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GLAUBER BATISTA MOREIRA SANTOS

ABORDAGEM METABOLÔMICA E QUIMIOMÉTRICA PARA AVALIAÇÃO DE ROTAS TECNOLÓGICAS APLICADAS NA PITAYA VERMELHA (*SELENICEREUS MONACANTHUS* (LEM.) D.R.HUNT)

FORTALEZA
2025

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Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, do Departamento de Engenharia de Alimentos da Universidade Federal do Ceará como requisito parcial para a obtenção do Título de Doutor em Ciência e Tecnologia de Alimentos. Área de concentração: Processamento de Alimentos de Origem Vegetal.

Orientadora: Profª. Drª. Dorasílvia Ferreira Pontes.

Co-orientadora: Profª. Drª. Ana Paula Dionisio.

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“Ele fez tudo apropriado a seu tempo.
Também pôs no coração do homem o anseio pela eternidade;
mesmo assim este não consegue compreender
inteiramente o que Deus fez.”
(Eclesiastes 3.11)

RESUMO

A pitaya vermelha (*Selenicereus monacanthus* (Lem.) D.R.Hunt, família *Cactaceae*) tem recebido destaque no mercado alimentício devido à sua capacidade de adaptação a variados climas, solos e ao seu potencial funcional, posicionando-se dentro do contexto da bioeconomia. Os efeitos metabólicos e quimiométricos de duas rotas tecnológicas aplicadas em pitaya vermelha foram estudados pela primeira vez em sucos tropicais e microfiltrados, objetivando agregar valor a matéria-prima. Para o suco tropical, a cromatografia líquida de ultra desempenho acoplada à espectrometria de massas com quadrupolo e tempo de voo em modo de aquisição com energia elevada (*UPLC-QTOF-MS*^{E}) e análises multivariadas foram utilizadas para analisar 64 compostos químicos relevantes, incluindo betalaínas e flavonoides. A avaliação sensorial com os testes *Check-All-That-Apply (CATA), Rate-All-That-Apply (RATA)* e afetivos revelaram que, nas formulações de pitaya vermelha com suco de caju, as amostras de sucos obtiveram as melhores percepções sensoriais dos consumidores não treinados, confirmando a influência do suco de caju para a aceitação sensorial das formulações. Além disso, as amostras com caju obtiveram as melhores intenções de compra na avaliação dos consumidores. Por outro lado, a microfiltração, sem tratamento enzimático, mostrou-se viável, pois a polpa obtida a partir do despolpamento com casca apresentou um fluxo de permeado interessante (48,12 ± 1,61 L h^{-1} m^{-2}), enquanto a polpa descascada manualmente apresentou maior permeabilidade na membrana (64,65 ± 5,5 L h^{-1} m^{-2}). Com isso, os valores de luminosidade das amostras de permeado foram superiores aos das amostras de polpa e retentado. As diferenças nos perfis metabólicos das frações microfiltradas, obtidas por *UPLC-QTOF-MS*^{E} e interpretadas por modelagem multivariada identificaram 45 metabólitos, incluindo carboidratos e isômeros de betalaínas. Este estudo destaca a importância da pitaya vermelha na indústria alimentícia, tanto em sucos tropicais de pitaya combinados com caju, quanto em sucos de pitaya clarificados por membrana. A pasteurização e a microfiltração são processos fundamentais para agregar valor aos produtos por meio da utilização sustentável da pitaya vermelha, contribuindo para práticas mais sustentáveis no sistema agroalimentar de produção no mercado que se apresenta ávido por produtos com potencial funcional.

Palavras-chave: sucos tropicais; sucos microfiltrados; análises multivariadas; sustentabilidade; potencial funcional.

ABSTRACT

The red pitaya (*Selenicereus monacanthus* (Lem.) D.R.Hunt, *Cactaceae* family) has gained prominence in the food market due to its ability to adapt to various climates and soils and its functional potential, positioning itself within the context of bioeconomy. The metabolic and chemometric effects of two technological routes applied to red pitaya were studied for the first time in tropical and microfiltered juices, aiming to add value to the raw material. For the tropical juice, ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry using elevated energy acquisition mode (UPLC-QTOF-MS^E) and multivariate analyses were used to evaluate 64 relevant chemical compounds, including betalains and flavonoids. Sensory evaluation with Check-All-That-Apply (CATA), Rate-All-That-Apply (RATA), and affective tests revealed that, in red pitaya formulations with cashew juice, the juice samples obtained the best sensory perceptions from untrained consumers, confirming the influence of cashew juice on the sensory acceptance of the formulations. Additionally, the samples with cashew received the highest purchase intentions in consumer evaluations. On the other hand, microfiltration, non-enzymatic treatment, proved viable, as the pulp obtained from depulping with peel showed an interesting permeate flux (48.12 ± 1.61 L h^{-1} m^{-2}), while manually peeled pulp showed higher membrane permeability (64.65 ± 5.5 L h^{-1} m^{-2}). Consequently, the luminosity values of the permeate samples were higher than those of the pulp and retentate samples. Differences in the metabolic profiles of the microfiltered fractions, obtained by UPLC-QTOF-MS^E and interpreted by multivariate modeling, identified 45 metabolites, including carbohydrates and betalain isomers. This study highlights the importance of red pitaya in the food industry, both in tropical pitaya juices combined with cashew and in pitaya juices clarified by membrane. Pasteurization and microfiltration are fundamental processes to add value to products through the sustainable use of red pitaya, contributing to more sustainable practices in the agro-food production system in a market eager for products with functional potential.

Keywords: tropical juices; microfiltered juices; multivariate analysis; sustainability; functional potential.

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1 INTRODUÇÃO

A cadeia de suprimentos do sistema global de alimentação tem sido cada vez mais desafiada, em relação à segurança, à distribuição e à produção, com novas tecnologias sustentáveis dos alimentos. Os principais motivos são os desperdícios de alimentos, as mudanças climáticas, as pandemias, os desastres naturais e os eventos da política global. Em adição, o aumento populacional global tem chegado a valores recordes e os consumidores estão cada vez mais exigentes quanto à qualidade dos alimentos, buscando em fontes exóticas características nutricionais e com potenciais benefícios à saúde (FAO, 2022; GHADGE *et al*., 2020; TAMASIGA *et al*., 2023; CARMEN *et al*., 2023).

A pitaya vermelha (*Selenicereus monacanthus* (Lem.) D.R.Hunt, família *Cactaceae*) (sin. *Hylocereus polyrhizus*), segundo Korotkova, *et al.* (2017), é uma cultura que apresenta a característica de baixa demanda hídrica e possui alto valor adicionado para comercialização. O mercado da Pitaya está em expansão não só na Ásia, mas também na Europa e na América. No Brasil, tem sido relatado sobre o seu cultivo em algumas regiões, como, por exemplo, em locais de clima semiárido, sendo importante para o desenvolvimento do agronegócio em regiões com solos pobres e com baixa disponibilidade de água para o plantio. Na literatura, há relatados sobre o potencial de aplicação industrial e aplicações relacionadas à atividade ansiolítica, ao controle do colesterol e ao tratamento do Diabetes. Além disso, as cascas e a polpa da pitaya são ricas em fitoquímicos e nutracêuticos (WU *et al*., 2019; LIMA *et al*., 2020; HOLANDA, *et al*., 2021; NABI *et al*., 2023).

Dentre os compostos de destaque para a pitaya vermelha, têm-se os compostos com potencial antioxidantes como, as betalaínas e os oligossacarídeos prebióticos. Portanto, é importante estudar os perfis químicos das amostras, através da abordagem metabolômica e a aplicação de diferentes tecnologias de processamento agroindustrial da pitaya, objetivando produzir novos produtos. Com isso, fomentar o uso intencional de ciência e tecnologia para beneficiar as pessoas e o planeta através da sustentabilidade (CHOO *et al*., 2016; THIRUGNANASAMBANDHAM; SIVAKUMAR, 2017; LIRA *et al*., 2019; SANTOS *et al*., 2024).

Devido ao alto valor de mercado da pitaya, com variação durante o período de safra que perdura entre os últimos e os primeiros meses do ano, tem-se buscado rotas tecnológicas para a comercialização de produtos que mantenham e agreguem benefícios à pitaya (LONE *et* *al*., 2020). Além disso, é sabido que devido a pouca familiaridade dos consumidores e o início recente de plantio de pitaya no Brasil existem poucos dados na literatura acadêmica de caráter conclusivo que contribuam com informações que combinem aplicações tecnológicas em planta-piloto industrial e perfil químico combinados com modelos de análise de dados multivariadas (LIRA *et al*., 2020; LIMA *et al*., 2020; SANTOS *et al*., 2020, 2024). Logo, a busca por inovações exige um processo de disrupção através do acompanhamento da evolução do conhecimento sobre o produto ao longo do tempo. Isso acontece, quando um produto que tem alto valor de mercado e é pouco acessível, torna-se com melhor custo-benefício, disponível para um público maior (CHRISTENSEN, 2012; CHRISTENSEN; RAYNOR; MCDONALD, 2015; CHRISTENSEN *et al*., 2020)

Nesse contexto, uma alternativa é a aplicação com formulação de sucos tropicais com adição de suco de caju, como alternativa para redução do custo e com a possibilidade de melhor aceitação pelos consumidores, visto que o caju é um fruto tradicional na região nordeste. Além disso, devido aos benefícios já relatados na literatura mais uma possibilidade é a aplicação da microfiltração tangencial. Uma operação unitária importante para a indústria de alimentos por agregar valor ao produto diminuindo a turbidez e produzindo um suco clarificado. A clarificação por membranas é um processo de filtração já utilizada em alguns vegetais como o caju, o yacon e a beterraba. Além disso, já é relatado na literatura sobre a microfiltração de polpa de pitaya para elaboração de corante. (NASCIMENTO, 2022; UROŠEVIĆ *et al*., 2017; SILVA *et al*., 2018; SOUSA *et al*., 2020; LIMA *et al*., 2020; SATYANNARAYANA, KUMAR, 2023).

O principal objetivo deste estudo foi examinar o impacto da aplicação de rotas tecnológicas na obtenção de suco misto tropical de pitaya vermelha com caju e de suco clarificado de pitaya vermelha por meio da caracterização físico-química, metabolômica (cromatografia líquida acoplada a espectrometria de massas e cromatografia gasosa acoplada a espectrometria de massas) e quimiométrica (integrando métodos supervisionados e não supervisionados). Além disso, obter a percepção sensorial dos consumidores, por meio dos testes *Check-All-That-Apply (CATA), Rate-All-That-Apply (RATA)* e afetivos nos blends de pitaya vermelha com caju.

O estudo é de grande importância para elevar o nível de prontidão tecnológica *(TRL)* visando uma futura aplicação na indústria de alimentos, pois será fundamental para preencher uma lacuna no mercado que se apresenta ávido por produtos com aplicação tecnológica e potencial funcional.

Esta tese está estruturada com uma revisão de literatura abrangente, algumas perspectivas futuras da pitaya vermelha e conta com dois apêndices complementares, o *APÊNDICE A - Metabolomic, chemometric and sensory characterization of tropical red pitaya and cashew apple juice blends: insights into processing and functional atributes e o APÊNDICE B - Metabolic and chemometric effects of red pitaya juice processing: a sustainable membrane clarification approach*, os quais, apresentam e desenvolvem rotas tecnológicas aplicadas à pitaya vermelha.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os impactos de duas rotas tecnológicas na agregação de valor à pitaya vermelha por meio de análises metabolômicas e quimiométricas.

2.2 Objetivos específicos

- Estabelecer o conhecimento amplo do perfil metabólico por meio de *UPLC-QTOF-MS*^{E} e *CG-MS* dos sucos pasteurizados e dos sucos microfiltrados de pitaya.
- Avaliar a composição química e físico-química dos sucos pasteurizados e dos sucos microfiltrados de pitaya.
- Obter informações a respeito das diferenças e semelhanças entre os perfis químicos, a partir dos dados de análise quimiométricas de componentes principais (*PCA*) nos sucos pasteurizados e nos sucos microfiltrados de pitaya.
- Determinar as variáveis ou compostos discriminantes, mediante a separação dos grupos através da análise discriminante de mínimos quadrados parciais ortogonais (*OPLS-DA*) e variáveis de importância em projeção (*S-Plot*) que influenciam a diferenciação nos sucos pasteurizados e nos sucos microfiltrados de pitaya.
- Conhecer o perfil sensorial das formulações dos sucos pasteurizados de pitaya vermelha e de pitaya vermelha com caju através das avaliações com grupo focal e análises *CATA*, *RATA* e testes afetivos.
- Estabelecer o fluxo de permeado dos sucos microfiltrados de pitaya.

3 REVISÃO DE LITERATURA

3.1 Potencial de aplicação da pitaya vermelha a partir da avaliação metabolômica e quimiométrica

A ideia de que o alimento poderia servir muito mais do que para nutrição dos indivíduos estava presente desde os primórdios da humanidade, visto que os seres humanos sempre usaram alimentos para prevenção de algumas doenças. De acordo coma Agência Nacional de Vigilância Sanitária, um alimento funcional é aquele que os benefícios não ficam restritos a funções nutricionais básicas, mas que também tem o potencial de contribuir com efeitos benéficos para o bem-estar e a saúde e para a redução dos riscos de algumas enfermidades. Logo, é importante que a rotulagem desses alimentos contenha as características funcionais e nutricionais, concomitantemente (BRASIL, 1999; WICHIENCHOT *et al.*, 2010)

A compreensão do perfil químico dos alimentos tem sido uma temática de intensa busca, pois é um campo que envolve análises qualitativas e quantitativas abrangentes de todos os metabólitos presentes em determinado organismo. O metaboloma é, portanto, o conjunto de metabólitos de massa molecular até 1500 Da. A metabolômica é considerada uma das mais avançadas abordagens sobre o mapeamento químico de um organismo, pois, é relatado que nos estudos metabolômicos, é priorizado a identificação do subconjunto de metabólitos que caracterizam as diferenças entre amostras distintas. Desse modo, os perfis metabólicos são obtidos a partir de métodos analíticos que revelem todas as características químicas inerentes a determinados padrões funcionais e estruturais das classes de produtos naturais (CANUTO *et al.*, 2018; GUEDES, 2018; CHEN *et al.*, 2023).

Logo, vários autores relatam sobre métodos e técnicas analíticas de determinação de metabólitos, como, a cromatografia líquida ou gasosa acoplada a espectrometria de massas (*LC-MS* ou *GC- MS*) e a ressonância magnética nuclear (*NMR*), pois esses métodos têm apresentado uma grande gama de aplicações e podem detectar a maioria dos compostos. Esses vários componentes avaliados são produtos da expressão gênica e podem fornecer uma compreensão abrangente do estado bioquímico das matérias-primas ainda mais se alinhados com análises multivariadas, entre elas, a avaliação não supervisionada mais empregada nos estudos metabolômicos, análise de componentes principais (*PCA*). Outras análises quimiométricas utilizando métodos supervisionados são executados posteriormente aos métodos não supervisionados, a análise de discriminante de mínimos quadrados parciais ortogonais (*OPLS-DA*) e as variáveis de importância em projeção dos marcadores (*VIP-SCORES*). Esses métodos são concebidos com o objetivo de melhorar a separação entre os grupos após as análises exploratórias. (MARACHIN *et al*., 2017; SUSSULINI, 2017; CANUTO *et al*., 2018; CHEN *et al*., 2023)

A *Selenicereus monacanthus* (Lem.) D.R.Hunt (*Cactaceae*) (KOROTKOVA *et al*., 2017) é descrita como uma fonte rica em oligossacarídeos prebióticos para ser usado como um substrato para o desenvolvimento de alimentos funcionais. A pitaya vermelha é considerada uma fruta muito nutritiva, possuindo alto teor de minerais, açúcares, vitamina C e baixo valor calórico, além de ser rica em pigmentos antioxidantes, derivados das betalaínas, flavonoides e proteína solúvel. Além do mais, a pitaya vermelha oferece benefícios econômicos notáveis aos agricultores locais, sendo uma cultura inteligente para o clima devido à sua tolerância à seca, a eficiência no uso da água, capacidade de crescimento em solos pobres, a riqueza genética e ao alto valor agregado no mercado internacional. Os consumidores estão cada vez mais interessados em consumir a pitaya vermelha, pois além das características com potencial benéfico na saúde, relatadas acima, a fruta do dragão possui sabor único, coloração atraente e formato exótico (WYBRANIEC *et al*., 2001, 2002, 2007, 2009, 2010, 2011, 2013; KHALILI *et al.,* 2014; GANDIA-HERRERO, F. ESCRIBANO, J. GARCIA-CARMONA, F., 2016; DASAESAMOH *et al*., 2016; KHAN, 2016, POLTURAK; AHARONI, 2018; LIMA *et al*., 2020; NABI *et al*., 2023)

A introdução de compostos funcionais como prebióticos na dieta parece ser uma alternativa atrativa para melhorar a qualidade de vida em diversas condições como, obesidade, câncer, hipersensibilidade, doenças vasculares e doenças degenerativas. Em virtude dessas possibilidades a pesquisa com bioativos tem ganhado grande impulso na comunidade científica, pois é necessário analisar os efeitos benéficos desses produtos para a saúde humana, bem como, as doses terapêuticas certificadas para cada posologia (WICHIENCHOT *et al*., 2010; FILHO *et al*., 2018; BANWO *et al*., 2021)

É relevante ressaltar que diversas condições técnicas laboratoriais têm sido utilizadas para extração dos componentes com potencial bioativo. Pode-se citar os extratos hidroalcoólicos (pH 2, pH 5,5), a extração por fluido supercrítico e o ultrassom (SAENJUM *et al*., 2021; FATHORDOOBADY *et al.,* 2019; RAMLI *et al*., 2014). Nesse contexto, é sabido que existe uma forte correlação entre os efeitos benéficos da pitaya à saúde e a abundância de seu metabólito, especialmente betalaínas. (OMIDIZADEH *et al*., 2014; DASAESAMOH *et al.,* 2016). A literatura tem apresentado dados metabolômicos sobre relação entre as moléculas presentes na polpa, casca e semente da pitaya e a redução da atividade ansiolítica, tratamento de diabetes e controle do colesterol a partir de ensaios em camundongos e *zebrafish*, ver Figura 1. Lira *et al.* (2020) avaliaram o perfil metabolômico da polpa e da casca de pitaya vermelha e identificaram 16 e 15 compostos na polpa e na casca, respectivamente. A polpa e a casca da pitaya não apresentaram toxicidade e a atividade ansiolítica foi verificada em ambos os materiais testados.

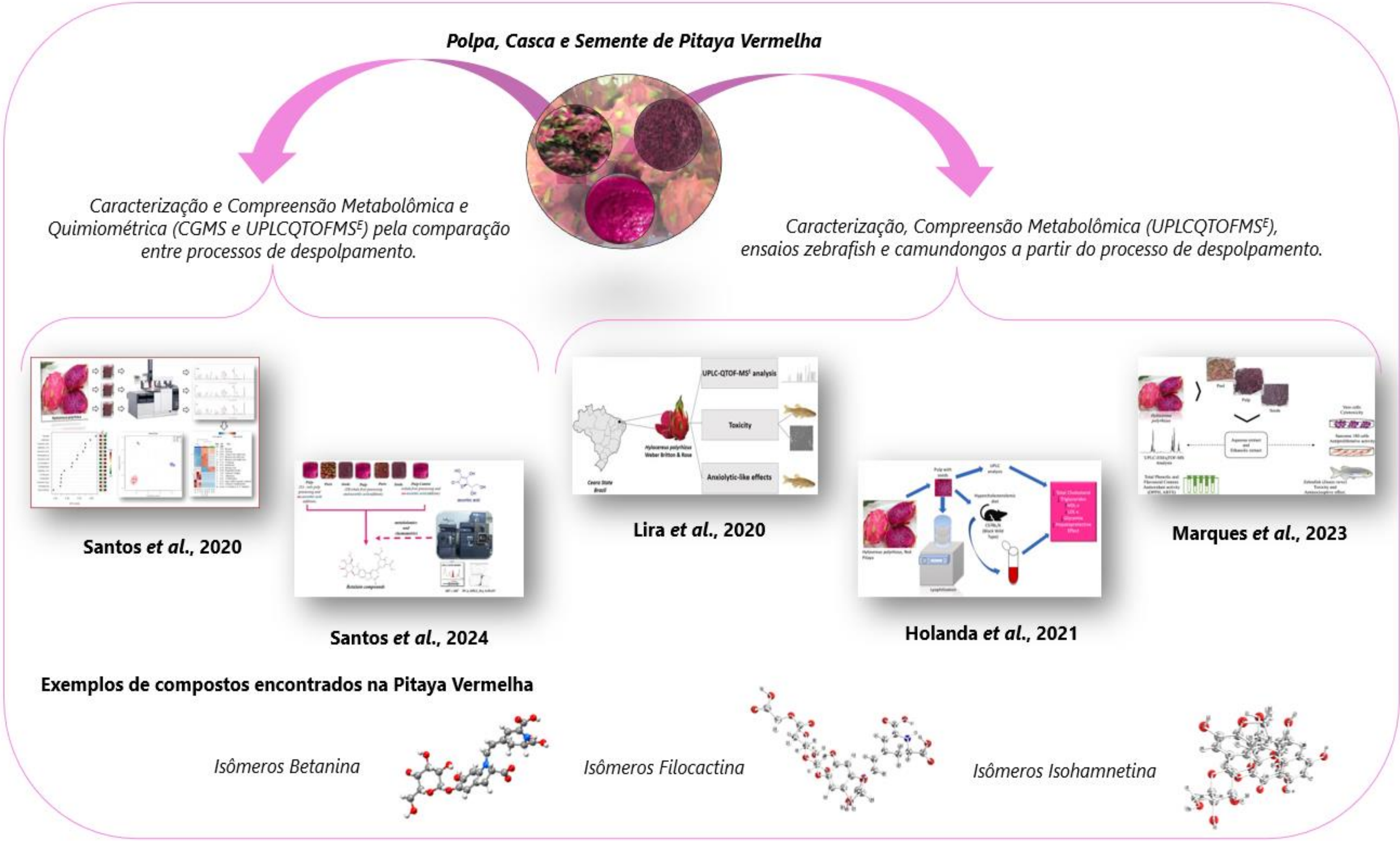
Holanda *et al.* (2021) avaliaram o perfil metabólico da pitaya e identificaram 22 compostos na polpa e na semente. O tratamento com pitaya vermelha foi eficaz na redução do colesterol, *LDL (Low-density lipoprotein)* e triacilgliceróis, elevando o *HDL* (*High-density lipoprotein)* demonstrando a atividade hipoglicemiante e hepatoprotetora e contribuindo para a redução do risco de doenças cardiovasculares. Assim, a pitaya vermelha se apresenta como uma alternativa alimentar promissora no auxílio do controle ou do tratamento da dislipidemia e da hiperglicemia. Além disso, Marques *et al.* (2023) identificaram vinte e cinco compostos nos extratos aquosos e etanólicos da pitaya vermelha ao avaliarem especificamente em modo de ionização negativo. O extrato etanólico da casca apresentou seletividade moderada à alta para células cancerígenas, enquanto os extratos etanólicos da polpa e das sementes possivelmente atuaram como citotóxicos ou citostáticos, ou mesmo ambos, pois apresentaram baixa seletividade sobre as células. O extrato etanólico das sementes atenuou a resposta inflamatória no peixe-zebra, que corresponde à segunda fase do comportamento nociceptivo induzido pela formalina. A polpa, a casca e a sementes da pitaya vermelha não apresentaram toxicidade contra o peixe-zebra.

