decarboxylation (–44 Da), yielding the diagnostic fragment at *m/z* 135. These hydroxycinnamic acids are widely recognized for their antioxidant properties and may significantly contribute to the radical-scavenging capacity of the extract.

Compounds 6–9 corresponded to quercetin- and kaempferol-derived glycosides. The identification was supported by neutral losses of 162 Da (hexose) and 308 Da (rutinoside), producing the respective aglycones at *m/z* 301 (quercetin) and *m/z* 285 (kaempferol). Additional fragments arising from Retro-Diels-Alder (RDA) cleavage further corroborated the structural assignment of these flavonols. The predominance of glycosylated flavonols reinforces the relevance of this class in the phytochemical profile of *H. acetosella* and supports its biological potential.

Overall, the use of high-resolution mass spectrometry ensured reliable structural annotation. The coexistence of hydroxycitric acid derivatives, hydroxycinnamic acids, and flavonol glycosides suggests a possible synergistic contribution to the bioactive potential of the extract, particularly regarding antioxidant mechanisms.

3.6 Quantitative Analysis by HPLC-DAD and Its Relationship with Antioxidant Activity

As shown in Table 5, caffeic acid concentration was strongly dependent on plasma voltage. Treatments at 18 kV resulted in the highest levels (2.27–2.74 mg/L) significantly higher than those observed in the control (0.071 mg/L) and in samples treated at 20 kV (0.015–0.060 mg/L). This marked reduction at 20 kV suggests oxidative degradation of caffeic acid or insufficient ester cleavage under these conditions. Samples treated at 22 kV showed intermediate concentrations (2.34–2.67 mg/L), indicating partial conversion.

Table 4: Total antioxidant activity and HPLC-DAD quantification of caffeic acid and 2-*O*-caffeoylhydroxycitric acid.

Sample	Antioxidant Activity (DPPH• µMTrolox/mL)	Caffeic Acid Concentration (mg/L) (9.45 min)	2-O-Caffeoylhydroxycitric Acid Concentration (mg/L) (9.77 min)
V18KV2	1382.6 ± 5.2	2.74 ± 0.03	2.07 ± 0.02
V18KV6	1476.8 ± 6.3	2.27 ± 0.02	1.75 ± 0.04
V18KV8	1427.6 ± 6.3	2.66 ± 0.03	2.03 ± 0.02
V20KV2	1157.6 ± 3.8	0.04 ± 0.001	8.66 ± 0.05
V20KV6	1129.3 ± 6.3	0.06 ± 0.001	9.38 ± 0.06
V20KV8	1091.8 ± 3.8	0.02 ± 0.001	8.45 ± 0.04
V22KV2	1361.8 ± 5.2	2.67 ± 0.03	2.19 ± 0.03
V22KV6	1348.5 ± 5.0	2.34 ± 0.02	1.75 ± 0.02
V22KV8	1296.0 ± 6.6	2.51 ± 0.03	1.97 ± 0.02

VP	1399.3 ± 3.8	0.071 ± 0.002	9.64 ± 0.05

VP = untreated sample (control). V18KV, V20KV, and V22KV indicate plasma treatments at 18, 20, and 22 kV, respectively, and the numbers 2, 6, and 8 correspond to exposure time (min).

The observed variation in antioxidant activity and phenolic compound concentrations can be explained by a biphasic kinetic mechanism mediated by reactive oxygen and nitrogen species (RONS) generated by cold plasma, as previously described in the literature (Heydari et al., 2023). Free caffeic acid, due to its ortho-diphenolic structure, is highly susceptible to oxidation by RONS (Suroowan et al., 2022). Consequently, two competing kinetic stages occur during plasma treatment: (i) formation, resulting from ester bond cleavage in 2-*O*-caffeoylhydroxycitric acid and predominant at early treatment times; and (ii) consumption, resulting from oxidative degradation of free caffeic acid into products with lower antioxidant potential. The 2-minute treatment at 18 kV represents a condition near the optimal balance between formation and degradation, whereas longer exposure times or higher voltages favor the oxidative stage, reducing the net concentration of free caffeic acid.