Atualmente, os principais compostos não voláteis registrados na literatura oriundos do processo de degradação dos macronutrientes da pitaya são: betanidina 5-*O*-*β*-glicosídeo (betanina), isobetanidina 5-*O*-*β*-glicosídeo (isobetanina), betanidina 5-*O*-(6́-*O*-malonil)-*β*-glicosídeo (filocactina), isobetanidina 5-*O*-(6́-*O*-malonil)-*β*-glicosídeo (isofilocactina), betanidina 5-*O*-(6́-*O*-3-hidroxi-butiril)-*β*-glicosídeo (isobutirilbetanina), decarboxi betanina 5-*O*-(6́-*O*-malonil)-*β*-glicosídeo, hilocerenina, betanidina 5-*O*-*β*-soforosídeo, 2'-apiosil-betanina, 2'-apiosil-filocatina, indicaxantina isoindicaxantina, miraxantina *V*, portulacaxantina *II*, Isoleucina-isoBx, isovulgaxantina *IV*, isoleucina-Bx, fenilalanina-isso-Bx, vulgaxantina IV, fenilalanina-Bx isorhamnetina glucosil ramnosil, isômeros de filocactina, 2′-*O*-apiosilfilocactina e 4′-*O*-malonil-betanina (WYBRANIEC *et al*., 2001, 2002, 2007, 2009, 2010, 2011, 2013, STINTZING *et al*., 2002, THU *et al*., 2019; WU *et al*., 2019; LIRA *et al.,* 2019; QIN *et al*., 2020; UTPOTT *et al*., 2020; LIMA *et al*., 2020; ARIVALAGAN *et al*., 2021).

Grupos de pesquisas estudaram o metabolismo de espécies de pitaya alinhado à visão de análise multivariada de dados químicos, Pasko *et al*. (2021a) relataram sobre as diferenças de citotoxidade e antioxidante ao serem comparados os frutos de pitaya de diferentes espécies e variedades. Pasko *et al*. (2021b) relataram sobre a comparação de espécies de pitaya plantadas na Tailandia e em Israel e constaram que o local de colheita e a variedade dos frutos podem ser importantes para a sua atividade biológica. Em uma perspectiva complementar foi discutido que as diferentes operações unitárias aplicadas no despolpamento de pitaya vermelha podem modificar os marcadores químicos na avaliação quimiométrica em cada fração de polpa, de casca e de semente. Por exemplo, Santos *et al*. (2020) alinharam a compreensão metabólica a uma visão quimiométrica para uma melhor compreensão e relataram sobre a compreensão dos compostos voláteis da polpa de pitaya aplicada a dois diferentes tipos de despolpamento. Em uma visão complementar Santos *et al*. (2024) constataram pela perspectiva dos compostos não voláteis que os diferentes tipos de despolpamento apresentaram a diferenciação clara nos perfis de betalaína observados entre as frações da casca e da semente do fruto da pitaya. Acrescenta-se que, foi elucidado por avaliação quimiométrica dos metabolomas que a adição de ácido ascórbico alterou o perfil de degradação dos metabólitos especializados e a ação das enzimas responsáveis pela atividade de escurecimento enzimático.

Esses dados reiteraram a importância de alinhar a rota tecnológica à compreensão metabólica dos marcadores químicos em potencial. Apesar da literatura também descrever uma abordagem metabólica com identificação de metabólitos voláteis e não voláteis presentes no corante multifuncional de pitaya vermelha e apresentar uma percepção sensorial de consumidores não treinados (LIMA *et al*., 2020), de uma forma geral, os dados relacionados à aplicação das rotas tecnológicas em pitaya vermelha fundamentados por análises metabolômicas e quimiométricas são escassos na literatura científica, o que reafirma a importância desse trabalho.

Figura 1**.** Caracterização do perfil químico da pitaya vermelha utilizando as análises metabolômica das frações polpa, casca e semente e exemplos de compostos estudados nos trabalhos citados em 3D.



Fonte: Adaptado de Santos *et al.* (2020); Lira *et al.* (2020); Holanda *et al.* (2021); Marques *et al.* (2023) e Santos *et al.* (2024)

3.2 Aplicação de rotas tecnológicas para agregar valor à pitaya vermelha

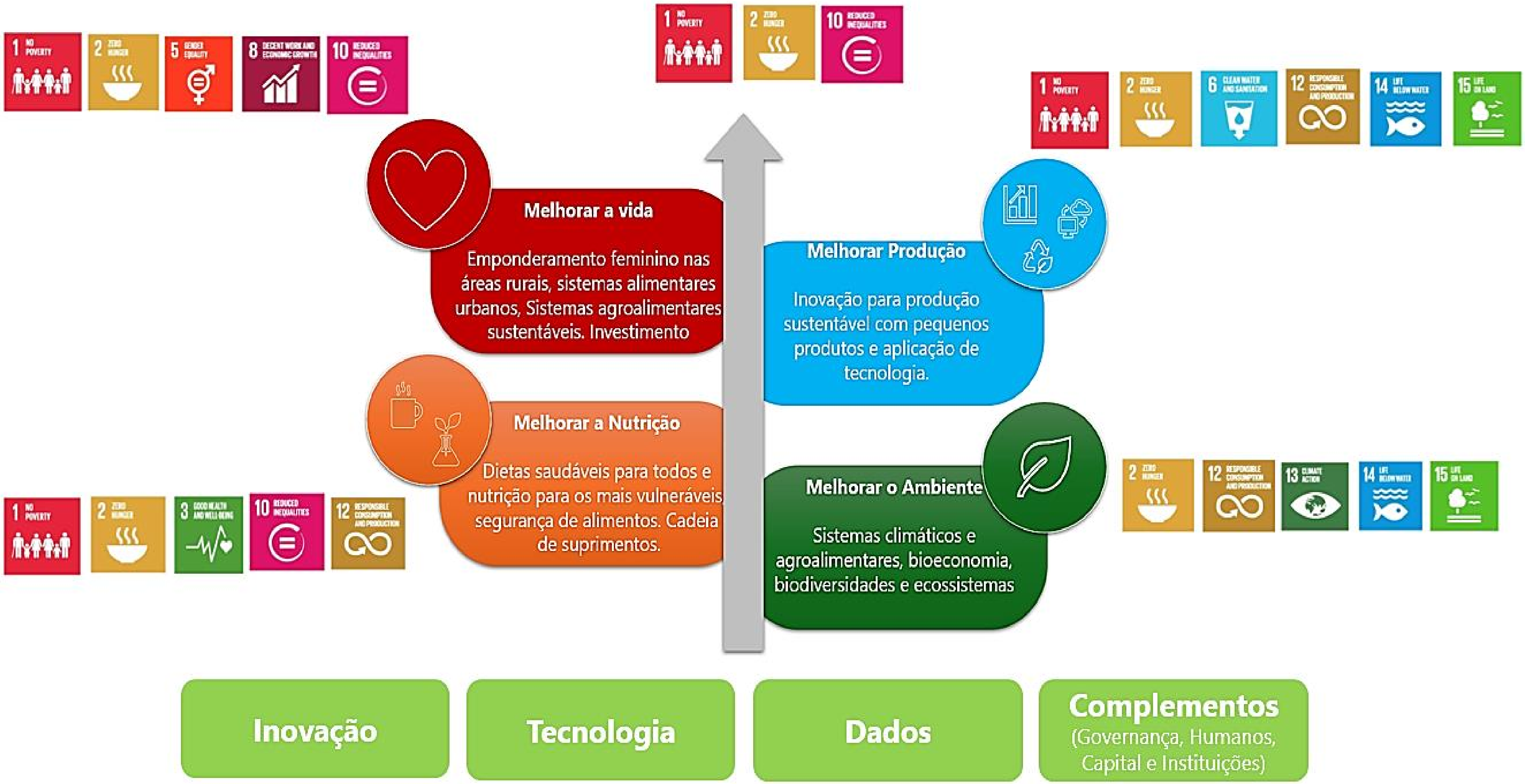
O contexto global da alimentação alinhado às crises na saúde, na sociedade e nas mudanças climáticas têm sofrido alterações intensas nos últimos anos. Os consumidores das diferentes gerações (x, y, z, α) têm procurado não somente alimentos seguros do ponto de vista microbiológico para a nutrição, mas que consigam também agregar potencial funcional em suas dietas, bem como a saúde planetária global, com a diminuição da utilização de defensivos agrícolas, melhoria das condições de trabalho no campo, diminuição da pegada de carbono, dentre outros. O consumo equilibrado dos alimentos é uma temática que as grandes indústrias de alimentos globais estão fomentando, visando à sustentabilidade de todo o processo de produção e consumo dos alimentos nesse contexto de aumento populacional (FAO, 2022).

A Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) relatou em 2022 uma grave dificuldade da cadeia de suprimentos, em que 13% dos alimentos globais são perdidos após a colheita e durante a logística de distribuição para os produtores. O equivalente a 931 milhões de toneladas ou 120 quilos (kg) per capita foram perdidos na cadeia de suprimentos após a colheita e antes de chegarem às prateleiras do varejo. Em 2022, estima-se que foram desperdiçadas 1,05 bilhão de toneladas de alimentos nos domicílios, nos serviços de alimentação e no varejo, equivalente a 132 kg per capita (UNEP, 2024).

Os desafios principais das metas globais da FAO incluem: Reduzir drasticamente as emissões de gases de efeito estufa dos sistemas agroalimentares e da economia, em parceria com outras agências; Tornar os sistemas agroalimentares mais resilientes a riscos climáticos; Usar e restaurar sustentavelmente os recursos naturais; Contribuir para erradicar a pobreza extrema; Assegurar estratégias que acabem com a fome e elimine a desnutrição a longo prazo; Adicionalmente, para uma melhor compreensão sobre o desperdício de alimentos é preciso relacionar dados sobre segurança alimentar, nutrição e sustentabilidade ambiental objetivos descritos pela organização das nações Unidas (ONU) de desenvolvimento sustentável, figura 2.

Desafios relativos aos gatilhos de mudança incluem: Fortalecer instituições, melhorar coordenação intersetorial, governança e estruturas legais. Aumentar a conscientização do consumidor sobre escolhas transformadoras. Abordar a distribuição de renda e riqueza dentro e entre países. Gerenciar tecnologias inovadoras e suas implicações para aumentar a produtividade alimentar e agrícola de forma sustentável (FAO, 2022).

Figura 2. A representação da estratégia da FAO sobre o sistema agroalimentar.



Fonte: Adaptado FAO, 2022.

De acordo como mesmo relatório da FAO (2022), as maiores porcentagens de perdas dos alimentos estão relacionadas às condições de armazenamento refrigerado ou processamentos inadequados, sendo as taxas mais elevadas quantidades relacionadas aos desperdícios de frutos e legumes. Dados do relatório anual da associação brasileira das indústrias de alimentos mostram que apenas 62% do que é produzido no agronegócio é processado pela indústria (ABIA, 2024). Nesse contexto e com um aumento populacional global, o custo de uma dieta equilibrada e saudável é elevado. Com isso, existe a visão do comercio global que os fornecedores de alimentos podem contribuir para a redução do desperdício de alimentos e consequentemente aumentar seus lucros.

No entanto, mesmo que esses fornecedores não consigam aumentar seus lucros inicialmente, os esforços de redução podem resultar em ganhos de produtividade que representam um benefício econômico para a sociedade em geral. Uma consequência do entendimento, durante o processo de inovação, com foco nas reais necessidades dos clientes em relação ao produto, é que uma empresa pode desenvolver produtos, serviços e marcas que os consumidores verdadeiramente valorizem (CHRISTENSEN, 2012; CHRISTENSEN; RAYNOR; MCDONALD, 2015; CHRISTENSEN *et al.*, 2020)

A agregação de valor aos produtos é uma estratégia crucial para acelerar o desenvolvimento global. No mercado de alimentos, países ao redor do mundo estão adotando modelos estratégicos que focam em alimentos industrializados de alto valor agregado. Esse sistema alimentar envolve recursos relacionados aos insumos, produção, transporte, indústrias de processamento e manufatura, varejo e consumo de alimentos, considerando ainda seus impactos ambientais, sanitários e sociais. Algumas macrotendências como "Sustentabilidade e Engajamento", e "Nutrição e Funcionalidade" têm descrito essa abordagem do mercado de alimentos a partir dos anseios dos consumidores com o olhar em novas e sustentáveis tecnologias (ITAL, 2023).

Seguindo essa perspectiva de alimentar de forma equilibrada o planeta a adição de rotas tecnológicas às matérias-primas são fundamentais, pois equilibram desde a agricultura ao consumo final. O desenvolvimento tecnológico sustentável promove uma maior produção de alimentos, com a otimização dos recursos naturais através do aumento de rendimento e como consequência a diminuição do custo operacional para o público em geral. Uma abordagem integrada na produção alimentar mundial, envolvendo diversos atores de forma coordenada e comprometida (GARCIA-GARCIA *et al*., 2019; CARMEN *et al*., 2023; KUWORNU *et al*., 2023).

Com isso, é necessário aumentar o nível de prontidão tecnológica das pesquisas científicas e a implementação no mercado de produtos derivados das matérias-primas, de forma sustentável, conforme ilustrado na figura 3, utilizando a escala de *TRL/MRL* (*Technology Readiness Levels/Manufacturing Readiness Levels*) para avaliar uma tecnologia de acordo com seu grau de desenvolvimento e seu enquadramento em níveis de maturidade tecnológica (EMBRAPA, 2018).

Figura 3. Escala de nível de maturidade tecnológica utilizada pela Embrapa.

Nível	Definição síntese	TRL - Tecnologia	MRL - Processo
1	Ideação	Princípios básicos observados e reportados	Princípios básicos observados e reportados
2	Concepção	Concepção tecnológica e/ou aplicação formulada	Conceito de manufatura definido
3	Prova de conceito	Prova de conceitos das funções críticas de forma analítica ou experimental	Processo de manufatura demonstrado (fazer funcionar)
4	Otimização	Validação em ambiente de laboratório de componentes ou arranjos experimentais básicos de laboratório	Capacidade de produzir a tecnologia em ambiente laboratorial (fazer funcionar apropriadamente)
5	Prototipagem	Validação em ambiente relevante de componentes ou arranjos experimentais com configurações físicas finais	Capacidade de produzir protótipo do componente do produto em ambiente relevante de produção
6	Escalonamento	Modelo do sistema/subsistema protótipo de demonstrador em ambiente relevante	Capacidade de produzir o produto ou seus subconjuntos em ambiente relevante de produção. A tecnologia está em fase de testes sem alcançar a escala final
7	Demonstração em ambiente operacional	Protótipo do demonstrador do sistema em ambiente operacional	Capacidade de produzir o produto ou seus subconjuntos em ambiente representativo de produção. Pode incluir testes operacionais e testes de fabricação, mas é testado usando modelos/simuladores inativos compatíveis com o produto final
8	Produção	Sistema completo, testado, qualificado e demonstrado	Implementação da produção e minimização dos custos
9	Produção continuada	Sistema já foi operado em todas as condições, extensão e alcance	Uso do produto em todo seu alcance e quantidade. Produção estabelecida

Fonte: Embrapa (2018)

Nesse contexto, as cactáceas têm chamado atenção do mercado e da ciência aplicada aos alimentos devidos às suas características agronômicas. Elas são de fácil plantio e adaptação em diversos climas e solos e enquadrando-se na bioeconomia. O mercado de diversos tipos de pitaya está em crescimento na Asia, Europa e América. Além de ser consumida *in natura*, o processamento de pitaya e utilização no desenvolvimento de produto representa um desafio devido ao seu alto teor de pectina, compostas principalmente de arabinose e galactose (JUNQUEIRA *et al*., 2010; LONE *et al*., 2020; JIANG *et al*., 2021; CARMEN *et al*., 2023).

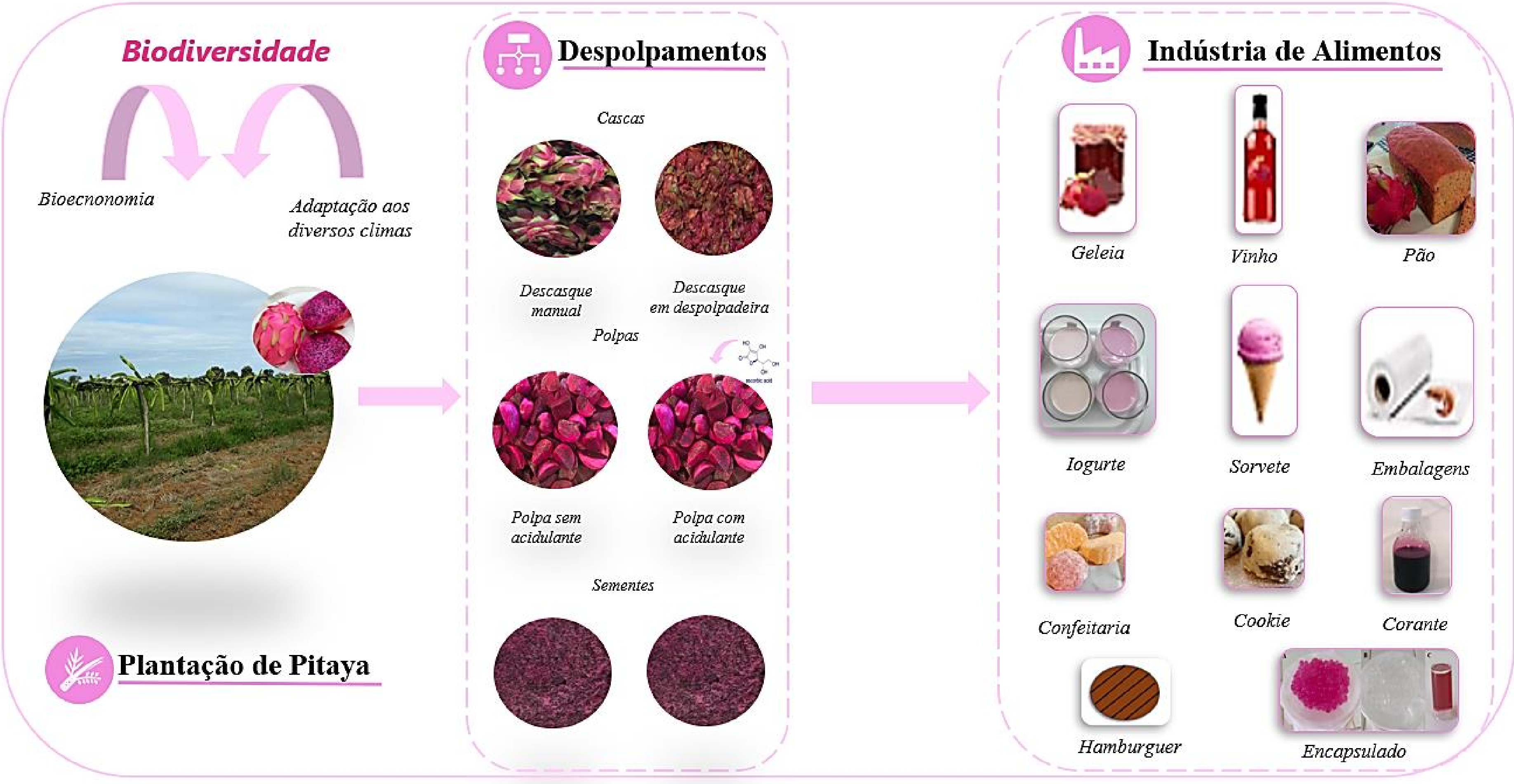
O elevado teor de umidade da pitaya vermelha é um dos responsáveis pela sua rápida deterioração pós-colheita (HUANG *et al*., 2021). Devido a isso, autores tem utilizado ácido ascórbico desde o processo de despolpamento na tentativa de obter maior aproveitamento e aumento da escala de produção de forma sustentável, mantendo ou gerando compostos químicos que agreguem valor para o consumidor (SANTOS *et al.,* 2020, 2024).