The modulation of 2-*O*-caffeoylhydroxycitric acid further supports this kinetic interpretation. This ester exhibited the highest concentrations in the untreated sample (9.64 mg/L) and in extracts treated at 20 kV (8.45–9.38 mg/L), while lower concentrations were found at 18 and 22 kV (1.75–2.19 mg/L)This inverse relationship between ester and free caffeic acid concentrations indicates that plasma treatment efficiently promotes ester bond cleavage, releasing caffeic acid as a reaction product, but excessive RONS exposure at higher voltages may accelerate subsequent oxidation, preventing accumulation of free caffeic acid.

Total antioxidant activity, measured by the DPPH• assay, followed a similar trend. The highest values were obtained at 18 kV, particularly at 6 min (1476.8 µMTrolox/mL),

highlighting the strong correlation between free caffeic acid concentration and antioxidant capacity. Treatments at 20 kV exhibited the lowest antioxidant activity, which decreased with increasing exposure time (1091.8–1157.6 μMTrolox/mL), reflecting the deleterious effect of excessive oxidative stress. Statistical analysis confirmed that voltage had a highly significant effect on caffeic acid concentration and antioxidant activity ($p < 0.001$), while treatment time alone did not show statistical significance ($p > 0.93$). Post-hoc analysis further revealed that treatments at 18 and 22 kV were significantly superior to 20 kV.

Overall, these findings indicate that modulation of the phenolic profile by cold plasma can be described as a two-factor optimization process. Voltage serves as the primary determinant initiating molecular ester cleavage, while treatment time modulates the accumulation of free caffeic acid, preventing its oxidative degradation. The data suggest that careful selection of both parameters is critical for maximizing phenolic release and antioxidant potential.

From a mechanistic standpoint, the biphasic kinetic model mediated by RONS explains why moderate plasma conditions enhance antioxidant activity, whereas excessive voltage or prolonged exposure may induce a net decrease in active phenolics. In practical terms, this emphasizes the importance of tuning plasma parameters to achieve a desirable balance between formation and consumption of key bioactive compounds. Such optimization is particularly relevant for functional food development and nutraceutical applications, where maximizing antioxidant capacity is crucial.

In conclusion, the results presented in Table 5 demonstrate that voltage is the primary factor driving phenolic transformation, while exposure time plays a modulatory role in balancing formation and degradation processes. By leveraging this understanding,

cold plasma treatment can be strategically applied to enhance the bioactive profile of *H. acetosella* extracts, ultimately improving their functional properties.

4. Conclusion

This study provides a comprehensive chemical and functional characterization of aqueous extracts from *Hibiscus acetosella* leaves, combining structural elucidation by NMR spectroscopy with quantitative determination by HPLC-DAD and evaluation of antioxidant activity. The integration of analytical approaches allowed the identification of major organic acids and phenolic derivatives, including citric acid, hydroxycitric acid, hydroxycitric acid lactone, caffeic acid, and 2-*O*-caffeoylhydroxycitric acid. Plasma treatment significantly modulated the phenolic profile of the extracts. Voltage was identified as the primary determinant of phenolic transformation, directly influencing ester bond cleavage and the release of free caffeic acid. In contrast, exposure time played a secondary yet modulatory role, affecting the balance between formation and oxidative degradation processes. The highest antioxidant activity was observed under intermediate plasma conditions, particularly at 18 kV, which promoted increased levels of free caffeic acid while minimizing oxidative degradation. The inverse relationship observed between 2-O-caffeoylhydroxycitric acid and caffeic acid concentrations supports a plasma-induced ester cleavage mechanism, likely mediated by reactive oxygen and nitrogen species (RONS). These findings suggest a bifactorial kinetic behavior, in which controlled plasma parameters optimize phenolic release and antioxidant performance. Overall, the results demonstrate that cold plasma is a promising green technology for tailoring phenolic composition and enhancing the antioxidant potential of plant matrices. The combined use of NMR and HPLC-DAD proved to be a robust strategy for correlating

structural modifications with functional properties, contributing to the development of value-added functional ingredients derived from *H. acetosella*.

Acknowledgements: The authors acknowledge the Support Program for Stricto Sensu Graduate Studies – POSGRAD (Fundação de Amparo à Pesquisa do Estado do Amazonas — FAPEAM) for providing the scholarship. The authors thank to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES. We also thank the Analytical Centers at IFAM-CMC and UFAM for their laboratory infrastructure support, as well as the Thematic Laboratory of Microscopy and Nanotechnology (LTMN/INPA) and the Samsung SRBR WTS Laboratory (Industrial District, Manaus, AM) for providing additional infrastructure and analytical assistance.

Funding Statement: The authors declare that this work received no financial support from public, private, or non-profit funding agencies.

Conflicts of Interest: The authors declare no conflict of interest.

References

Amiri, M., Arab, M., & Mortazavian, A. M. (2025). The effect of cold plasma treatment on the phenolic and flavonoid content and antioxidant activity of whole buckwheat grain and flour. *Scientific Reports*, *15*(1), 1–8. https://doi.org/10.1038/s41598-025-10281-x

Amorim, D. S., Amorim, I. S., Chisté, R. C., Fernandes, F. A. N., Zanatta, F. B., Godoy, H. T., & Teixeira Filho, J. (2024). Effect of cold plasma on carotenoid content and instrumental color of carrot juice: a systematic review and meta-analysis. *Food Science and Technology*, *44*(1), 1–7. https://doi.org/10.5327/fst.00279

Bezerra, J. de A., Lamarão, C. V., Sanches, E. A., Rodrigues, S., Fernandes, F. A. N., Ramos, G. L. P. A., Esmerino, E. A., Cruz, A. G., & Campelo, P. H. (2023). Cold plasma as a pre-treatment for processing improvement in food: A review. *Food Research International*, *167*(3), 1–9. https://doi.org/10.1016/j.foodres.2023.112663

Birania, S., Attkan, A. K., Kumar, S., Kumar, N., & Singh, V. K. (2022). Cold plasma in food processing and preservation: A review. *Journal of Food Process Engineering*, *45*(9), 1–19. https://doi.org/10.1111/jfpe.14110

Brandenburg, R., Ehlbeck, J., Stieber, M., Woedtke, T. V., Zeymer, J., Schlüter, O., & Weltmann, K. D. (2007). Antimicrobial treatment of heat sensitive materials by means of atmospheric pressure Rf-driven plasma jet. *Contributions to Plasma Physics*, *47*(1–2), 72–79. https://doi.org/10.1002/ctpp.200710011

Bursać Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, *190*(1), 317–323. https://doi.org/10.1016/j.foodchem.2015.05.099

Dantas, A. M., Batista, J. D. F., dos Santos Lima, M., Fernandes, F. A. N., Rodrigues, S., Magnani, M., & Borges, G. da S. C. (2021). Effect of cold plasma on açai pulp: Enzymatic activity, color and bioaccessibility of phenolic compounds. *Lwt*, *149*(June). https://doi.org/10.1016/j.lwt.2021.111883

de Castro, D. R. G., Mar, J. M., da Silva, L. S., da Silva, K. A., Sanches, E. A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F. A. N., & Campelo, P. H. (2020). Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, *131*(January), 109044. https://doi.org/10.1016/j.foodres.2020.109044

de Souza Carvalho, L. M., Lemos, M. C. M., Sanches, E. A., da Silva, L. S., de Araújo Bezerra, J., Aguiar, J. P. L., das Chagas do Amaral Souza, F., Alves Filho, E. G., & Campelo, P. H. (2020). Improvement of the bioaccessibility of bioactive compounds from Amazon fruits treated using high energy ultrasound. *Ultrasonics Sonochemistry*, *67*(April), 105148. https://doi.org/10.1016/j.ultsonch.2020.105148