Essas informações são importantíssimas, pois é relatado que a utilização de maior quantidade do mesocarpo pode ser considerada como um mecanismo de maior aproveitamento, redução de custos e desenvolvimento de uma cultura industrial mais sustentável, pois cerca de 33% do peso de diversos tipos de pitaya seria subproduto que atualmente é destinado a ração animal ou descarte. Mas ainda há um longo caminho pela frente, pois diferentes rotas tecnológicas podem ser desenvolvidas e aplicadas na pitaya conforme também descrito por Magalhães et al. (2022); Ferreira et al. (2023), Carmen et al. (2023).

Alguns relatos de aplicações de rotas tecnológicas alimentícias para gerar produtos a partir da polpa, casca e semente da pitaya são citadas na literatura, entre eles, corante, confeitaria, cookies, hamburguer sorvete, vinho, iogurte, encapsulado para sucos, pão e novas embalagens (LIMA *et al.,* 2020*;* GENGATHARAN *et al., 2021;* THAIUDOM *et al*., 2021; HUANG *et al., 2021;* BEZUS *et al., 2022;* FERREIRA *et al., 2023).* Ver figura 4.

Todavia, uma comparação entre dois diferentes métodos de processamento da polpa de pitaya e a produção de sucos mistos tropicais pasteurizados e de sucos clarificados por membranas da pitaya e, consequentemente, a compreensão do ambiente químico sustentado por avaliação metabólica e quimiométrica são escassos na literatura, o que reitera a importância do presente estudo para uma melhor compreensão dessas e outras rotas tecnológicas aplicadas à pitaya vermelha.

Figura 4. Rotas tecnológicas aplicadas na Pitaya vermelha.



Fonte: Adaptado de Santos *et al.* (2020, 2024); Lima *et al.* (2020); Huang *et al.* (2021); Gengatharan *et al.* (2021); Bezus *et al.* (2022); Ferreira *et al.* (2023).

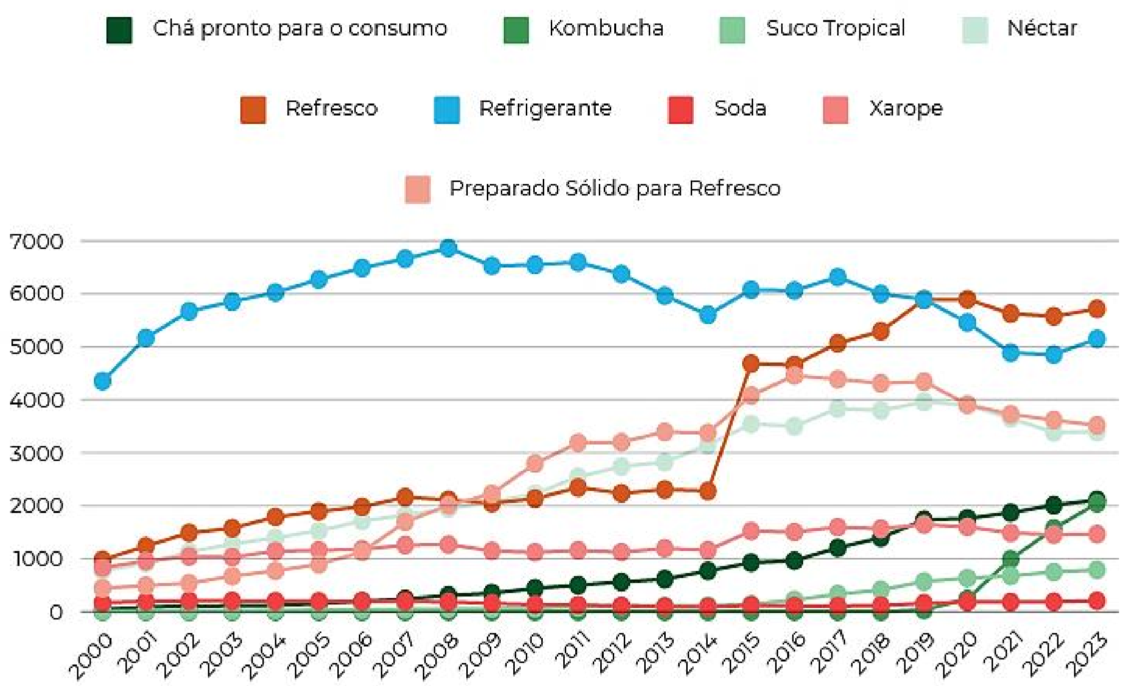
3.3 Suco tropical de pitaya vermelha e caju.

O Brasil ocupa a terceira posição no ranking mundial de produção de frutas, atrás somente da China e da Índia. O setor representa 16% da mão de obra do agronegócio nacional. Em 2023, as exportações de fruticultura alcançaram US$ 1,35 bilhão, sendo o maior valor registrado na série histórica. Desde 2019, as vendas externas brasileiras de frutas ultrapassam US$ 1 bilhão, com um crescimento de 24,5% em 2023. O principal destino das frutas brasileiras é a União Europeia, que recebe aproximadamente metade do valor total exportado (Brasil, 2024). Os plantios de frutos exóticos, entre eles a pitaya estão aumentando no Nordeste, mas a estabilidade do fruto *in natura* ainda seria uma barreira para expansão do mercado e o consumidor não está familiarizado com o sabor característico desses frutos (HUANG *et al*., 2021).

Esses frutos apresentam custo elevado no mercado nacional, sendo a rota tecnológica dos sucos tropicais mistos uma importante alternativa. O Suco Tropical Misto é definido como a bebida obtida pela dissolução, em água potável ou em suco clarificado de fruta tropical, da mistura de polpas de frutas polposas de origem tropical, por meio de processo tecnológico adequado, não fermentada, devendo ter cor, aroma e sabor característicos das frutas, submetido a tratamento que assegure a sua apresentação e conservação até o momento do consumo (BRASIL, 2009).

O mercado de sucos continua com ótimas perspectivas no cenário nacional. Dados publicados pelo anuário das bebidas não alcoólicas revelam que o suco tropical é a categoria com maior aumento no número de produtos registrados no Brasil, partindo de 9 produtos em 2000 para alcançar 789 produtos em 2023, representando um crescimento maior que 8.000%. Além disso, quando avaliado o volume de produção declarado de suco tropical, ele atinge nacionalmente o montante maior que 2.8 milhões de litros (BRASIL, 2024) conforme mostra a figura 5.

Figura 5. Apresentação do anuário das bebidas não alcoólicas 2024 sobre o número de produtos registrado no Brasil.



Fonte: Brasil (2024)

O setor comercial global de sucos tem se desenvolvido, procurando agradar a um público cada vez mais exigente. Devido à sua coloração e aos seus benefícios, a pitaya vermelha tem atraído o olhar do consumidor. Entretanto, o suco de pitaya vermelha não é um produto amplamente industrializado. É visto em alguns produtos a utilização de aromatizantes, conferindo “sabor pitaya” na denominação regulatória para a venda. A indústria alimentícia tem procurado inovações em relação a estabilidade dos compostos, sabores e aromas através de bebidas mistas (BEZUS *et al*., 2022).

Pesquisadores têm afirmado que o suco de pitaya vermelha é um produto saudável e com uma cor natural diferenciada. Porém, a estabilidade química das betalaínas no suco de pitaya é um ponto que merece atenção. No experimento relatado por Wong e Siow (2014), em que eles expuseram as betalaínas do suco de pitaya (*Brix* 60) vermelha à luz branca (5000k) por um período de cinco semanas e as compararam com amostras armazenadas no escuro, eles concluíram que a degradação da via metabólica das betalaínas apresentou maior mudança nas amostras submetidas à luz. As vantagens desses sucos estão relacionadas a alternativas para agregar valor através da união de características nutricionais, funcionais e sensoriais (BEZUS *et al*., 2022).

Uma possibilidade que poderia agregar valor à pitaya vermelha seria adicionar suco de caju (*Anacardium occidentale L.,*). O cajueiro é uma planta tropical natural da América do Sul, mais precisamente no Brasil, estando presente em quase todo o território nacional. A castanha, o fruto, é o principal produto de exportação e já há vários dados técnicos relacionados às suas aplicações, à sua estabilidade química e aos seus possíveis benefícios à saúde (ZANQUI *et al*., 2020, USLU, ÖZCAN, 2019; LEAL *et al*., 2023).

O pseudofruto, o pedúnculo, pode ser aplicado em sucos, em polpas, em doces, em compotas, em sorvetes e em refrigerantes, sendo uma matéria-prima promissora e renovável que pode ser valorizada para produzir uma ampla gama de produtos de valor agregado. Além das propriedades citadas, foi observado os benefícios das propriedades sensoriais conferidas aos alimentos onde o caju já foi agregado, devido ao *flavour* característico do produto (QUEIROZ *et al*., 2011; MENDES, 2017; REINA *et al*., 2022).

Vários estudos *in vitro* e *in vivo* já foram realizados em relação à presença de compostos químicos benéficos no caju, sendo detectada a presença de vários ácidos com atividade antioxidante como, ácido quínico, ácido cafeíco, ácido ferúlico, ácido *p*-cumárico e ácido salicílico. Além disso, o ácido anacárdico presente no caju tem chamado bastante atenção devido à sua associação com vários efeitos benéficos à saude entre eles, propriedades antioxidantes e anti-inflamatórias, ação antimicrobiana e antifúngica e propriedades antitumorais (QUEIROZ *et al*., 2011 SOUSA *et al*., 2020).

A aplicação de tratamento térmico é uma etapa fundamental para avaliação dos componentes químicos em sucos de frutas tropicais como, por exemplo, açaí e acerola. (FILHO *et al*., 2018; WURLITZER *et al.,* 2019; LINHARES *et al*., 2020; BEZUS *et al*., 2022), diminuindo a atividade dos microrganismos. Estudos mostram que a polpa de pitaya vermelha apresenta valores de pH e de acidez intermediários, enquanto a polpa de caju apresenta baixos valores de pH e altos valores de acidez, o que poderá contribuir para a conservação dos *blends*, utilizando a pasteurização branda e dispensando um tratamento térmico mais intenso como a esterilização (QUEIROZ *et al*., 2011; MENDES, 2017; REINA *et al*., 2022).

Entender a percepção e as expectativas dos consumidores sobre o produto é essencial para evitar fracassos na indústria de alimentos. Porém, é fundamental destacar que os dados relacionados ao processamento térmico dos *blends* de pitaya com caju alinhados à avaliação de componentes metabólicos, análise multivariadas e dados sensoriais correlacionados são praticamente inexistentes na literatura.

3.4 Suco clarificado de pitaya vermelha

A filtração é uma operação unitária muito utilizada na indústria de alimentos com o objetivo de separar componentes. A separação através de membranas pode ocorrer através de alguns métodos como, microfiltração, ultrafiltração, nanofiltração, osmose reversa e diálise. A membrana é uma barreira seletivamente permeável feita de materiais naturais ou sintéticos que separa a alimentação em duas frações impulsionadas pela pressão. (MULDER, 1996; MCCABE, SMITH, HARRIOTT, 2005; MOHAMMAD *et al.*, 2015)

O permeado ou clarificado atravessa a membrana e o retido ou retentado é rejeitado pela membrana. O sistema de fluxo tangencial funciona por diferença de pressão, onde o fluido é alimentado e passará através do meio filtrante. As principais vantagens da utilização dos processos de clarificação por membranas estão relacionadas à alta seletividade, à ausência de perda de substâncias aromáticas voláteis, à ausência de adição química exclusivamente na etapa de clarificação e ao baixo consumo de energia (BEVILACQUA *et al.*, 2018; BHATTACHARJEE *et al.*, 2018; LU *et al.*, 2021).

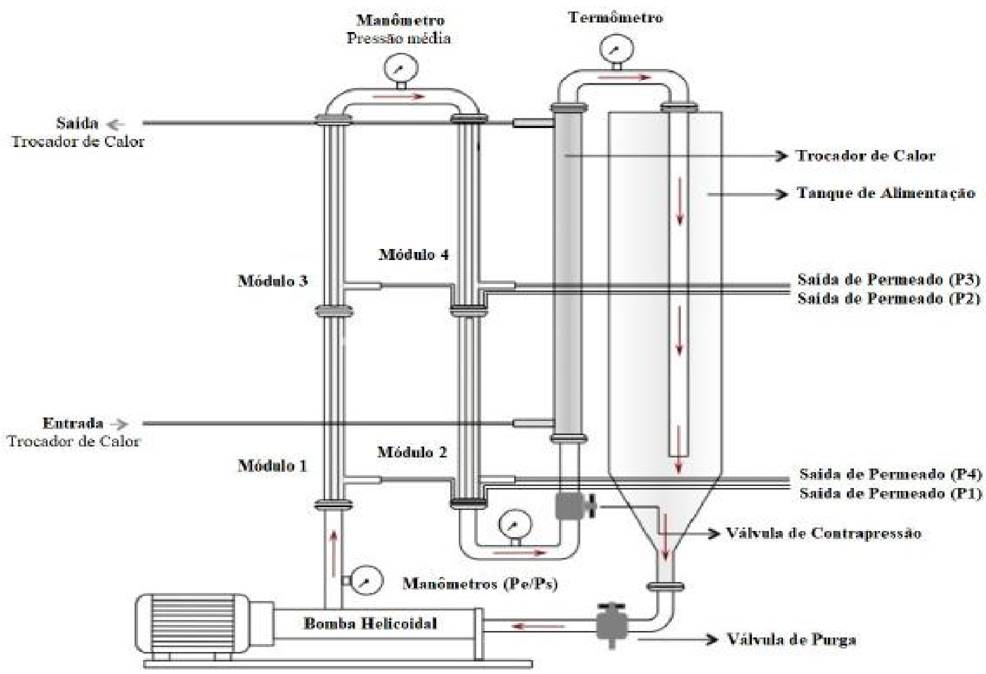
No Processo de clarificação através de uma barreira seletivamente permeável feita de materiais naturais ou sintéticos, a membrana, pode ser avaliado a sua seletividade, utilizando basicamente dois parâmetros: o fator de separação e a retenção. Enquanto isso, a avalição do fluxo de permeado da membrana é feita, utilizando a massa de amostra permeada dividida pela massa específica da água. Também é realizada a multiplicação pelo tempo e a área específica de filtração da membrana (VAILLANT *et al.*, 2001; VERGARA *et al.*, 2015; GHOSH *et al.*, 2017; LU *et al.*, 2021).

Os sistemas de clarificação por membranas, especialmente aqueles que utilizam microfiltração, geralmente possuem poros com tamanhos variando entre 100 e 10.000 nanômetros (0,1 a 10 µm). Esses poros permitem a passagem de água e outros componentes menores, enquanto retêm sólidos, bactérias e partículas em suspensão, promovendo a clarificação. Quando se utiliza membranas com poros inferiores a 200 nanômetros, a microfiltração pode ser comparada a um processo de conservação não térmico, denominado pasteurização a frio, favorecendo à estabilidade de características importantes do ambiente químico. Em relação aos materiais da membrana, a literatura aponta uma grande variedade de materiais, entre eles, cerâmicas e polímeros são os mais utilizados (VERGARA *et al*., 2015; UROŠEVIĆ *et al*., 2017; SATYANNARAYANA, KUMAR, 2023).

O processo de filtração pode ser usado para obtenção de vários produtos alimentícios, como, por exemplo, sucos clarificados, sucos mistos, bebidas gaseificadas e licores. Vários autores relataram a clarificação de vários sucos de frutas, usando a tecnologia de membrana. Comparada aos tratamentos térmicos, a filtração por membrana geralmente requer menos energia, menos mão de obra, menos tempo e custo, ao mesmo tempo que fornece suco de qualidade superior (LU *et al*., 2021; SATYANNARAYANA, KUMAR, 2023).

Abreu *et al*., (2013) adaptou o método de microfiltração para ser realizado em um sistema em planta piloto com quatro membranas de óxido de alumínio, onde o fluído é submetido a uma bomba de deslocamento positivo, diâmetro médio dos poros de 200 nanômetros e com área de contato de 0,0055 m^2, ver figura 6. Esse modelo foi utilizado para clarificação de beterraba, de caju, e usado como parte do processo de obtenção do xarope de yacon e do corante multifuncional de pitaya vermelha. Com isso, foi observado uma diminuição da turbidez dos materias, utilizando uma baixa fonte energética.

Figura 6. Sistema de Clarificação por membranas em planta piloto.



Fonte: Abreu *et al.* (2013)

A literatura relata que a polpa da pitaya possui alta turbidez e viscosidade (NUR'ALIAA *et al.*, 2008). O equipamento foi de fácil utilização e não afetou negativamente os componentes termossensíveis presentes nos materiais clarificados (SILVA *et al.*, 2018; SOUSA *et al.*, 2020; LIMA *et al.*, 2020).

A aplicação do processo de microfiltração da pitaya foi estudada por Viana et al. (2022) e por Silva et al. (2024) em que dois processos de despolpamento, sendo submetidos ao tratamento enzimático por 60 minutos e obtendo melhor elucidação da vazão de permeado e *fouling* em membrana de óxido de alumínio. Foi observado que o processamento tradicional (separação manual da casca) com maceração enzimática obteve a melhor performance dos fluxos de permeado (±25% maiores que o processo com casca) e menor gasto energético. Os autores concluíram que a partir desses dados existe a possibilidade de aplicação em maior escala industrial.

Lima et al. (2020) também utilizou de forma combinada maceração enzimática e sistema de clarificação por membranas para obter o corante de pitaya com a perspectiva de substituição futura dos corantes comerciais usados atualmente. Além disso, obteve um interessante perfil metabólico de componentes não voláteis na amostra concentrada e aplicada em iogurte. Porém, tem sido relatado sobre possíveis influências nas características da pitaya relacionada à aplicação de métodos de produção combinados com tratamento enzimático conforme descrito por Nur'aliaa *et al.* (2008), Schweiggert *et al.* (2009), Nur'aliaa *et al.* (2011), Pui & Abdul Kalam Saleena, (2023) e Kallel *et al.* (2023). Entretanto, a literatura apresenta uma escassez de estudos aprofundados sobre o perfil químico dos sucos de frutas clarificados.

A aplicação direta da microfiltração de pitaya vermelha sem a etapa da maceração enzimática por dois diferentes processos de obtenção e a elucidação por potenciais marcadores em projeção dos componentes não voláteis e voláteis por dados metabolômicos e quimiométricos são relativamente ausentes de dados substanciais na literatura, sendo uma importante lacuna para o prosseguimento das pesquisas, alinhando dados de processo e do ambiente químico para aumento sustentável de escala da rota tecnológica.

4 CONCLUSÕES

A compreensão do estudo realizado foi fundamental para concluir sobre a importância da pitaya vermelha como uma cultura de alto valor agregado para o mercado de alimentos, destacando seu potencial de desenvolvimento sustentável. A revisão de literatura enfatiza sobre os vários desafios de explorar novas rotas tecnologias de processamento com o objetivo de potencializar o perfil funcional da pitaya vermelha, como a formulação de sucos tropicais em *blends* com caju com foco na percepção dos consumidores e a aplicação da clarificação por membranas.

Essas rotas tecnológicas podem representar um significativo aumento de escala para futura produção na indústria de alimentos, pois contribuem diretamente para a sustentabilidade e a inovação na produção de pitaya vermelha. As análises metabolômicas e quimiométricas fornecem uma compreensão detalhada dos compostos não voláteis presentes na pitaya, abrindo caminho para futuras otimizações e aplicações industriais que agreguem valor através da potencialização das suas propriedades nutricionais e funcionais.

Com isso, essa pesquisa constitui uma importante alternativa com a perspectiva de aumentar o nível competitividade de novos produtos inovadores derivados da pitaya vermelha, de forma sustentável, a partir da aplicação de rotas tecnológicas e avaliação do perfil metabólico das amostras que favoreçam a estabilidade durante toda a cadeia de suprimentos, garantindo aos consumidores produtos com qualidade assegurada, contribuindo para a segurança alimentar.