Deng, S., Deng, Z., Fan, Y., Li, J., Liu, R., & Xiong, D. (2009). Application of high-speed counter-current chromatography coupled with high performance liquid chromatography for the separation and purification of Quercetin-3-O-sambubioside from the leaves of Nelumbo nucifera. *Frontiers of Chemical Engineering in China*, *3*(4), 375–382. https://doi.org/10.1007/s11705-009-0247-9

dos Santos Silva, L. Y., da Silva Ramos, A., Cavalcante, D. N., Kinupp, V. F., da Silva Rodrigues, J. V., Ventura, B. M. L., de Oliveira Mendes, T. A., Sanches, E. A., Campelo, P. H., & de Araújo Bezerra, J. (2023). Hibiscus acetosella: An Unconventional Alternative Edible Flower Rich in Bioactive Compounds. *Molecules*, *28*(12), 1–13. https://doi.org/10.3390/molecules28124819

Fernandes, L. H., Alvarenga, C. W., Dos Santos, L. L., & Pazin-Filho, A. (2014). The

need to improve health care in prisons. *Revista de Saude Publica*, *48*(2), 275–283. https://doi.org/10.1590/S0034-8910.2014048004934

Fernández, A., & Thompson, A. (2012). The inactivation of Salmonella by cold atmospheric plasma treatment. *Food Research International*, *45*(2), 678–684. https://doi.org/10.1016/j.foodres.2011.04.009

Grzegorczyk-Karolak, I., Krzemińska, M., Kiss, A. K., Olszewska, M. A., & Owczarek, A. (2020). Phytochemical profile and antioxidant activity of aerial and underground parts of salvia bulleyana diels. Plants. *Metabolites*, *10*(12), 1–18. https://doi.org/10.3390/metabo10120497

Hemmati, V., Garavand, F., Goudarzi, M., Sarlak, Z., Cacciotti, I., & Tiwari, B. K. (2021). Cold atmospheric-pressure plasma treatment of turmeric powder: microbial load, essential oil profile, bioactivity and microstructure analyses. *International Journal of Food Science and Technology*, *56*(5), 2224–2232. https://doi.org/10.1111/ijfs.14838

Hemmati, V., Garavand, F., Khorshidian, N., Cacciotti, I., Goudarzi, M., Chaichi, M., & Tiwari, B. K. (2021). Impact of cold atmospheric plasma on microbial safety, total phenolic and flavonoid contents, antioxidant activity, volatile compounds, surface morphology, and sensory quality of green tea powder. *Food Bioscience*, *44*(PA), 101348. https://doi.org/10.1016/j.fbio.2021.101348

Heydari, M., Carbone, K., Gervasi, F., Parandi, E., Rouhi, M., Rostami, O., Abedi-Firoozjah, R., Kolahdouz-Nasiri, A., Garavand, F., & Mohammadi, R. (2023). Cold Plasma-Assisted Extraction of Phytochemicals: A Review. In *Foods* (Vol. 12, Issue 17). https://doi.org/10.3390/foods12173181

Hou, Y., Wang, R., Gan, Z., Shao, T., Zhang, X., He, M., & Sun, A. (2019). Effect of cold plasma on blueberry juice quality. *Food Chemistry*, *290*(October 2018), 79–86. https://doi.org/10.1016/j.foodchem.2019.03.123

Ikeda, T., Sugahara, T., Kanamitsu, H., Nakashima, K., Onda, H., Yoshino, N., Nishi, K., & Ishida, M. (2023). Isolation and Quantification of 2-O-Caffeoyl-hydroxycitric Acid, an Active Component in Hot Water Extracts of Coriander (Coriandrum sativum L.), Which Inhibits Degranulation of RBL-2H3 Cells. *Natural Product Communications*, *18*(7), 1–11. https://doi.org/10.1177/1934578X231183603