5 PERSPECTIVAS FUTURAS

- Otimização das formulações dos blends de sucos de pitaya vermelha e caju com a descrição das análises de monitoramento da estabilidade do perfil metabólico, físico-químico, microbiológico, sensorial e de compostos voláteis e não voláteis dos sucos durante o período de armazenamento;

- Estabelecimento do perfil metabólico, físico-químico, microbiológico, sensorial, de compostos voláteis e não voláteis dos blends clarificados de pitaya com caju;

- Isolamento e purificação de oligossacarídeos;

- Prototipagem e ampliação de escala de produção do clarificado concentrado de pitaya vermelha para ambiente relevante (*TRL* 6 – 9);

- Atividade biológica dos *blends* de pitaya com caju;

- Atividade biológica do clarificado concentrado de pitaya vermelha;

- Avaliação da viabilidade econômica e regulatória dos produtos gerados.

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APÊNDICE A - METABOLOMIC, CHEMOMETRIC AND SENSORY CHARACTERIZATION OF TROPICAL RED PITAYA AND CASHEW APPLE JUICE BLENDS: INSIGHTS INTO PROCESSING AND FUNCTIONAL ATTRIBUTES

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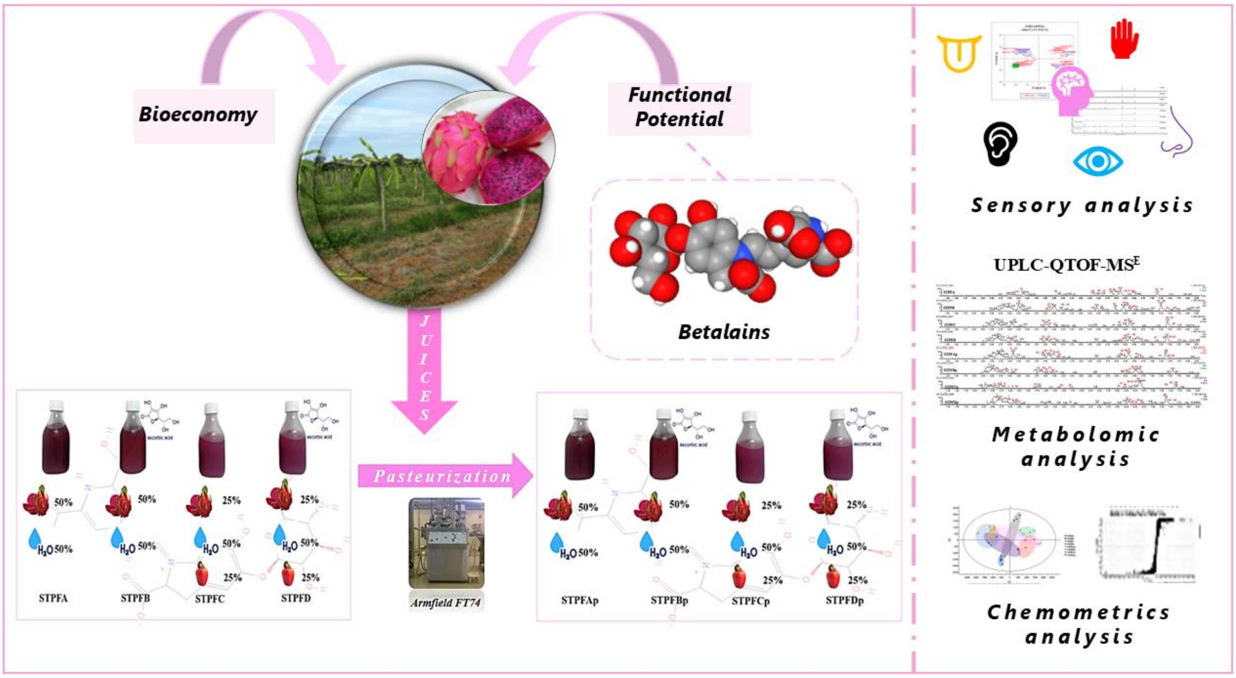
Graphical abstract



Fonte: Author (2025)

Abstract

This is the first study evaluates the effects of pasteurization, integrative analysis combining multivariate, sensory, and metabolomics data on red pitaya and cashew juice blends. Red pitaya has attracted the attention of the food market due to its adaptation to various climates and soils, fitting into the bioeconomy. The application of red pitaya in blends with cashew apple is presented as a technological alternative to enhance functionality, add sensory value, and reduce costs related to sustainable production in the supply chain. A total of 64 compounds were identified in blends using ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry using elevated energy acquisition mode. Multivariate statistical analyses identified key chemical markers, including betalains and flavonoids. Sensory evaluation Check-All-That-Apply, Rate-All-That-Apply and and affective tests revealed that blends with cashew apple juice achieved acceptance scores within the favorable range, particularly for attributes such as cashew apple flavor, sweet taste and fruit flavor. In addition, more than 70% of consumers expressed a positive response to red pitaya and cashew apple blends, reflecting an interesting purchase intent. These findings offer valuable insights for optimizing tropical juice formulations, contributing to sustainable food industry practices.

Keywords: *tropical juices, red pitaya, pasteurization, multivariate analysis, cashew apple.*

1 Introduction

Sustainable food consumption is an important issue that focuses on optimizing raw materials from planting, during consumption, and in the disposal and reuse of waste. Therefore, there is a strong appreciation for applying technological processes aligned with data science that can add value to natural products. Fruit juices processing is an example of practical and simple technological routes for maintaining nutritional and functional characteristics and are gaining prominence in the daily diet due to changes in consumption patterns (FAO, 2022, Ital, 2023, Ferreira *et al*., 2023)

In this context, red pitaya (*Selenicereus monacanthus* (Lem.) D.R.Hunt, *Cactaceae* family) has attracted attention due to its sensory characteristics and its potential beneficial health effects listed in the literature by Holanda *et al*. (2021), Bezus *et al*. (2022), Marques *et al*. (2023). Also, the sustainable development of new products based on the maximum use of pulp, peel and seeds has been sought. However, although the pulp has a high market value, high humidity and instability of the chemical profile due to biochemical and enzymatic reactions have also been reported (Wybraniec *et al*., 2013, Khan, 2016, Santos *et al*., 2024). Furthermore, it was previously reported by Santos *et al*. (2024) that evaluation of the chemical profile of pitaya red fruit pulp based on robust multivariate analyses confirmed the presence of betalain and flavonoid isomers and the influence of processing on molecular markers.

Therefore, it is important to establish technological routes to guarantee quality and maximize nutrition and bioactivity to help reduce food production's negative impact on the environment and promote social well-being (Huang *et al*., 2021, Ferreira *et al*., 2023). The application of red pitaya in juices combined with cashew apple (*Anacardium occidentale* L.) presents itself as a technological alternative to enhance functionality, add sensory value, and reduce costs related to sustainable production in the supply chain.

Cashew apple juice is obtained from used the pseudofruit of the cashew apple tree, a native South American plant of economic importance. This pseudofruit is rich in bioactive compounds, mainly phenolics, such as flavonoids, and has several applications already consistently reported in the literature by Abreu *et al*. (2013), Sousa *et al*. (2020), Alves *et al*. (2023). However, these processes can modify the sensory properties of juice blends, which play an important role in consumer acceptance. Therefore, the literature has highlighted the importance of consumer sensory perception in blends juices with cashew apple, and used robust sensory techniques such as Check-All-That-Apply (CATA) and Rate-All-That-Apply (RATA) to establish the sensory profile, optimizing the development of products for the market (Martins *et al*., 2022, Prado *et al*., 2022).

However, the literature lacks comparative assessments between the metabolic profile and the consumer's perspective on red pitaya and cashew apple juices from two pulping processes and subjected to pasteurization. Therefore, this study aimed to evaluate the metabolic and sensory impacts of different pulping and pasteurization conditions on tropical juice blends formulated with red pitaya and cashew apple through using ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry using elevated energy acquisition mode (UPLC-QTOF-MS^E), multivariate PCA analysis (principal component analysis) and OPLS-DA (orthogonal partial least squares-discriminant analysis), along with sensory and physicochemical evaluation.

2 Materials and methods

2.1 Raw material

Fully ripe red pitaya fruits, weighing 300-400 g and with intact peel, were harvested from the Jaguaribe Valley in Ceará, Brazil (5° 53′ 27″ S, 38° 37′ 19″ W). After collecting the pitaya, the fruits were processed at the Agroindustrial Processes Laboratory of Embrapa Agroindustry Tropical (Fortaleza-CE) to obtain the pitaya pulp. The cashew apple juice was supplied by the company Natvita from the Eusebio, in Ceará, Brazil. Both materials were stored at -18 °C, then thawed at 5 °C for 24 hours before use.

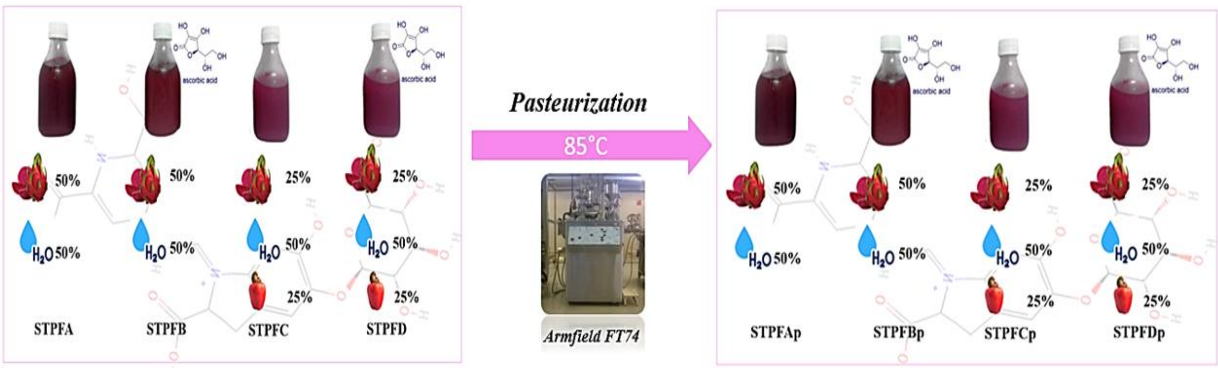
2.2 Preparation of formulations and pasteurization processing tropical juice

The pitaya was subjected to two types of pulping treatments, following a methodology from Santos *et al*. (2024), using a pulper (ITAMETAL/BONINA 0.25 DF). Two pulp samples were generated: Treatment A, in which the pulp was manually separated from the peel and subjected to pulping on a 0.8 mm sieve; Treatment B, in which 500 mg kg^{-1} of ascorbic acid were added to minimize oxidative reactions during processing and the pitaya red was fractionated into four parts and subjected to pulping with 2.5 mm and 0.8 mm sieves.

The fractions of treatments A and B produced tropical blended juices. The juices were prepared with different concentrations of pitaya pulp, with the addition of cashew apple juice. The formulations before pasteurization were: **STPFA** - 50% pitaya pulp A treatment/ 50% water; **STPFB** - 50% pitaya pulp B treatment/ 50% water; **STPFC** - 25% pitaya pulp A treatment/ 50% water/ 25% cashew apple; **STPFD** - 25% pitaya pulp B treatment/ 50% water/ 25% cashew apple, see Figure 1.

After the formulations, the juices were homogenized in a colloidal mill and taken to a thermal treatment (UHT/HTST system) using an ARMFIELD FT74 (Ringwood, England). The pasteurization temperature was 85 °C, with controlled flow to obtain a retention time of 30 seconds. Hot filling followed in 200 mL glass bottles, previously sanitized and closed with a screw-on plastic cap. The bottles were laid down to pasteurize the cap for 3 min, cooled in a water bath, and stored under refrigeration (5 ± 2 °C) until the analysis, following a methodology from Wurlitzer *et al*. (2019). The pasteurized formulations were: **STPFAp** - 50% pitaya pulp A treatment/ 50% water; **STPFBp** - 50% pitaya pulp B treatment/ 50% water; **STPFCp** - 25% pitaya pulp A treatment/ 50% water/ 25% cashew apple; **STPFDp**- 25% pitaya pulp B treatment/ 50% water/ 25% cashew apple, see Figure 1.

Figure 1. Tropical juices before and after thermal processing*.



*Formulations before pasteurization: STPFA - 50% pitaya pulp A treatment/ 50% water; STPFB - 50% pitaya pulp treatment B/ 50% water; STPFC - 25% pitaya pulp A treatment/ 50% water/ 25% cashew apple; STPFD - 25% pitaya pulp treatment B/ 50% water/ 25% cashew apple. Pasteurized formulations: STPFAp - 50% pitaya pulp W treatment/ 50% water; STPFBp - 50% pitaya pulp treatment B/ 50% water; STPFCp - 25% pitaya pulp A treatment/ 50% water/ 25% cashew apple; STPFDp - 25% pitaya pulp treatment B/ 50% water/ 25% cashew apple.

Fonte: Author (2025)

2.3 Microbiological Analysis

The microbiological quality of the juices were assessed by counting filamentous fungi and yeasts (Tournas *et al*., 2001), total coliforms and *E. coli* (Method 991.14) and *Salmonella spp.* (Method 989.13) according to the methodology described in the FDA's Bacteriological Analytical Manual (Bam) and the Official Method, Association of Official Analytical Chemists International (AOAC, 2005). To check whether the juices were suitable for human consumption, microbiological analysis was performed before sensory analyses.

2.4 Physical-chemical evaluation

The pH was determined through direct reading of the samples in a potentiometer (AOAC 981.12, METTLER TOLEDO FE28, Switzerland). The titratable acidity was carried out with the aid of the automatic potentiometer (HI 901/HI 902 – Hanna instruments) on a sample with NaOH 0.1 mol L^{-1} solution up to pH 8.1 (AOAC 942.15), expressed as % malic acid. The soluble solids were determined using a digital refractometer (ATAGO pocket palm-3, Japan). Color determination was carried out (L*, a* and b* values), using colorimeter (KONIKA MINOLTA CR-400, Japan) with D65 illuminant and 10° observer (AOAC, 2005).

2.5 Analysis of the chemical profiles of the extracts by UPLC-QTOF-MS^E

2.5.1 Extraction procedure for analysis by UPLC-QTOF-MS^E and chromatographic conditions

The eight samples were tested to establish the chemical profile of the bioactive compounds in each material. The juices obtained were freeze-dried (LIOTOP LP 510), stored in a freezer at -18°C, and extracted in biological quintuplets. The extractions were performed using the liquid-liquid extraction method, previously described by Chagas-Paula *et al*. (2015) and Guedes *et al*. (2018). Initially, 50 mg of the sample were weighed separately and then transferred to a test tube. Then, 4 mL of hexane (95% purity) were added in a vortex system (1 min). The nonpolar protection was performed in an ultrasonic bath with a fixed power of 135 W for 20 min. The polar compounds were then extracted in an ethanol/water solution (70:30, v/v) under the same conditions as in the previous procedure.

The mixture obtained in the test tube was then homogenized again in a vortex system and placed in an ultrasonic bath for 20 min. The test tubes were then centrifuged for 10 min (3000 rpm) to remove the insoluble part of the material. At the end of the procedure, the 1 mL aliquot of the lower polar phase (ethanol/water) was removed with a Pasteur pipette and supplied to a syringe coupled to a 0.22 µm PTFE filter. The filtered aliquot was collected in vials and stored at -80°C until UPLC-QTOF-MS^E analysis. The extracts were analyzed using the Acquity UPLC system (Waters, Milford, MA, USA) coupled with the Quadrupole/TOF system (QTOF, Waters, Milford, MA, USA). A Waters Acquity UPLC BEH column (150 × 2.1 mm, 1.7 µm) was used, with the column temperature set at 40 °C. The binary gradient elution system consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The UPLC elution conditions were optimized as follows: linear gradient from 2% to 95% B (0–15 min), 100% B (15–17 min), 2% B (17.01), 2% (17.02–19.01 min), flow rate of 0.4 mL min-1, and sample injection volume of 5 µL.

The chemical profiles of the juice samples were determined by coupling a Waters ACQUITY UPLC system to a QTOF mass spectrometer (Waters, Milford, MA, USA) in positive ionization mode with an electrospray ionization (ESI) interface. ESI^+ modes were acquired in the range of 110–1180 Da in MS and 50–1180 in MS/MS, with a fixed source temperature of 120 °C and desolvation temperature of 350 °C. A desolvation gas flow of 350 L h^{-1} was used for the ESI^+ mode. The capillary voltage was 3 kV. Leucine enkephalin was used as the lock mass. The spectrometer operated with MS^E centroid programming using a voltage ramp from 20 V to 40 V. The instrument was controlled by Mass Lynx 4.1 software (Waters Corporation, USA). The solvents used were purchased from LiChrosolv® of the Sigma-Aldrich Chemical Company (Oakville, ON, Canada). High-purity Milli-Q water (Billerica, MA, USA) was used in all analyses. The genistein acquired from Sigma Aldrich was used as an internal standard. The other reagents used (hexane, ethanol and acetonitrile) were from analytical grade.

2.5.2 Metabolites annotation and chemometric data analysis

To annotate the metabolites, the dataset obtained from UPLC-QTOF-MS^E was imported into the Mass Spectrometry - Data Independent Analysis (MS-DIAL 3.82) software. Therefore, spectral deconvolution, alignment, and peak filtering were performed (Lai *et al*., 2018; Tsugawa *et al*., 2015, 2019). Subsequently, the unknown metabolites could be identified by their elemental formulas and mass spectral fragmentation on silica with MS-FINDER 4.24 (Lai *et al*., 2018; Tsugawa *et al*., 2015). Structural elucidation and metabolite identification were based on molecular formula (MS) and fragmentation (MS/MS) with heuristic rules activated (Kind, Fieh, 2007). Metabolite annotation was performed by comparing the data with database information such as KNApSAcK Core System, PubChem and ChemSpider. Following the parameters of the metabolic standards initiative (MS^1) level 2.1, presenting a putative identification, including molecular formula and fragment ions. Also, it is important to mention that metabolite annotation was based on family, genus and species.

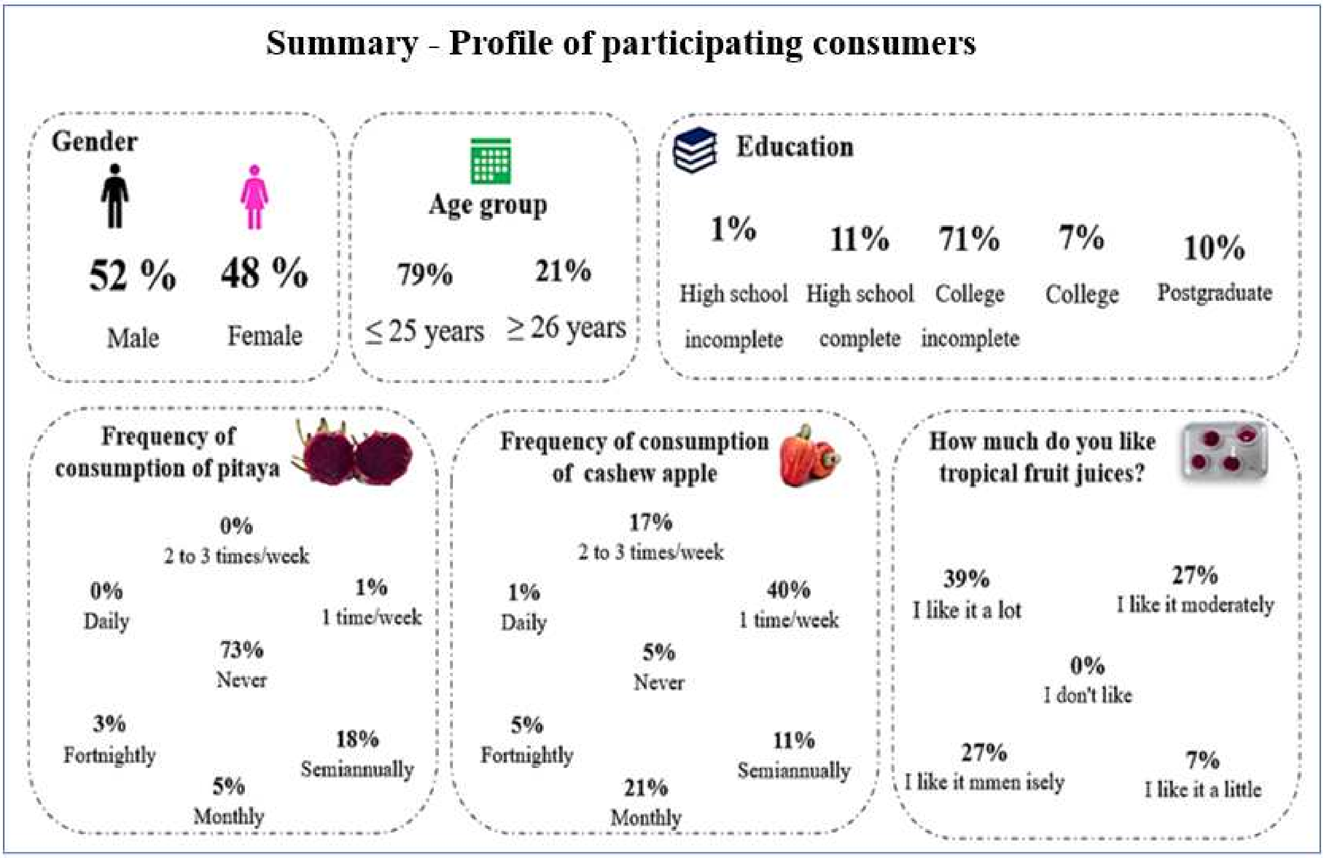
UPLC-QTOF-MS^E data of all samples were analyzed using MarkerLynx XS (Waters, v4.1) to identify potential discriminatory chemical markers in different extracts. For data collection, the method parameters were defined as retention time range from 0.80 to 17.0 min and mass range from 110 to 1180 Da. For data analysis, a list composed of the identities of the detected peaks was generated using retention time (t_R) - mass data (*m/z*) pairs as the identifier for each peak. An arbitrary ID was assigned to each of these t_R - *m/z* pairs based on their elution order from the UPLC system. Ion identification was based on the t_R and *m/z* values. Peaks were aligned, normalized to total intensity, and Pareto-scaled. PCA and OPLS-DA models were built in SIMCA-P (v16.0, Umetrics) with 7-fold cross-validation. Model validity was assessed via permutation testing (n = 100). VIP scores >1.0 and $p < 0.05$ (ANOVA) defined significant markers.