Jeong, C. H., Jeong, H. R., Choi, G. N., Kim, D. O., Lee, U., & Heo, H. J. (2011). Neuroprotective and anti-oxidant effects of caffeic acid isolated from Erigeron annuus leaf. *Chinese Medicine*, *6*(1), 25. https://doi.org/10.1186/1749-8546-6-25

Ji, Y., Hu, W., Liao, J., Jiang, A., Xiu, Z., Gaowa, S., Guan, Y., Yang, X., Feng, K., & Liu, C. (2020). Effect of atmospheric cold plasma treatment on antioxidant activities and reactive oxygen species production in postharvest blueberries during storage. *Journal of the Science of Food and Agriculture*, *100*(15), 5586–5595. https://doi.org/10.1002/jsfa.10611

Keshavarzi, M., Najafi, G., Ahmadi Gavlighi, H., Seyfi, P., & Ghomi, H. (2020). Enhancement of polyphenolic content extraction rate with maximal antioxidant activity from green tea leaves by cold plasma. *Journal of Food Science*, *85*(10), 3415–3422. https://doi.org/10.1111/1750-3841.15448

Köstereli, Z., & Severin, K. (2015). Selective detection of Al3+ and citric acid with a fluorescent amphiphile. *Organic and Biomolecular Chemistry*, *13*(1), 252–257. https://doi.org/10.1039/c4ob02095b

Lyu, J. Il, Ryu, J., Jin, C. H., Kim, D., Kim, J. M., Seo, K., Kim, J., Kim, S. H., Ahn, J., Kang, S., & Kwon, S. (2020). Phenolic Compounds in Extracts of. *Molecules*, *25*(1), 1–12.

Mak, J. Y. W. (2022). Determination of Sample Concentrations by PULCON NMR

Spectroscopy. *Aust J Chem.*, *75*(2), 1–15. https://doi.org/10.1071/ch21149.Determination

Mar, J. M., da Silva, L. S., Lira, A. C., Kinupp, V. F., Yoshida, M. I., Moreira, W. P., Bruginski, E., Campos, F. R., Machado, M. B., de Souza, T. P., Campelo, P. H., de Araújo Bezerra, J., & Sanches, E. A. (2020). Bioactive compounds-rich powders: Influence of different carriers and drying techniques on the chemical stability of the Hibiscus acetosella extract. *Powder Technology*, *360*, 383–391. https://doi.org/10.1016/j.powtec.2019.10.062

Mar, J. M., Frota, R., Ramos, S., Kinupp, V. F., Sanches, E. A., Campelo, P. H., & Ara, J. De. (2023). Enhancing Bioactive Compound Bioaccessibility in Tapirira guianensis Juices through Ultrasound-Assisted Applications. *Process*, *11*(2718), 1–16.

Marques Mandaji, C., da Silva Pena, R., & Campos Chisté, R. (2022). Encapsulation of bioactive compounds extracted from plants of genus Hibiscus: A review of selected techniques and applications. *Food Research International*, *151*(11), 1–16. https://doi.org/10.1016/j.foodres.2021.110820

Mathew, S., Abraham, T. E., & Zakaria, Z. A. (2015). Reactivity of phenolic compounds towards free radicals under in vitro conditions. *Journal of Food Science and Technology*, *52*(9), 5790–5798. https://doi.org/10.1007/s13197-014-1704-0

Mir, S. A., Siddiqui, M. W., Dar, B. N., Shah, M. A., Wani, M. H., Roohinejad, S., Annor, G. A., Mallikarjunan, K., Chin, C. F., & Ali, A. (2020). Promising applications of cold plasma for microbial safety, chemical decontamination and quality enhancement in fruits. *Journal of Applied Microbiology*, *129*(3), 474–485. https://doi.org/10.1111/jam.14541

Moreira, J., Souza, L., Picanço, W., & Bezerra, A. (2021). Edible flowers from Theobroma speciosum: Aqueous extract rich in antioxidant compounds. *Food Chemistry*, *356*(March). https://doi.org/10.1016/j.foodchem.2021.129723