2.6 Sensory evaluation

The focus group, containing 15 participants, was selected from among the postgraduate students in Food Science and Technology of the Federal University of Ceará, where four different formulations of pitaya juice were presented – STPFAp, STPFBp, STPFCp and STPFDp. The microbiological analysis confirmed that the samples were suitable for consumption. The session lasted approximately one hour, during which the moderator led the session in which sensory descriptors were generated from the focus group.

The formulations validated by the focus group were prepared in a pilot plant and subjected to sensory tasting. This sensory evaluation was approved by the Research Ethics Committee the Federal University of Ceará (Institute of Culture and Art - 56393715.5.0000.5054 - Ethics presentation certificate number). The research was conducted with 100 volunteer and untrained tasters in controlled lighting, noise, and temperature environments. The individuals evaluated were divided between 52% male and 48% female. Most participants were aged ≤ 25 years (79%) and had incomplete higher education (71%), recruited according to their interest in participating and their consumption habits around red pitayas and cashews, see Figure 2.

Figure 2. Profile of the 100 untrained sensory evaluators.



Fonte: Author (2025)

The sensory evaluation was carried out with the participation of untrained consumers. The 100 untrained sensory evaluators were asked about their gender, age group and education. The samples were served to the tasters in 50 mL plastic cups coded with three-digit random numbers. A balanced complete block design was used. At the end of each sample evaluation, the amount of mineral water available for consumption was indicated. At the end of the initial tests, the evaluators were invited to complete the CATA questionnaire, which presented descriptive terms related to the sensory characteristics of the samples of tropical pitaya juice and tropical pitaya and cashew apple juice ("red color", "pink color", "purple color", "homogeneous", "heterogeneous", "shiny", "particles", "clear", "sedimented", "viscous", "sweet aroma", "acidic aroma", "astringent aroma", "fruit aroma", "pitaya aroma", "cashew apple aroma", "green leaf aroma", "no aroma", "sweet taste", "acidic taste", "astringent taste", "fruit flavor", "pitaya fruit flavor", "cashew apple flavor", "beetroot flavor", "fermented flavor", "root flavor", "cashew apple aftertaste" and "beetroot flavor"). Sensory panelists were instructed to mark on the form with the letter "X" the terms describing the samples' sensory characteristics. In addition, descriptive terms were evaluated about the ideal scale (Meyners & Castura, 2014; Vidal *et al*., 2015). The RATA, in turn, was used to quantify these descriptors using a numerical intensity scale (1 - little/weak to 5 - very/strong) (Ares, 2014; Giacalone & Hedelund, 2016).

In addition, the samples were evaluated according to overall evaluation, appearance and flavor. A hedonic scale was used, structured with 9 points (1 = extremely disliked and 9 = extremely liked). In addition, the purchase intention was evaluated and structured with 5 points (1 = certainly would not buy and 5 = certainly would buy) (Meilgaard, Civille & Carr, 2007).

2.7 Volatile metabolic composition

The volatile metabolic composition of pitaya samples was determined for each treatment using the methods that Santos *et al.* (2020) and Lima *et al.* (2020) described. 1.24 g of NaCl was added to 4 g of each sample material for the analysis. The volatile compounds were isolated from the matrix by headspace solid-phase microextraction (HS-SPME) utilizing DVB/CAR/PDMS 50/30 µm fiber (Supelco, Bellefonte, PA, USA). Before analysis, the fiber was conditioned according to the manufacturer's recommended temperature. Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using an Agilent Technologies chromatograph (Palo Alto, CA, USA) equipped with a mass spectrometer (model

5977A). The chromatographic column employed was RTX-5 (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). The temperature program included a ramp of 4 °C/min from 40 °C to 110 °C, followed by 16 °C/min to 200 °C, held for 10 min, and then 7 °C/min to 230 °C. The interface temperature between the chromatograph and the mass-selective detector was set to the recommended value. Ionization was performed by electron impact at 70 eV, with the ion source maintained at 150 °C.

2.8 Statistical analysis

The data from the accessibility tests were evaluated by ANOVA, followed by comparisons of the means of the hedonic values using the Tukey test at 5% probability. The Cochran Q test was performed to identify significant differences for each attribute of the CATA method (Ares & Jaeger, 2015). The frequency count was conducted in the CATA table to understand the positioning of the four pitaya juice samples according to the consumer's perception and ideal sample. The results were also evaluated through the principal component analysis (PCA) with two-dimensional graphs and the mean tests presented in tabular and graphical form. Also, the peak areas of volatile compounds obtained from their chromatograms were normalized by the treated sum and scaled accordingly with autoscaling.

The other data obtained from the chemical, physicochemical, and affective sensory analyses were subjected to the Tukey test Tukey test with a significance level of 5% to compare means and standard deviations using the XLSTAT (Addinsoft, 2024; New York, NY, USA) statistical program.

3 Results and discussion

3.1 Physicochemical, chemical and microbiological results of the delivery of tropical juice

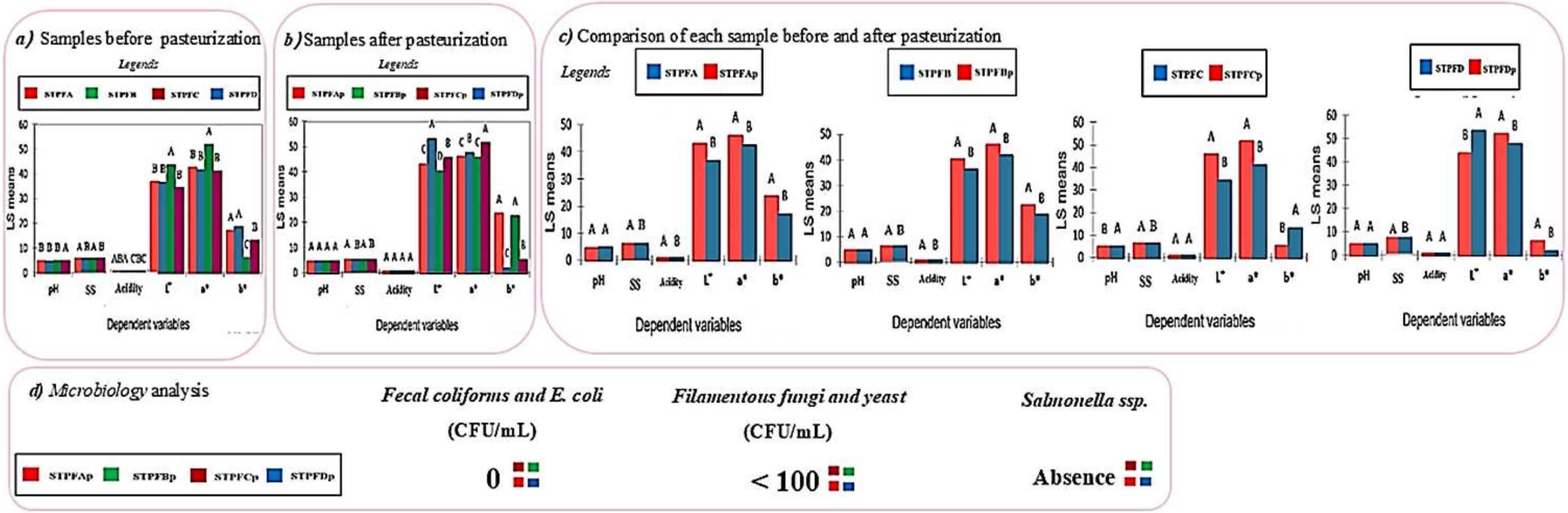
The different chemical and physicochemical compositions resulting from the different formulations of tropical juice are presented in Figure 3. The data were statistically presented to evaluate whether there is a significant difference between the samples before pasteurization (STPA, STPB, STPC, and STPD), and it can be inferred that there are significant differences in all analyses with and without the addition of cashew apple in the formulations. On the other hand, when evaluating the differences after pasteurization (STPFAp, STPFBp, STPFCp, and STPFDp), it was found that in the analyses of pH and titratable acidity, despite having low values (Wong & Siow, 2014; Khan, 2016), no significant difference was observed.

Additionally, Santos *et al.* (2020) reported that pitaya pulp causes changes in pH and soluble solids when subjected to the acidification process. Therefore, this acidification of the medium is recommended to improve the accumulation of betalains and prevent their degradation. These significant differences are possibly related to the treatments during pulping and the different formulations with and without the addition of cashew. Furthermore, the influence of pasteurization on the data studied was assessed, and it can be inferred that STPFA x STPFAp and STPFB x STPFBp did not present significant differences in pH. In contrast, STPFC x STPFCp did not present significant differences in acidity, and STPFD x STPFDp did not significantly affect pH and acidity.

Therefore, all other parameters made a significant difference, which may indicate an influence of the pasteurization process on the physicochemical and chemical composition. For example, Martins *et al.* (2022) compared a thermal and non-thermal method in tropical juices and found that pasteurization caused greater changes in the sensory characteristics of mixed tropical juice. As previously reported by Filho *et al.* (2018),Wurlitzer *et al.* (2019), Linhares *et al.* (2020) and Bezus *et al.* (2022), the application of processing and temperature in fruit juices açaí, camu-camu, acerola, cashew, cajá and pineapple can change the responses of the chemical environment, accelerating the activation energy of responses, such as deglycosylation, decarboxylation and isomerization that can result in color changes and in this research a difference can be seen as significant ($p < 0.05$) in the parameters L*, a* and b*, between the samples before and after pasteurization (see Figure 3). However, this detailed evaluation of the effect of pasteurization on the metabolic profile of the formulations were described in the following sections.

Microbiological analyses showed that the juices were suitable for consumption. The four pasteurized juice formulations (STPFAp, STPFBp, STPFCp and STPFDp) presented results of Fecal Coliforms and *E. Coli* 0 CFU/mL, Filamentous fungi and yeast values lower than 10^2 and in relation to the absence of Salmonella per 25 mL. The results were within expectations for tropical juices, as the literature reports that microbial counts above approximately 10^7cfu/mL of product could alter the food matrix and consequently the sensory aspects. In addition, the low acidity and pH values of the pasteurized samples, around 0.2 and 4.5, hinder microbial growth (Ribeiro *et al.*, 2018; Lima *et al.*, 2020).

Figure 3. Results expressed as mean and standard deviation; Means with the same letters, on the same line, do not vary, at the 5% significance level for the Tukey test*.



* *Results expressed as mean and standard deviation; Means with the same letters, on the same line, do not vary*, at the 5% significance level for the Tukey test.

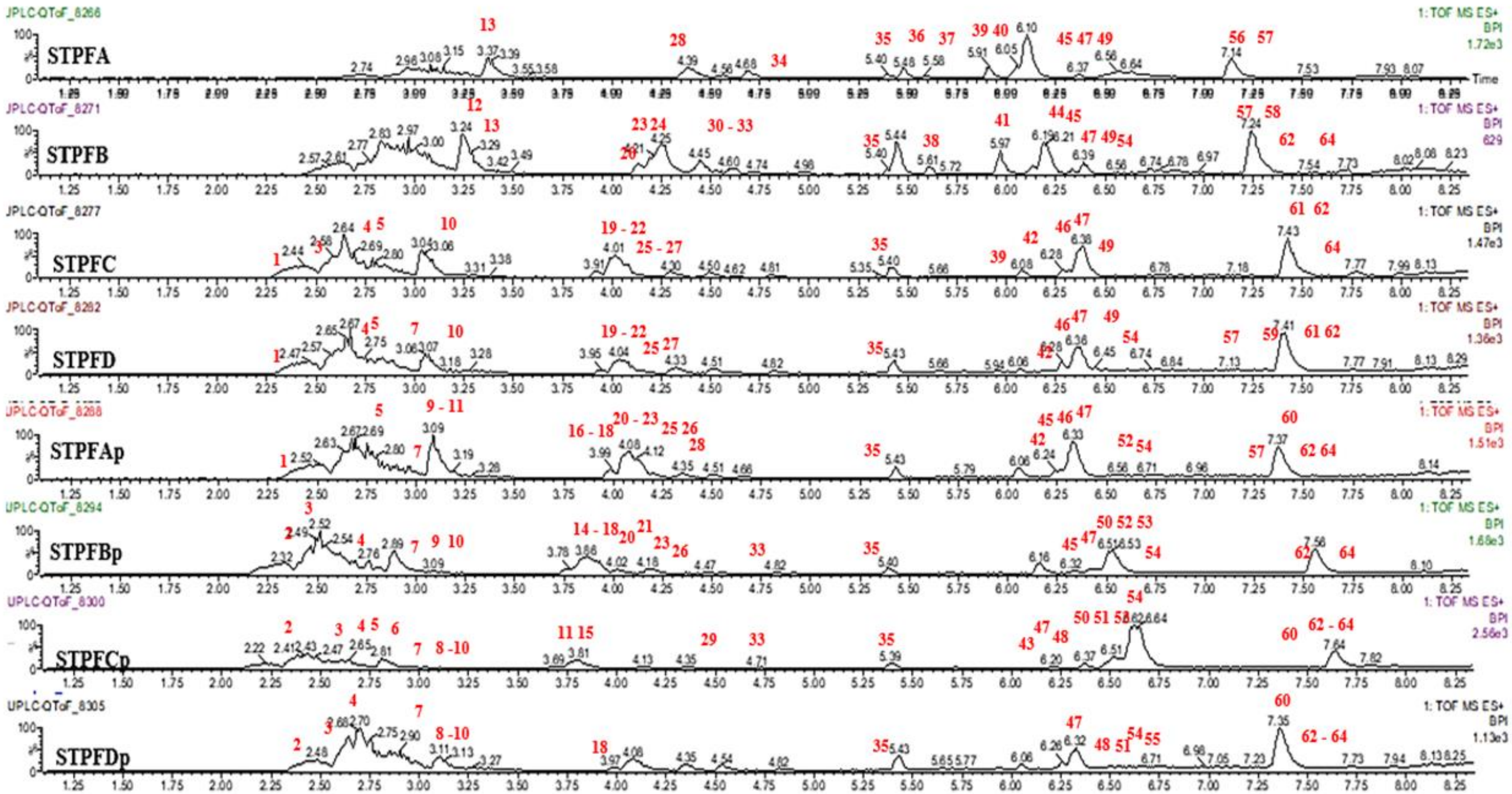
Fonte: Author (2025)

3.2 Metabolites annotation in the extracts

Metabolic profiling of the red pitaya was investigated using an untargeted global metabolomics approach by UPLC-QTOF-MS^E analysis. The ethanol extracts of 8 samples of tropical pitaya juices (Figure 4) were obtained in quintuplicate following as described in section **2.5.1**. Fragmentation mechanisms and the Scifinder, ChemSpider and Pubchem databases were used. All samples were tested in positive ionization mode, and a comparative analysis was performed between the tropical juices before pasteurization (STPFA, STPFB, STPTFC and STPTFD) and after pasteurization (STPFAp, STPFBp, STPTFCp and STPTFDp). Previously, the literature recorded research identified pathways of betalains and other pitaya components, as highlighted by Santos *et al*. (2024), Lira *et al*. (2020) and Lima *et al*. (2020).

Table 1 provides a concise summary of the UPLC-QTOF-MS^E chromatographic and MS data, which includes information such as retention time, theoretical mass, molecular formula, observed mass (MS), as well as fragments ions (MS/MS). Therefore, the annotation of the compounds was conceived through the correlation of the precursor ions (protonated ions) observed in the MS spectra with their respective product ions (fragment ions) observed in the MS/MS spectra.

Figure 4. Chromatograms corresponding to the formulations before pasteurization: STPFA, STPFB, STPFC, STPFD and after pasteurization: STPFAp, STPFBp, STPFCp, STPFDp profiles in positive ionization mode (ESI^+).



Fonte: Author (2025)

Table 1. Chromatographic and UPLC-Q-TOF-MS^E mass spectrometry data of tropical juices in positive ion mode*

N°	***t$_R$*** *(min)*	*Adduct*	*MS (m/z)* Observed	*MS (m/z)* Calculated	*Mass error (Ppm)*	*Fragment Ions (MS/MS)*	*Empirical formula*	*Annotated metabolites*	*Juices* *STPFA*	*STPFB*	*STPFC*	*STPFD*	*STPFA$_P$*	*STPFB$_P$*	*STPFC$_P$*	*STPFD$_P$*	*References*
1	2.26	$[M+H]^+$	869.8564	-	-	640.5025 524.2045 410.1941 336.1891 296.1864 222.1536	$C_{61}H_{1}0_{9}N_{2}$	unknown	-	-	✓	✓	✓	-	-	-	-
2	2.30	$[M+H]^+$	869.8564	-	-	640.5191 524.2055 410.1946 336.1890 296.1868	$C_{6}1H_{1}0_{9}N_{2}$	unknown	-	-	-	-	-	✓	✓	✓	-
3	2.48	$[M + K + H]^+$	705.1833	705.1850	-2.41	543.1318 399.0982 219.0515 203.0510	$C_{24}H_{42}O_{21}K$	maltotetraose	-	-	✓	-	-	✓	✓	✓	(Dasaesamoh *et al.*, 2016; Khalili *et al.*, 2014, Lira *et al.*, 2020 e Lima *et al.*, 2020; Santos *et al.*, 2024)
4	2.66	$[M + K + H]^+$	543.1327	543.1322	0.92	399.0982 219.0515 203.0510	$C_{18}H_{32}O_{16}K$	maltotriose	-	-	✓	✓	-	✓	✓	✓	(Dasaesamoh *et al.*, 2016; Matros *et al.*, 2015)
5	2.69	$[M+(K+H_2O)]^+$	399.0877	399.0899	-5.51	219.0510	$C_{12}H_{22}O_{11}$	sucrose	-	-	✓	✓	✓	-	✓	-	(Hua *et al.*, 2018; Lira *et al.*, 2020; Lima *et al.*,

																	2020; Santos *et al.*, 2024)
6	2.86	M^+	637.1512	637.1517	-0.78	593.1580 551.1521	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin Isomers (15*S*/15*R)*	-	-	-	-	-	-	✓	-	(Suh *et al*., 2014; Szot, 2015; Lira *et al*., 2020 e Lima *et al*., 2020; Santos *et al*., 2024)
7	2.90	M^+	769.1968	769.1956	1.17	637.1577 593.1586 551.1515	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15*R*/15*S*-phyllocatin Isomers	-	-	-	✓	✓	✓	✓	✓	(Wybraniec *et al*., 2001, 2010; Lira*et al*., 2020; Lima *et al*., 2020; Santos ., 2024)
8	3.06	-	182.0792	182.0805	-6.6	-	$C_9H_{12}NO_3$	unknown	-	-	-	-	-	-	✓	✓	-
9	3.08	M^+	769.1970	769.1956	1.82	637.1577 593.1590 551,1520	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15*R*/15*S*-phyllocatin isomers	-	-	-	✓	✓	✓	✓	✓	(Wybraniec *et al*., 2001, 2010; Lira *et al*., 2020; Santos *et al*., 2024)
10	3.08	M^+	637.1498	637.1517	-2,98	389.0998	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R)*	-	-	✓	✓	✓	✓	✓	✓	(Santos *et al*., 2024)
11	3.09	-	483.2050	483.2072	-4.55	-	$C_{33}H_{27}N_2O_2$	unknown	-	-	-	-	✓	-	-	-	-

12	3.24	M^+	637.1515	637.1517	-0.31	593.1601 551.1522	$C_{27}H_{28}N_2O_{16}$	4'-O-malonyl-isobetanina (15*S*/15*R*)	-	✓	-	-	-	-	-	-	(Wybraniec *et al.*, 2001, 2010; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)
13	3.28	$[M+H]^+$	483.2090	483.2072	3.72	-	$C_{33}H_{27}N_2O_2$	unknown	✓	✓	-	-	✓	-	-	-	-
14	3.74	M^+	551.1499	551.1513	-2.54	389.1010	$C_{24}H_{27}N_2O_{13}^+$	betanin isomers	-	-	-	-	-	✓	-	-	(Lira *et al.*, 2020)
15	3.76	M^+	551.1541	551.1513	5.08	389.0994	$C_{24}H_{27}N_2O_{13}^+$	betanin	-	-	-	-	-	✓	✓	-	(Wybraniec *et al.*, 2001, 2010; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)
16	3.86	M^+	637.1522	637.1517	0.78	389.0997	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R)*	-	-	-	-	✓	✓	-	-	(Santos *et al.*, (2024))
17	3.89	M^+	463.1709	463.1717	-1.72	301.1183	$C_{22}H_{27}N_2O_9^+$	2,17-bidecarboxy-betanin/ isobetanin	-	-	-	-	✓	✓	-	✓	(Starzak *et al.*, 2021)
18	3.89	-	483.1954	-	-	-	$C_{15}H_{35}N_2O_{15}$	unknown	-	-	-	-	✓	✓	-	✓	-
19	3.95	M^+	551.1566	551.1582	-3.62	389.1028	$C_{24}H_{26}N_2O_{13}$	isobetanin	-	-	✓	✓	-	-	-	-	(Fathordoobady *et al.*, 2016;

																Santos *et al.*, 2024)
20	4.00	M^+	637.1521	637.1517	0.63	389.1006	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R)*	-	✓	✓	✓	✓	✓	-	-	(Santos *et al.*, 2024)
21	4.03	M^+	637.1564	637.1531	5.18	593.1503 551.1600 389.1432	$C_{27}H_{28}N_2O_{16}$	2'- apiosyl-15*R/*15*S*-phyllocatin isomers	-	-	✓	✓	✓	-	✓	-	(Wybraniec *et al.*, 2010; Lira *et al.*, 2020 e Lima *et al.*, 2020; Santos *et al.*, 2024)
22	4.08	M^+	769.1929	769.1956	-	683.1539 637.1521 593.1601 551.1522 389.0910	$C_{32}H_{37}N_2O_{20}^+$	2'-O-2'-apiosyl-15*R*/15*S*-phyllocactin isomers	-	-	✓	✓	✓	-	-	-	*(Wybraniec et al.*, 2010)
23	4.09	$[M+H]^+$	483.2050	483.2072	- 4.55	-	$C_{33}H_{27}N_2O_2$	unknown	-	✓	-	-	✓	✓	-	-	-
24	4.13	M^+	551.1500	551.1508	-1.47	389.0921	$C_{24}H_{26}N_2O_{13}$	betanin isomers	-	✓	-	-	-	-	-	-	(Stintzing *et al.*, 2002)
25	4.16	M^+	637.1521	637.1517	-2.98	593.1622 551.1516 389.0999	$C_{27}H_{28}N_2O_{16}$	phyllocactin isomers (15*S*/15*R)*	-	-	✓	✓	✓	-	-	-	(Fathordoobady *et al.*, 2016; Kumorkiewiczand Wybraniec, 2017; Lira *et al.,*2020; Lima *et al.*, 2020)
26	4.20	M^+	637.1509	637.1517	-1.25	593.1698 551.1502 389.1011	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R)*	-	-	✓	-	✓	✓	-	-	(Fathordoobady *et al.*, 2016; Kumorkiewiczand, Wybraniec, 2017; Lira *et al.*,

																	2020 e Lima *et al.*, 2020; Santos *et al.*, 2024)
27	4.25	M^+	637.1534	637.1517	2.67	593.1081 551.1600 389.0944	$C_{27}H_{28}N_2O_{16}$	phyllocactin isomers (15*S*/15*R*)	-	-	✓	✓	-	-	-	-	(Wybraniec *et al.*, 2010; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*,2024)
28	4.29	$[M+H]^+$	483.2051	483.2072	-4.24	-	$C_{33}H_{27}N_2O_2$	unknown	✓	-	-	-	✓	-	-	-	-
29	4.40	$[M+H]^+$	637.1651	637.1665	-2.2	538.1154	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R*)	-	-	-	-	-	-	✓	-	(Santos *et al.*, 2024)
30	4.49	M+	637.1502	637.1517	-2.35	389.1101	$C_{27}H_{29}N_2O_{16}^+$	phyllocactin isomers (15*S*/15*R*)	-	✓	-	-	-	-	-	-	(Santos *et al.*, 2024)
31	4.52	$[M+H]^+$	483.1940	483.2072	-3.3	-	$C_{15}H_{35}N_2O_{15}$	unknown	-	✓	-	-	-	-	-	-	-
32	4.68	M+	795.2013	795.2037	-3.0	679.0210 637.1516	$C_{41}H_{35}N_2O_{15}$	unknown	-	✓	-	-	-	-	-	-	-
33	4.69	$[M+H]^+$	625.1735	625.1763	-4.47	479.1188 317.0655	$C_{28}H_{32}O_{16}$	Isorha-mnetin glicosyl rhamnosyl	-	✓	-	-	-	✓	✓	-	(Antunes-Ricardo *et al.,* 2017, 2015; Ginestra *et al.,* 2009)
34	5.40	$[M+H]^+$	224.2389	224.2378	4.90	-	$C_{15}H_{30}N$	unknown	✓	-	-	-	-	-	-	-	-
35	5.43	$[M+H]^+$	271.0616	271.0607	3.31	-	$C_{15}H_{11}O_5$	apigenin isomers	✓	✓	✓	✓	✓	✓	✓	✓	(Omidizadeh *et al.*, 2014)

36	5.48	$[M+H]^+$	271.0602	271.0607	-1.50	-	$C_{15}H_{11}O_5$	apigenin isomers	✓	-	-	-	-	-	-	-	(Omidizadeh *et al.*, 2014)
37	5.58	$[M+H]^+$	274.2758	274.2746	4.40	-	$C_{16}H_{36}NO_2$	unknown	✓	-	-	-	-	-	-	-	-
38	5.62	$[M+H]^+$	274.2744	274.2746	-0.70	-	$C_{16}H_{36}NO_2$	unknown	-	✓	-	-	-	-	-	-	-
39	5.91	$[M+H]^+$	504.3825	504.3842	-3.40	459.1190	$C_{34}H_{50}NO_2$	unknown	✓	-	✓	-	-	-	-	-	-
40	5.95	$[M+H]^+$	318.2995	318.3008	-4.08	-	$C_{18}H_{40}NO_3$	unknown	✓								-
41	5.97	$[M+H_2O+H]^+$	435.1555	435.1575	-4.60	385.1475 318.1509 300.1519	$C_{12}H_{27}N_4O_{13}$	unknown	-	✓	-	-	-	-	-	-	-
42	6.08	$[M+H]^+$	575.5939	575.5939	0,00	318.1510 282.1431	$C_{37}H_{67}O_4$	unknown	-	-	✓	✓	✓	-	-	-	-
43	6.15	$[M+H]^+$	575.5941	575.5939	0.35	318.1515 282.1468	$C_{37}H_{67}O_4$	unknown	-	-	-	-	-	-	✓	-	-
44	6.19	$[M+H]^+$	878.5649	878.5683	3.90	820.4950 504.0000 459.0000	$C_{54}H_{76}N_3O_7$	unknown	-	✓	-	-	-	-	-	-	-
45	6.21	$[M+H]^+$	504.3795	504.3801	-1.19	-	$C_{29}H_{50}N_3O_4$	unknown	✓	✓			✓	✓			-
46	6.27	$[M+H]^+$	459.3211	459.3223	-2.6	403.0000 375.0000	$C_{27}H_{43}N_2O_4$	unknown	-	-	✓	✓	✓	-	-	-	-
47	6.30	$[M+H]^+$	459.3256	459.3282	-5.66	-	$C_{20}H_{47}N_2O_9$	unknown	✓	✓	✓	✓	✓	✓	✓	✓	-
48	6.34	$[M+H]^+$	504.3862	504.3842	4.0	459.0000	$C_{34}H_{50}NO_2$	unknown	-	-	-	-	-	-	✓	✓	-
49	6.38	$[M+H]^+$	387.1805	387.1808	-0.8	331.0000	$C_{22}H_{27}O_6$	unknown	✓	✓	✓	✓	-	-	-	-	-
50	6.52	$[M+H]^+$	504.3825	504.3842	-2.6	459.0000 409.0000 387.0000 331.0000	$C_{34}H_{50}NO_2$	unknown	-	-	-	-	-	✓	✓	-	-
51	6.52	M+	505.1449	505.1458	-1.78	-	$C_{23}H_{25}N_2O_{11}^+$	decarboxy-neobetanin	-	-	-	-	-	-	✓	✓	(Mokoena *et al.*, 2023)
52	6.54	M+	504.2021	504.2022	-0.2	-	$C_{29}H_{30}NO_7^+$	unknown	-	-	-	-	✓	✓	-	-	-

53	6.55	$[M+H]^+$	504.3827	504,3842	-3.0	459.0000	$C_{34}H_{50}NO_2$	unknown	-	-	-	-	-	✓	✓	-	-
54	6.55	$[M+H]^+$	459.3265	459.3282	-3.70	-	$C_{20}H_{47}N_2O_9$	unknown		✓	✓	✓	✓	✓	✓	✓	-
55	6.63	$[M+H]^+$	504.3848	504.3842	1.20	459,0000	$C_{34}H_{50}NO_2$	unknown	-	-	-	-	-	-	-	✓	-
56	7.14	$[M+H]^+$	457.2794	457.2801	-1.5	457.0000 435.0000 317.0000	$C_{24}H_{41}O_8$	unknown	✓	-	-	-	-	-	-	-	-
57	7.20	$[M+H]^+$	457.2745	457.2761	-3.49	-	$C_{19}H_{41}N_2O_{10}$	unknown	✓	✓		✓	✓			✓	-
58	7.24	$[M+H]^+$	457.2799	457.2801	-1.3	457.0000 435.0000 317.0000	$C_{24}H_{41}O_8$	unknown	-	✓	-	-	-	-	-	-	-
59	7.37	$[M+H]^+$	457.2801	457.2801	0,0	457.0000 435.0000 317.0000	$C_{24}H_{41}O_8$	unknown	-	-	-	-	✓	-	✓	✓	-
60	7.40	$[M+H]^+$	473.2556	473.2539	1.2	457.0000 435.0000 317.0000	$C_{27}H_{37}O_7$	unknown	-	-	-	✓	-	-	-	-	-
61	7.42	$[M+H]^+$	473.2556	473.2539	3.6	435.0000 317.0000 155.0000	$C_{27}H_{37}O_7$	unknown	-	-	✓	✓	-	-	-	-	-
62	7.49	$[M+H]^+$	457.2747	457.2761	-3.06	-	$C_{19}H_{41}N_2O_{10}$	unknown	-	✓	✓	✓	✓	✓	✓	✓	-
63	7.54	$[M+H]^+$	457.2814	457.2801	2.8	435.2823 317.1965 155.0742	$C_{24}H_{41}O_8$	unknown	-	-	-	-	-	-	✓	✓	-
64	7.63	$[M+H]^+$	457.2749	457.2761	-2.62	-	$C_{19}H_{41}N_2O_{10}$	unknown	-	✓	✓		✓	✓	✓	✓	-

* The symbols (✓) or (–), which represent the confirmation or denial of the existence of that compound, were assigned in the Table 1 to each metabolite and tropical juice of pitaya.

Fonte: Author (2024)

Carbohydrates

In the first-order mass spectrum (MS) for Compound **3** (t_R = 2.48 min), the ion [M + K + H]$^+$ was observed at *m/z* 705.1850 and was tentatively identified as Maltotetraose. The MS^2 spectra revealed fragment ions at *m/z* 543.1318, 399.0982, 219.0515, and 203.0510. The fragmentation pattern of this compound followed the typical profile for carbohydrates, as previously reported by authors such as Matros *et al.* (2015), Dasaesamoh *et al.* (2016), Lira *et al.* (2020), Lima *et al.* (2020), and Santos *et al.* (2024). This pattern is characteristic of glycosidic cleavages, a potassium ion's presence, and water molecules' involvement.

Furthermore, in compound **4** (t_R = 2.66 min), the ion [M + K + H]$^+$ was observed at *m/z* 543.1327, and the fragmentation pattern in the MS^2 spectrum showed peaks *at m/z* 399.0982, *m/z* 219.0515 [543 − (2 × 162)]$^+$, and *m/z* 203.0510 ([hexose + Na]$^+$). These fragments resulted from the typical loss of two hexoside units (*m/z* 162 each), with the *m/z* 203.0510 fragment corresponding to a hexose molecule with sodium adduct. Based on the collected data, compound 4 was identified as Maltotriose, confirming the findings of Lima *et al.* (2020).

Compound **5** (t_R = 2.69 min) was tentatively identified as sucrose, with the ion [M + (K + H_2O)]$^+$ observed at *m/z* 399.0877. The MS^2 spectra showed a fragment ion at *m/z* 219.0510, corresponding to [glucose + K]$^+$, resulting from losing a hexoside unit (such as glucose) from the parent ion. This fragmentation profile is consistent with the observations reported by Lima *et al.* (2020).

Betalains

The eleven isomeric forms of 15*S*/15*R*-phyllocactin ($C_{27}H_{28}N_2O_{16}$) were observed at different retention times: **6** (t_R = 2.86 min), **10** (t_R = 3.08 min), **12** (t_R = 3.24 min), **16** (t_R = 3.86 min), **20** (t_R = 4.00 min), **21** (t_R = 4.03 min), **25** (t_R = 4.16 min), **26** (t_R = 4.20 min), **27** (t_R = 4.25 min), **29** (t_R = 4.40 min), and **30** (t_R = 4.49 min), respectively. In the MS^2 spectra, the following fragment ions were specifically observed: *m/z* 86 (637 → 551), *m/z* 162 (551 → 389), and *m/z* 44 (637 → 593), corresponding to the loss of malonyl, glucosyl, and carboxylic groups, respectively. These fragmentation patterns are consistent with those previously reported by

Wybraniec *et al.* (2001, 2007, 2010), Suh *et al.* (2014), Szot (2015), Fathordoobady *et al.* (2016), Lira *et al.* (2020), Lima *et al.* (2020), and Santos *et al.* (2024).

The MS^1 spectra of compounds **7** (t_R= 2.90 min) and **9** (t_R = 3.08 min) were tentatively identified as 2'-apiosyl-15R/15S-phyllocactin isomers ($C_{32}H_{37}N_2O_{20}^+$) with *m/z* values of 769.1968 and 769.1070, respectively. In the MS^2 spectrum, typical fragment ions were observed at *m/z* 637, *m/z* 593, and *m/z* 551, corresponding to the loss of apiosyl (769 → 637) Da, CO_2 (637 → 593) Da, and betanin ($[C_{24}H_{26}N_2O_{13} + H]^+$) Da, respectively.

Peak **22** (t_R = 4.08 min) was tentatively identified as 2'-O-2'-apiosyl-15*R*/15*S*-phyllocactin isomers at *m/z* 769.1973. The MS^2 spectra demonstrated fragments at 683.1539 Da, 637.1521 Da $[683 – CO_2H_2]^+$, 593.1601 Da $[637 – CO_2]^+$, 551.1522 Da [637 – malonyl group]$^+$, 389.0910 Da (betanidin) (Wybraniec *et al.*, 2001, 2010, Santos *et al.*, 2024).

In addition, compounds **14** (t_R = 3.74 min), **15** (t_R = 3.76 min), **19** (t_R = 3.95 min), and **24** (t_R = 4.13 min) were tentatively identified as isomers of betanin (15*R*/15*S*), all with the molecular formula $C_{24}H_{26}N_2O_{13}$, showing [M^+] ions at *m/z* 551.1499, *m/z* 551.1541, *m/z* 551.1566. Also, the MS^2 spectrum shows the presence of betanidin fragments (*m/z* 389), formed due to loss of sugar unit (162 Da) (Stintzing *et al.*, 2002, Wybraniec *et al.*, 2007, 2010, Lima *et al.*, 2020, Santos *et al.*, 2024).

Peak **17** (t_R = 3.89 min) was tentatively identified as 2,17 - bidecaboxi-betanin/isobetanin **(**$C_{22}H_{27}N_2O_9^+$) at *m/z* 465.1709. The MS^2 spectra demonstrated a fragment at 301.1183, consistent with findings from studies by Starzak *et al.* (2021). In addition, the mass spectrum of peak **51** (t_R = 6.52 min) was tentatively assigned to decarboxy-neobetanin, which has the molecular formula $C_{23}H_{25}N_2O_{11}^+$**.** In the MS^1 spectrum, it appeared at *m/z* 505.1449**.** While no significant fragment ions were observed in the MS^2 spectrum, similar findings have been documented in previous studies, such as those by Mokoena *et al.* (2023).

The formation of decarboxylated betacyanin is reported in the literature as being associated with processes that use high temperatures of betalains (Wybraniec, 2005, Herbach, Stintzing, & Carle, 2006). Also, it was described that heat treatment at 85 °C for 3 hours degrades betanin in red pitaya juice (Herbach, Stintzing, & Carle, 2004). However, it was found that pasteurization did not negatively affect the concentration of components with functional potential derived from betalains, corroborating the data described on thermal application in juices at 85°C/30 seconds by Wurlitzer *et al.* (2019). Furthermore, Santos *et al.* (2024), Khan, (2016) and Herbach, Stintzing, & Carle, (2006) have already reported that the addition of ascorbic acid is directly related to the increase in the stability of the chemical profile of betalain isomers, see Figure 4. Additionally, Holanda *et al.* (2021), Lira *et al.* (2020, 2023), and Marques *et al.* (2023) described, based on tests on mice and zebrafish model, functional benefits related to the presence of betalains in red pitaya, including anxiolytic capacity and dyslipidemia, but the evaluation of the possible impacts of temperature on the metabolic fingerprint will be discussed based on chemical markers in next section of the document.

Flavonoids

Compound **33** (t_R = 4.69 min) was identified as glycosyl rhamnosyl isorhamnetin, with the protonated molecular ion $[M + H]^+$ observed at *m/z* 625.1735. The MS^2 spectrum showed fragments at 479.1188 Da and 317.0655 Da, which have been previously reported in the literature by Ginestra *et al.* (2009), Polturak & Aharoni, (2018), and Santos *et al.* (2024). These fragments are specific for the loss of rhamnosyl and glycosyl groups and are considered anti-inflammatory components in Antunes-Ricardo *et al.* (2015, 2017). Compounds **35** (t_R = 5.43 min) and **36** (t_R = 5.48 min) were investigated as isomers of apigenin, as previously reported by Omidizadeh *et al.* (2014). Their molecular formulas are $C_{15}H_{11}O_5$, and the protonated molecular ions $[M + H]^+$ were observed at *m/z* 271.0616 and *m/z* 271.0602, respectively.

Other compounds

The peaks **1** (t_R = 2.26 min), **2** (t_R = 2.30 min), **8** (t_R = 3.06 min), **11** (t_R = 3.09 min), **13** (t_R = 3.28 min), **18** (t_R = 3.89 min), **23** (t_R = 4.09 min), **28** (t_R = 4.29 min), **31** (t_R = 4.52 min), **32** (t_R = 4.68 min), **34** (t_R = 5.40 min), **37** (t_R = 5.58 min), **38** (t_R = 5.62 min), **39** (t_R = 5.91 min), **40** (t_R = 5.95 min), **41** (t_R = 5.97 min), **42** (t_R= 6.08 min), **43** (t_R = 6.15 min), **44** (t_R = 6.19 min), **45** (t_R = 6.21 min), **46** (t_R = 6.27 min), **47** (t_R = 6.30 min), **48** (t_R = 6.34 min), **49** (t_R= 6.38 min), **50** (t_R = 6.52 min), **52** (t_R = 6.54 min), **53** (t_R= 6.55 min), **54** (t_R = 6.55 min), **55** (t_R = 6.63 min), **56** (t_R = 7.14 min), **57** (t_R = 7.20 min), **58** (t_R = 7.24 min), **59** (t_R = 7.37 min), **60** (t_R = 7.40 min), **61** (t_R = 7.42 min), **62** (t_R = 7.49 min), **63** (t_R = 7.54 min) and **64** (t_R = 7.63 min) are compounds that have not been annotated. Since there was no discernible manifestation between the ions detected in the MS spectra and the ion fragments presented in the MS/MS spectra, it was impossible to identify any of these compounds based on the metabolites described in the various databases consulted.