Moszczyńska, J., Roszek, K., & Wiśniewski, M. (2023). Non-Thermal Plasma Application in Medicine—Focus on Reactive Species Involvement. *International Journal of Molecular Sciences*, *24*(16), 1–19. https://doi.org/10.3390/ijms241612667

Nwabor, O. F., Onyeaka, H., Miri, T., Obileke, K., Anumudu, C., & Hart, A. (2022). A Cold Plasma Technology for Ensuring the Microbiological Safety and Quality of Foods. *Food Engineering Reviews*, *14*(4), 535–554. https://doi.org/10.1007/s12393-022-09316-0

Oliveira, A. F. A., Mar, J. M., Santos, S. F., Júnior, J. L. da S., Kluczkovski, A. M., Bakry, A. M., Bezerra, J. de A., Nunomura, R. de C. S., Sanches, E. A., & Campelo, P. H. (2018). Non-thermal combined treatments in the processing of açai (Euterpe oleracea) juice. *Food Chemistry*, *265*(April), 57–63. https://doi.org/10.1016/j.foodchem.2018.05.081

Parveen, I., Winters, A., Threadgill, M. D., Hauck, B., & Morris, P. (2008). Extraction, structural characterisation and evaluation of hydroxycinnamate esters of orchard grass (Dactylis glomerata) as substrates for polyphenol oxidase. *Phytochemistry*, *69*(16), 2799–2806. https://doi.org/10.1016/j.phytochem.2008.08.019

Ramezan, Y., Kamkari, A., Lashkari, A., Moradi, D., & Tabrizi, A. N. (2024). A review on mechanisms and impacts of cold plasma treatment as a non-thermal technology on food pigments. *Food Sci Nutr.*, *12*(1), 1502–1527.

Seethapathy, G. S., Tadesse, M., Urumarudappa, S. K. J., Gunaga, S. V., Vasudeva, R., Malterud, K. E., Shaanker, R. U., De Boer, H. J., Ravikanth, G., & Wangensteen, H. (2018). Authentication of Garcinia fruits and food supplements using DNA

barcoding and NMR spectroscopy. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-28635-z

Suroowan, S., Llorent-Martínez, E. J., Zengin, G., Dall'Acqua, S., Sut, S., Buskaran, K., Fakurazi, S., & Mahomoodally, M. F. (2022). Phytochemical Characterization, Anti-Oxidant, Anti-Enzymatic and Cytotoxic Effects of Artemisia verlotiorum Lamotte Extracts: A New Source of Bioactive Agents. *Molecules*, *27*(18), 1–21. https://doi.org/10.3390/molecules27185886

Wu, J., Gong, J., Chen, Q., Hao, W., He, J., Wang, M., & Zhou, Q. (2024). Unveiling kaempferol glycosides as the key antiglycative components in butterfly pea (Clitoria ternatea) flower. *Current Research in Food Science*, *9*(August), 100896. https://doi.org/10.1016/j.crfs.2024.100896

Xi, J., Wang, Y., Zhou, X., Wei, S., & Zhang, D. (2024). Cold plasma pretreatment technology for enhancing the extraction of bioactive ingredients from plant materials: A review. *Industrial Crops and Products*, *209*(August 2023), 117963. https://doi.org/10.1016/j.indcrop.2023.117963

Xiao, Z., He, L., Hou, X., Wei, J., Ma, X., Gao, Z., Yuan, Y., Xiao, J., Li, P., & Yue, T. (2021). Relationships between structure and antioxidant capacity and activity of glycosylated flavonols. *Foods*, *10*(4), 1–14. https://doi.org/10.3390/foods10040849

Zargarchi, S., Hornbacher, J., Afifi, S. M., Saremnezhad, S., Günal-Köroğlu, D., Capanoglu, E., & Esatbeyoglu, T. (2024). Exploring the impact of cold plasma treatment on the antioxidant capacity, ascorbic acid, phenolic profile, and bioaccessibility of fruits and fruit juices. *Food Frontiers*, *5*(3), 1108–1125. https://doi.org/10.1002/fft2.372