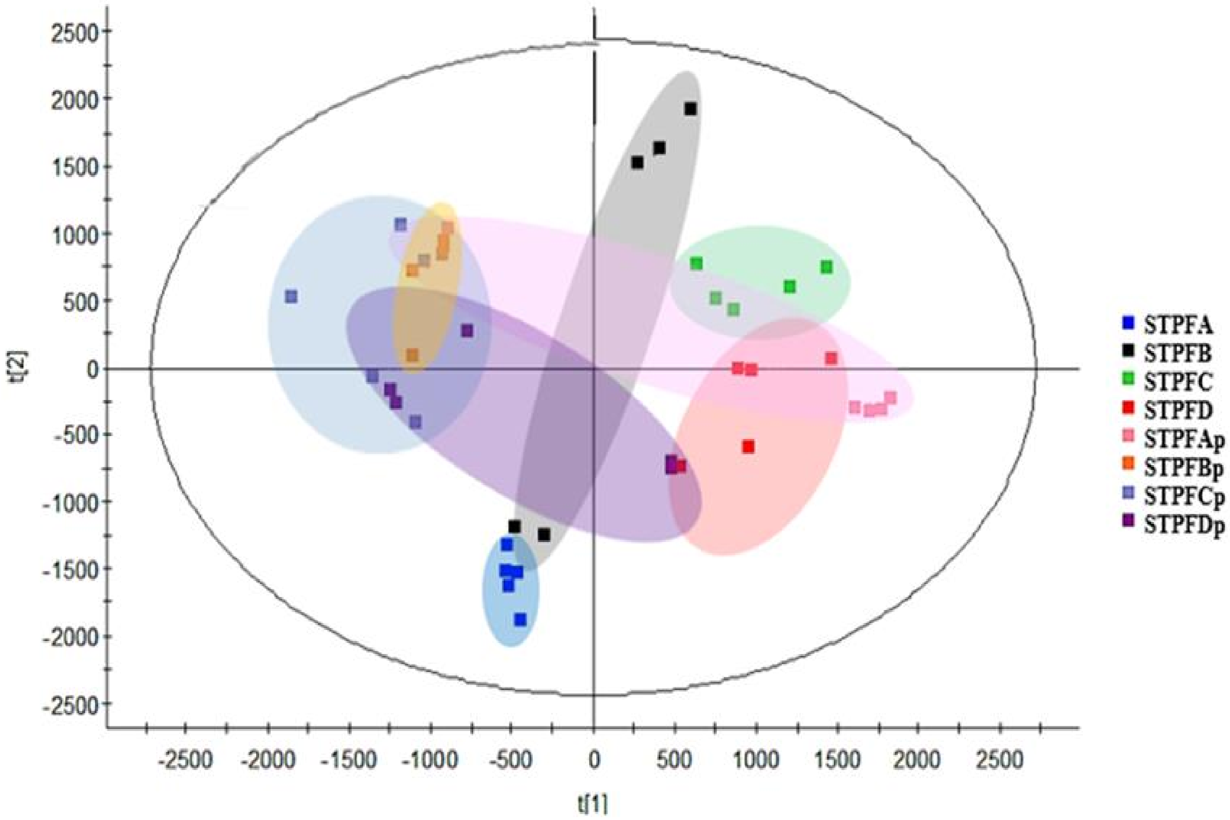
Furthermore, the positive mode evaluation did not detect the presence of compounds originating from the cashew apple chemical profile. The literature reported identifying a metabolic profile of cashew apple compounds in the negative ionization mode (Cunha *et al.*, 2017, Sousa *et al.*, 2020, Alves *et al.*, 2023). Therefore, this underscores the necessity of meticulously analyzing the chemical markers, which will be elaborated upon in the following section.

3.3 Multivariate analysis UPLC-QTOF-MS^E

The samples' principal component analysis (PCA) demonstrated the importance of applying an unsupervised method to increase the understanding of the data set, examining the presence or absence of natural groupings among the samples. In the PCA analysis, Figure 5, observing the different behaviors of the trained formulations is possible. In addition, PCA evaluation is used in this study as the first step in the chemometric analysis to visualize clustering trends in samples with many variables (Total of 64 compounds x 8 chromatograms = 512 variables) in UPLC-QTOF-MS^E. In this case, the PCA score plots were derived from the non-target metabolite profile analysis to understand the relationship between the formulations and the annotated metabolites. The generated model presented discrimination of the equal samples (R2XCum = 0.6433). The PCA graphs showed that the four different formulations with and without the addition of cashew apple obtained from two different treatments, with and without ascorbic acid, and present differences in the metabolites since the samples are specific and grouped based on chemical similarities. Therefore, the data corroborate the data presented by Santos *et al.* (2024), who found that adding ascorbic acid in two different treatments of pitaya pulp altered the metabolomic profile.

In addition, it can be inferred that there is a difference in the distribution of the quintuplicates between the samples before and after pasteurization. Still, we will report on this in greater depth in evaluating the possible chemical markers since no information regarding the identity of the samples has been taken into account. Therefore, the graphs of analysis of main compounds do not refer to conclusive data on the chemical differences.

Figure 5. Analysis of main compounds from red pitaya processing: before pasteurization: STPFA, STPFB, STPFC, STPFD and after pasteurization: STPFAp, STPFBp, STPFCp, STPFDp.



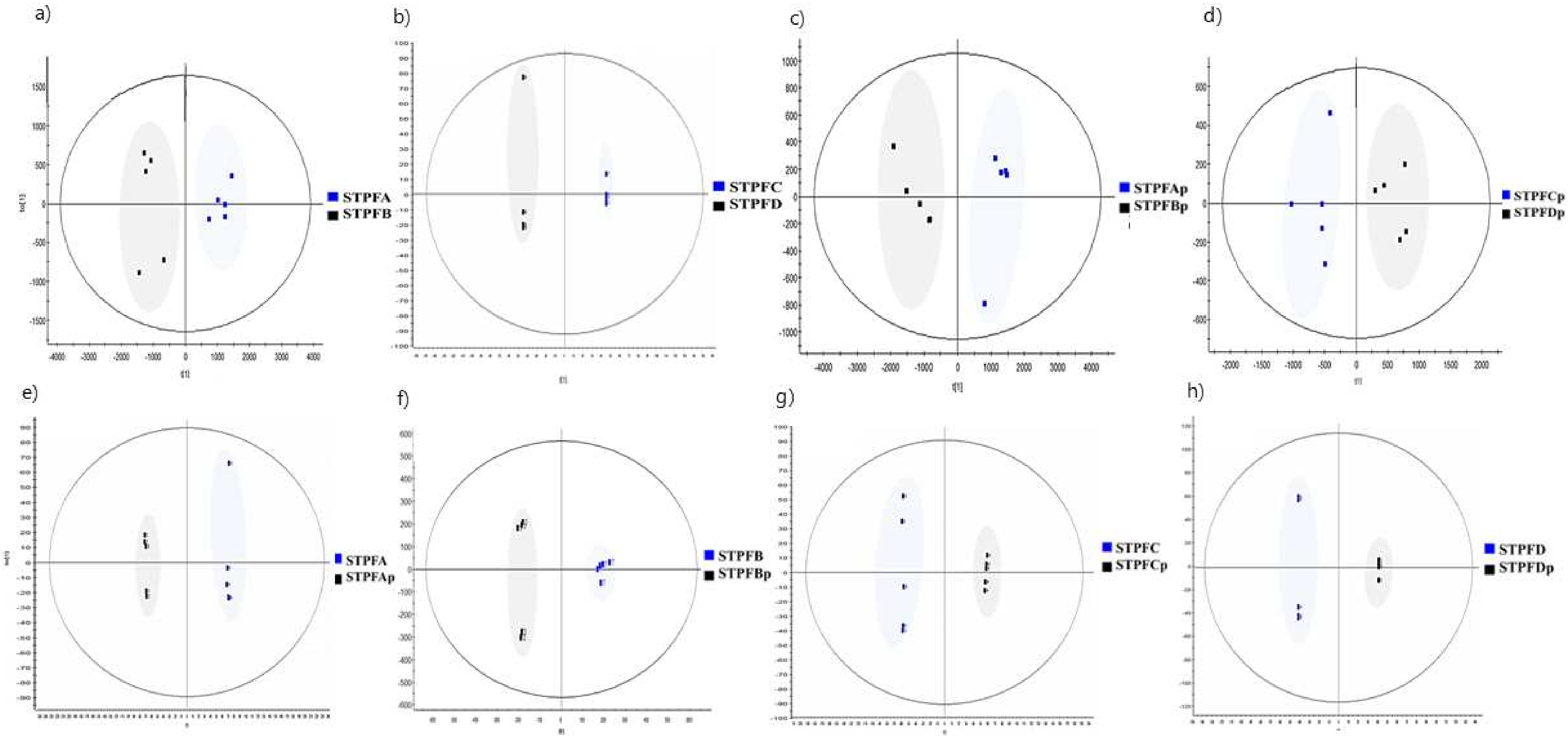
Fonte: Author (2025)

3.4.1 Potential chemical markers

To better elucidate the chemometric data, analyses were performed using OPLS-DA, S-Plot and graphs with the discriminants of the samples of the four formulations evaluated before and after pasteurization. In the OPLS-DA graphs, the algorithm tends to improve the predictive capacity, due to the orthogonal signal correction to distinguish the variations in the data that are important for predicting a response.

Figure 6 shows two blocks of comparisons. The first are the models of the comparisons (a) STPFA x STPFB, (b) STPFC x STPFD, (c) STPFAp x STPFBp, (d) STPFCp x STPFDp with discrimination of the same samples: R2XCum[1] = 0.9551, R2XCum[1] = 0.7023, R2XCum[1] = 0.7525, R2XCum[1] = 0.5293, respectively. These comparisons describe a metabolic behavior with a statistically significant difference, as they confirm that the different formulations of red pitaya juices with and without the addition of cashew apple juice and the treatments interfere with the profile of the metabolic compositions, maintaining the chemical profile of behavior after pasteurization. The data from Santos *et al.* (2024) show similarity in behavior when treatment with ascorbic acid is applied to red pitaya pulps. Furthermore, in Figure 6, the second comparison block shows the influence of the pasteurization process on the chemical profile of the following samples: (e) STPFA x STPFAp, (f) STPFB x STPFBp, (g) STPFC x STPFCp and (h) STPFD x STPFDp with the following sample discriminations: R2XCum[1] = 0.6164, R2XCum[1] = 0.7723, R2XCum[1] = 0.5336, and R2XCum[1] = 0.7463 respectively. The metabolite groupings are explained forming two clusters in different quadrants. Therefore, we can infer that pasteurization also significantly influences the differences between the composition of the metabolites studied in the same formulations. The data presented a robust technique for the inferences described for the first time in the literature on the formulations of tropical red pitaya juices, and tropical red pitaya juice with cashew apple submitted to different treatments in a pilot manufacturing plant and submitted to the pasteurization process.

Figure 6. Graphs of OPLS-Da analyses of the formulations: (a) STPFA x STPFB, (b) STPFC x STPFD, (c) STPFAp x STPFBp, (d) STPFCp x STPFDp, (e) STPFA x STPFAp, (f) STPFB x STPFBp, (g) STPFC x STPFCp and (h) STPFD x STPFDp.



Fonte: Author (2025)

Figure 7 shows the variation of the chemical markers distributed in the tropical juice formulations. With this, we can see what was predicted by the S-Plot and trend graphs, where we can observe and determine the chemical markers associated with the compositions of the pitaya juices with and without the addition of cashew apple juice. The chemical markers of the STPFA and STPFB treatments can be observed in Figure 7 (a). Compounds **20** (phyllocactin isomers), **16** (phyllocactin isomers), **41** (unknown), **47** (unknown), **54** (unknown) and **23** (unknown) are responsible for the differentiation of STPFA from STPFB. However, components **13** (unknown), **57** (unknown) and **45** (unknown) were responsible for the differentiation of STPFB in relation to STPFA. This difference in the chemical markers of the juices from the two processing processes corroborates the report by Santos *et al.* (2024) and may be related to the compounds obtained by the type of pulping and addition of ascorbic acid.

Other markers are found in the pasteurized samples STPFAp and STPFBp (see Figure 7 [c]) and are responsible for the differentiation of STPFAp from STPFBp, which are: **54** (unknown), **16** (phyllocactin isomers), **18** (unknown), **6** (phyllocactin isomers) and **64** (unknown). On the other hand, when evaluating the markers responsible for the differentiation of STPFBp from STPFAp, the following compounds were highlighted: **20** (phyllocactin isomers), **23** (unknown), **11** (unknown), **57** (unknown), **47** (unknown) and **10** (phyllocactin isomers). This change in the marker profile may be related to the influence of the pulping and pasteurization step.

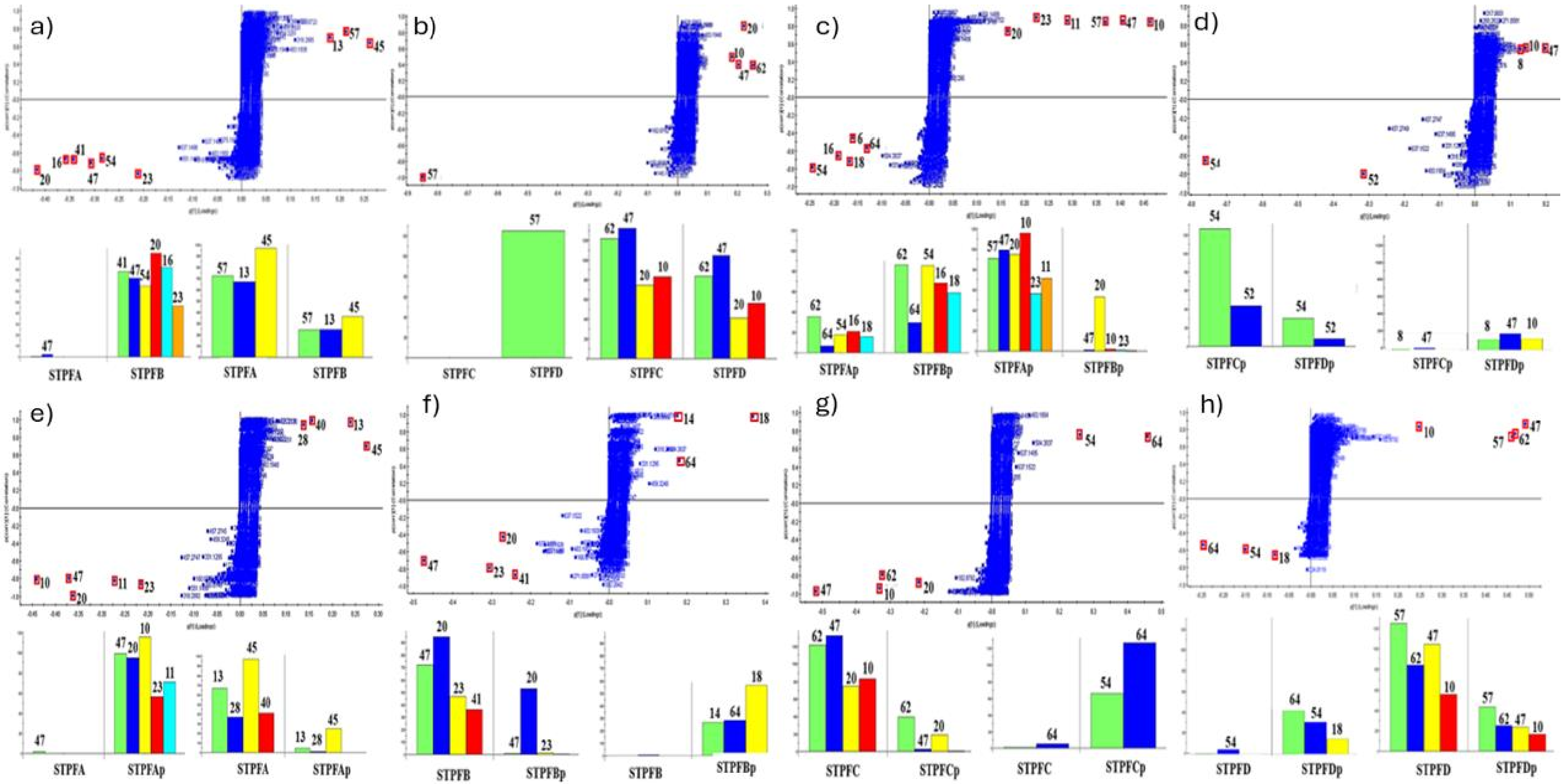
When evaluating the STPFC and STPFD formulations with the addition of cashew apple in two different processing processes (see Figure 7 [b]), the following marker responsible for the differentiation of STPFC from STPFD was observed: compound **57** (unknown). Furthermore, compounds **20** (phyllocactin isomers), **10** (phyllocactin isomers), **47** (unknown) and **62** (unknown) are the markers responsible for the classification of STPFD from STPFC. When these formulations were evaluated after pasteurization STPFCp and STPFDp, the following markers were assigned: **54** (unknown), **52** (unknown), responsible for the differentiation of STPFDp from STPFCp, and compounds **8** (unknown), **10** (phyllocactin isomers) and **47** (unknown), which are responsible for the differentiation of STPFDp from STPFCp (see Figure 7 [d]). This difference in the marker profile may also be related to the interaction of the chemical compounds of pitaya juice and cashew apple juice. For a better understanding of the influence of pasteurization on each formulation, an S-Plot analysis was performed, and the bar graphs showed the discriminant compositions (see Figure 7 [e, f, g, h]).

The chemical marker stands out in red. In addition, it contains the same comparative numbering in the bar graph.

Regarding the markers responsible for the differentiation of STPFA from STPFAp, phyllocactin isomers (**10** and **20**) and unknown compounds (**47**, **11** and **23**) were observed. In turn, the S-Plot and the bar graphs showed some more discriminant compositions of STPFAp from STPFA: the unknown compounds **28**, **40**, **13** and **45** (see Figure 7 [e]). A similar behavior was observed when evaluating the organic components responsible for the differences between STPFB and STPFBp, in which compounds **47** (unknown), **25** (phyllocactin isomers), **20** (phyllocactin isomers) and **41** (unknown) were reported. In addition, other compounds differentiating STPFBp from STPFB were described: compound **14** (betanin isomers) and unknown compounds **64** and **18**, as shown in Figure 7 (f).

To conclude the evaluation, the samples were analyzed in different processing processes with the addition of cashew apple juice subjected to pasteurization. The result also showed an evident difference in the profile of metabolic groups before and after the application of the pasteurization process. The differentiation compounds **47** (unknown), **62** (unknown), **10** (phyllocactin isomers) and **20** (phyllocactin isomers) of the STPFC formulations from STPFCp. On the other hand, only two compounds were found for differentiation of STPFCp from STPFC, compounds **54** and **64**, which were not identified (see Figure 7 [g]). Similar to Figure 7 (h), differentiation of the STPFD samples from STPFDp were through three compounds that were not identified: **64**, **54** and **18.** In addition, for differentiation of STPFDp from STPFD, four chemical markers were presented: **10** (phyllocactin isomers), **57** (unknown), **62** (unknown) and **47** (unknown). In general, it can be inferred that pasteurization directly altered the composition profile of chemical markers in all formulations. This reinforces the importance of evaluating consumer perceptions of pasteurized formulations in the following sections.

Figure 7. Graphs of the SPLOT analyses of the formulations: (a) STPFA x STPFB, (b) STPFC x STPFD, (c) STPFAp x STPFBp, (d) STPFCp x STPFDp, (e) STPFA x STPFAp, (f) STPFB x STPFBp, (g) STPFC x STPFCp and (h) STPFD x STPFDp.



Fonte: Author (2025)

3.5 Sensorial analysis

3.5.1 *Focus group*

Reports in the literature on red pitaya juices and red pitaya juices with cashew apple are scarce in the literature. Then, a focus group methodology with questions and brainstorming were applied related to the four formulations. The participants' suggestions were essential to choose the 29 attributes that would best describe the four juice formulations. Regarding appearance, the predominant pitaya's red color in the formulations was one of the relevant points of the section, as all participants stated that color is an important factor in evaluating a juice purchase. Regarding aroma and taste, sensory memory related to the characteristic *aftertaste of cashew apple* in the formulations was predominant in samples that contained cashew. On the other hand, in samples that contained only red pitaya, a fruity aroma and sweet flavor were found.

For a robust view of these observations, sensory analyses of CATA, RATA, hedonic and purchase intention were carried out, which will be described in detail below.

3.5.2 *CATA (Check-All-that-apply) and RATA (Rate-all-that-apply)*

The CATA test is important for understanding and discriminating samples that differ from each other (Jaeger *et al.*, 2018). In this study, as formulas for four different juice recipes, the understanding of the attributes related to juices is presented for the first time in red pitaya juices. Table 2 shows the 29 attributes and the results of the CATA test in the juice formulations evaluated sensorially. Of these, 14 attributes showed significant differences ($p < 0.05$), including *homogeneous, presence of particles, clear, sedimented, viscous, sweet aroma, fruit aroma, pitaya aroma, cashew apple aroma, green leaf aroma, no aroma, cashew apple flavor, root flavor,* and *cashew apple aftertaste*. In addition, the following attributes do not show a 5% significance difference: *red color, pink color, purple color, heterogeneous, shiny, fermented, acidic aroma, astringent aroma, sweet taste, acidic taste, astringent taste, fruit flavor, pitaya fruit flavor, beetroot flavor, fermented flavor*.

Table 2. *Check-All-that-apply (CATA*) frequency using the Cochran test to compare juice formulations*.

Attributes	**STPFAp**	**STPFBp**	**STPFCp**	**STPFDp**	***p-values***
Red color	0.990^{a}	1^{a}	1^{a}	1^{a}	0.392
Pink color	0.690^{a}	0.700^{a}	0.750^{a}	0.810^{a}	0.031
Purple color	0.460^{a}	0.450^{a}	0.500^{a}	0.430^{a}	0.392
Homogeneous	0.620^{ab}	0.510^{a}	0.650^{ab}	0.700^{b}	0.002
Heterogeneous	0.310^{a}	0.430^{a}	0.320^{a}	0.280^{a}	0.009
Shiny	0.580^{a}	0.500^{a}	0.600^{a}	0.590^{a}	0.207
Presence of Particles	0.540^{a}	0.700^{b}	0.460^{a}	0.520^{a}	0.000
Limpid	0.360^{ab}	0.280^{a}	0.470^{b}	0.360^{ab}	0.001
Fermented	0.180^{a}	0.230^{a}	0.170^{a}	0.210^{a}	0.201
Sedimented	0.190^{ab}	0.300^{b}	0.160^{a}	0.200^{ab}	0.000
Viscous	0.390^{b}	0.570^{c}	0.220^{a}	0.290^{ab}	0.000
Sweet aroma	0.380^{ab}	0.320^{a}	0.490^{b}	0.480^{b}	0.002
Acidic aroma	0.240^{a}	0.250^{a}	0.200^{a}	0.250^{a}	0.488
Astringent aroma	0.250^{a}	0.360^{a}	0.260^{a}	0.280^{a}	0.057
Fruit aroma	0.360^{b}	0.240^{a}	0.490^{b}	0.470^{b}	0.000
Pitaya aroma	0.390^{b}	0.330^{ab}	0.270^{ab}	0.220^{a}	0.000
Cashew apple aroma	0.260^{a}	0.230^{a}	0.680^{b}	0.640^{b}	0.000
Green leaf aroma	0.350^{b}	0.380^{b}	0.180^{a}	0.210^{a}	0.000
No aroma	0.300^{b}	0.290^{b}	0.190^{ab}	0.170^{a}	0.002
Sweet taste	0.420^{b}	0.260^{a}	0.480^{b}	0.510^{b}	0.000
Acidic taste	0.280^{a}	0.280^{a}	0.260^{a}	0.290^{a}	0.880
Astringent taste	0.290^{a}	0.350^{a}	0.300^{a}	0.320^{a}	0.615
Fruit flavor	0.420^{a}	0.310^{a}	0.440^{a}	0.410^{a}	0.030
Pitaya fruit flavor	0.370^{a}	0.360^{a}	0.350^{a}	0.330^{a}	0.843
Cashew apple flavor	0.280^{a}	0.220^{a}	0.650^{b}	0.640^{b}	0.000
Beetroot flavor	0.360^{b}	0.340^{b}	0.200^{a}	0.190^{a}	0.000
Fermented flavor	0.210^{a}	0.230^{a}	0.190^{a}	0.180^{a}	0.422
Root flavor	0.480^{b}	0.420^{b}	0.190^{a}	0.210^{a}	0.000
Cashew apple aftertaste	0.260^{a}	0.290^{ab}	0.430^{b}	0.410^{b}	0.000

*Results expressed as mean and standard deviation; for means with the same letters, in the same line, there was not significant difference ($p < 0.05$) for the Cochran test. Pasteurized formulations: STPFAp - 50% pitaya Pulp A treatment/ 50% water; STPFBp - 50% pitaya pulp treatment B/ 50% Water; STPFCp - 25% pitaya Pulp A treatment/ 50% water/ 25% cashew apple; STPFDp - 25% pitaya pulp treatment B/ 50% water/ 25% cashew apple.

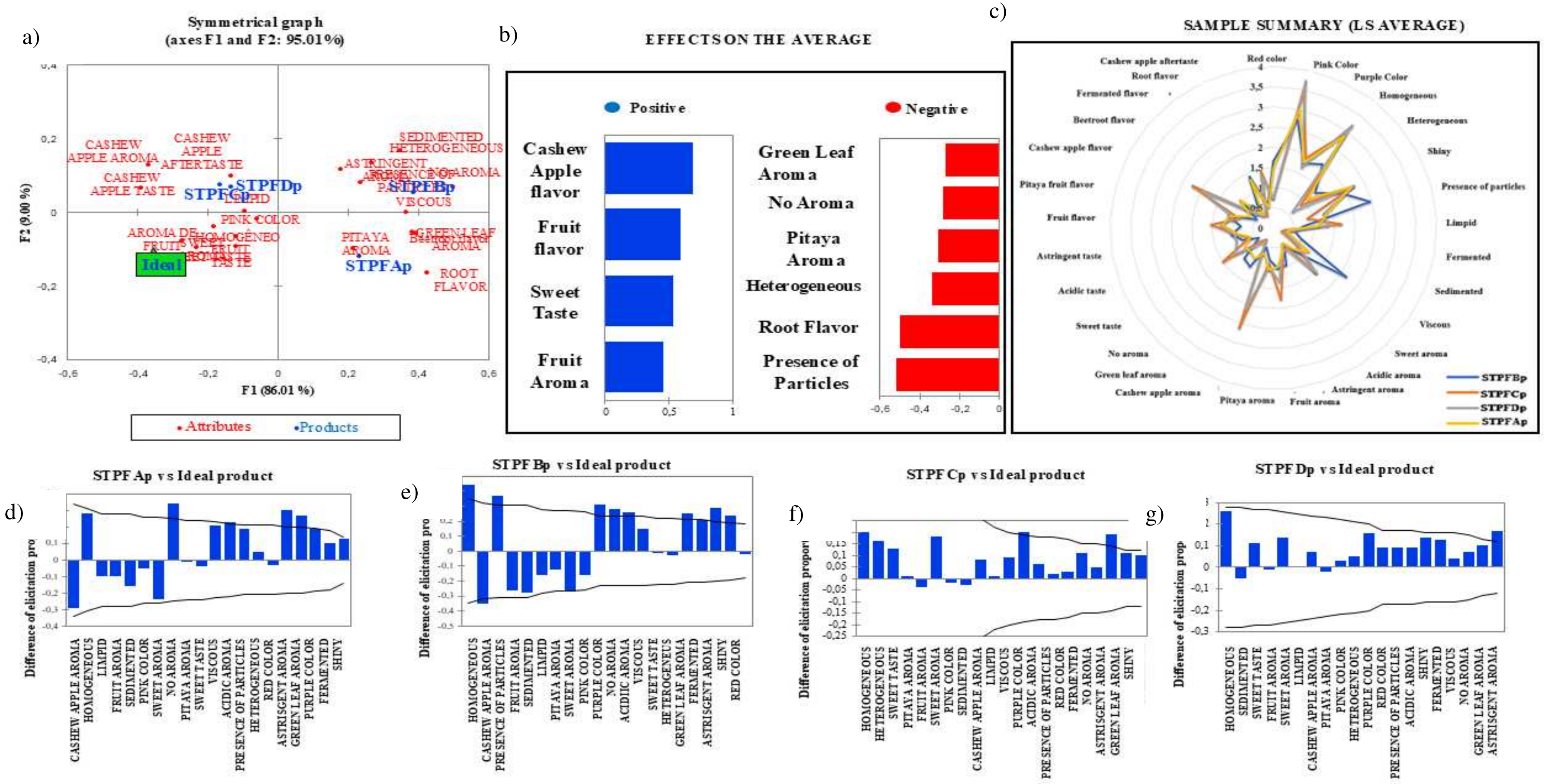
Fonte: Author (2025)

In general, these attribute response profiles are explained due to four different formulations obtained from two different pulping processes and pitaya, cashew apple and water. A difference in the presence of specific particles in the samples may be linked to treatment B, STPFBp and STPFDp, which during pulping removes the pulp adhered to the peel. STPFCp and STPFDp samples contain 25% cashew apple juice, which is related to the difference in the cashew apple aftertaste descriptor. As noted in section 3.3, flavonoids might enhance fruity notes, seen in STPFCp. The sweet taste is likely due to maltotetraose (3), maltotriose (4) and sucrose (5), highlighting its role in fruit product acceptance.

Section **3.4** mentions markers that phyllocactin isomers (**6**, **10**, **16** and **20**) and betanin isomers (**14**), originating from betalains, contribute to the intense color of all formulations and do not present a significant difference in the *colors red*, *pink* and *purple* in the perception of consumers. Maintaining the same proportion of water in all formulations may be another determining factor for these results. The literature describes that the use of water provides greater efficiency in the solubility of betalain-derived components. In addition, samples with low acidity and pH values provide greater stability to the pigment compared to different types of formulations (Wybraniec, 2005, 2013; Khan, 2016; Polturak & Aharoni, 2018).

Furthermore, with the averages obtained in the CATA test, the principal components were analyzed (Figure 8a), highlighting the main characteristics attributed to each formulation. In the analysis of the main compounds, the influence and distribution of the terms cited by the tasters as characteristics present in the samples were evaluated, with 95.01% of the variation between the data, being 86.1% in $\mathbf{F_1}$ and 9% in $\mathbf{F_2}$, how much a certain characteristic was perceived or not about the others.

Figure 8. Principal Component Analysis (PCA) for CATA (Check-All-That-Apply) and ideal attributes, (b) – Average impact of the main sensory profiles evaluated in the samples, (c) Quantification of the intensity of the descriptors (RATA) and evaluation of the elicitation difference: (d) STPAp x Ideal Product, (e) STPBp x Ideal Product, (f) STPCp x Ideal Product, (g) STPDp x Ideal Product



Fonte: Author (2025)

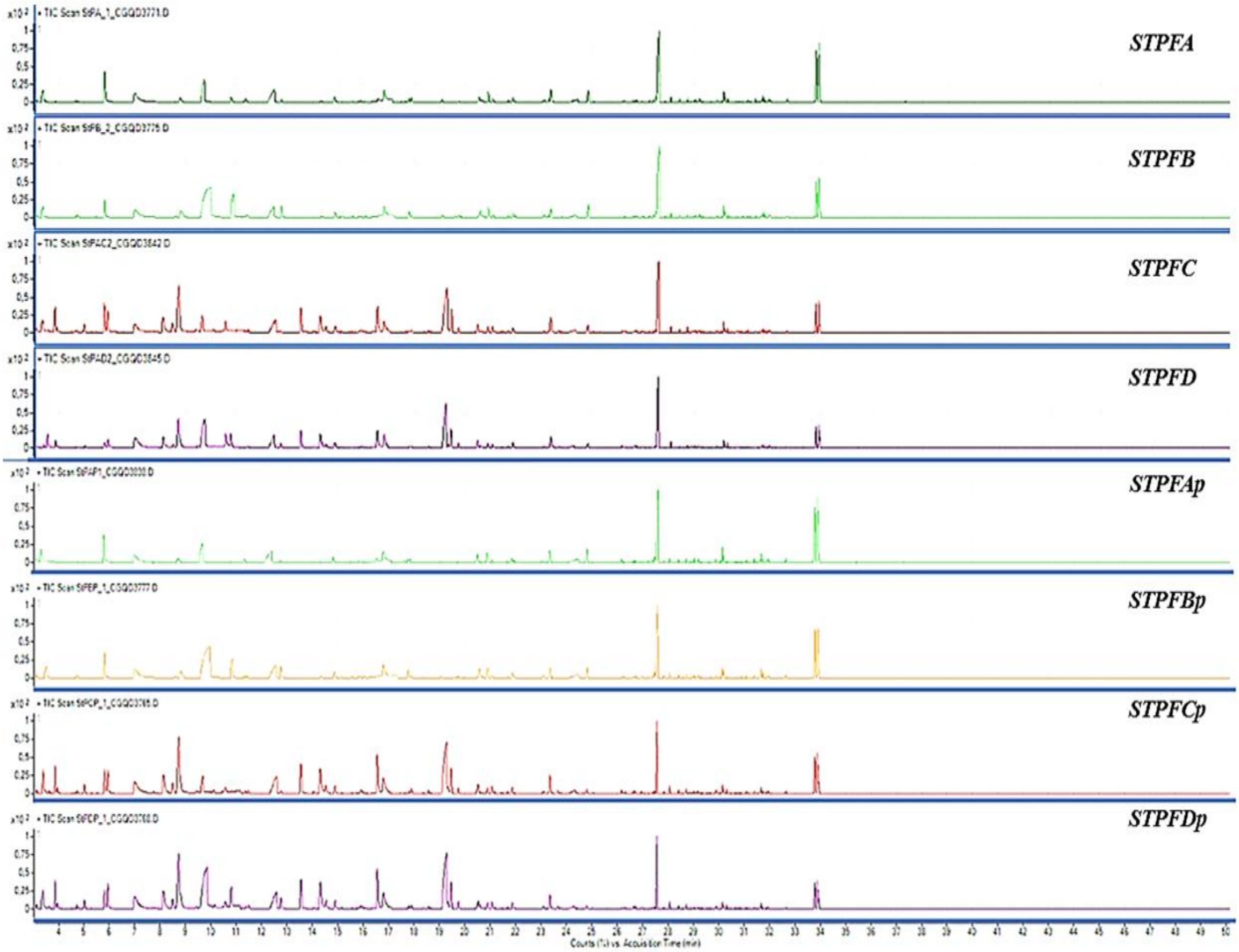
It is important to highlight that there was an association between the STPFCp and STPFDp samples about the descriptors in F_1, including *cashew apple flavor, fruit flavor, sweet flavor, and fruit aroma*, which were also presented in Graph 4b, in positive values, the average impact of the main sensory profiles demonstrating the expected attributes for the juice samples. To reinforce these results, the profile of volatile compounds was determined (see Figure 9), in which the difference between the peaks in the samples with cashew apple is also observed. In addition, the report by Santos *et al.* (2020) describes 80 different volatile metabolites present in the red pulp of pitaya, including alcohols and esters, with the presence of fruity odor as descriptor. In addition, it is possible to infer the influence of cashew apple juice in improving consumer sensory acceptance, confirming the results of the hedonic tests reported in the previous section. Meanwhile, the characteristics described –*green leaf aroma, no aroma, pitaya aroma, heterogeneous, root flavor,* and *presence of particles* –represent the negative mean increase (red bar), showing sensory attributes that untrained consumers do not expect of the juice samples. The positive or negative terms that most characterize the samples must be considered when preparing the product to optimize it in the future.

Regarding the analysis of the averages obtained in the RATA test (Figure 8[c]), in the evaluation of the intensity for the sensor descriptors, we can observe that the four formulations obtained averages without significant difference for the attributes studied: *purple color, bright, fermented, acidic aroma, astringent aroma, pitaya aroma, acidic taste, astringent flavor, pitaya flavor,* and *fermented flavor*. The other 19 items showed significant differences. However, it is important to emphasize that the descriptors present in the STPFCp and STPFDp samples present a certain similarity in the results, justifying the use of the RATA test since Giancalone and Hedelund (2016) state that this test is more indicated when the difference between the samples is more subtle. The data presented specificity and consistency in the transparency between the terms to establish the relationship between the means of the sensory descriptors and the samples with better discriminative capacity for similar perceptions (Ares, 2014).

Then, a comparison of the elicitation rates of each product and the ideal product was performed. This analysis was essential to elucidate which attributes are within the expected limits for the ideal product, and it was possible to infer that the samples presented the following behaviors: STPFAp reported the attributes no aroma, astringent aroma and green leaf aroma; STPFBp was described as *homogeneous characteristics, cashew apple aroma, presence of particle, no aroma, acidic aroma, purple color, green leaf aroma, astringent aroma*; STPFCp presented only two attributes that were outside the limits: *purple color and astringent aroma.*

Similar behavior was observed in the STPFDp sample, where only one attribute was outside the ideal astringent aroma. See figures 8 (d), 8 (e), 8 (f) and 8 (g). Therefore, it can be inferred that the use of cashew apple stalk juice improves sensory acceptance by the consumer, mitigating the attributes previously reported with negative means of delivering products to consumers with profiles close to ideal (see Figure 8 [b]). That confirms the information previously obtained during the focus group related to sensory acceptance.

Figure 9. Representative chromatograms of volatile compounds. The formulations before pasteurization: STPFA, STPFB, STPFC, STPFD and after pasteurization: STPFAp, STPFBp, STPFCp, STPFDp profiles.



Fonte: Author (2025)

3.5.3 *Affective tests*

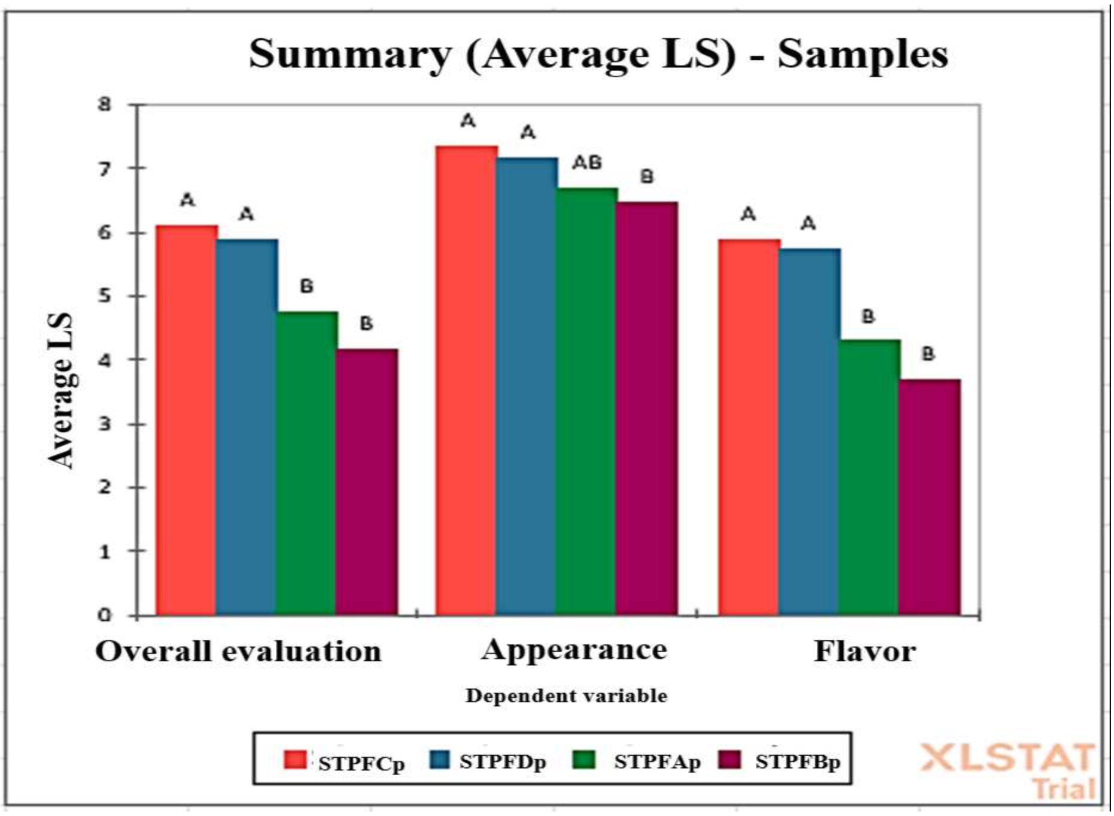
The culture of pitaya and cashew apple consumption has been reported in the northeast region (Abreu *et al.*, 2013, Hua *et al.*, 2018). This characteristic can be seen as positive by consumers, as reported by Włodarska *et al.* (2019) who observed that consumers may have a greater preference for locally produced foods. The data in Figure 2, section 2.6, confirm this profile of the participating consumers. In the apparent attribute for all products, the averages were above 6.0, falling within the accessibility zone that varies from 6.0 (I Like slightly) to 9.0 (I liked it extremely), with greater emphasis on the STPFCp and STPFDp samples, which had averages of 7.3 and 7.1, respectively (Figure 10).

These evaluations confirm that pitaya with its red-purple coloration presented good accessibility for consumers. Lima *et al.* (2020), when evaluating pitaya dye, found an improvement in color without altering the sensory characteristics of yogurts. A similar behavior was observed when evaluating the average flavor scores in the STPFCp and STPFDp samples. Meanwhile, the STPFAp and STPFBp samples obtained averages below the accessible sensory zone. The STPFCp and STPFDp samples did not differ statistically at the 5% level by the Tukey test for the two attributes reported. The sensory results of pitaya juices with cashew apple in the treatments used were presented for the first time in the literature.

In addition, consumer behavior studies are essential to understand what leads to the consumption or non-consumption of a given product and what factors are involved in the process of purchasing a food, since the intention to purchase may increase if a health benefit is expected by the consumer, as in the case of fruit juices (Santos *et al.*, 2018). Thus, when consumers were asked to express their purchase intention, Formulations C and D exhibited over 70% purchase responses in the neutral to positive region ("I have doubts if I would buy" + "probably would" + "certainly would buy"), while Formulations A and B did not reach 50%.

These sensory results are an important indicator for optimizing red pitaya juice formulations. Thus, based on all the results (physicochemical, metabolomic, chemometric and sensorial), the formulations with tropical pitaya juice with cashew apple present interesting metabolic profiles due to the preservation or formation of compounds, such as betalain derivatives, and better sensorial acceptability, which is expected for the food industry.

Figure 10. Overall evaluation, appearance and flavor average (different letters statistically supervised at the 5% level by the Tukey test).



Fonte: Author (2025)

4 Conclusion

The combination of red pitaya pulp with cashew apple juice (tropical red pitaya and cashew apple juice blends) proved to be a promising alternative for adding value to pitaya pulp, a high-cost and chemically unstable product. Sensory results indicated that formulations containing cashew apple (STPFCp and STPFDp) juice obtained interesting acceptance rates, mainly for attributes such as cashew flavor and sweet flavor, and absence of undesirable characteristics, such as particles and vegetal notes. These results are corroborated by CATA and RATA analyses, which demonstrated the positive influence of cashew apple on consumers' sensory perception.

Regarding the chemical identification of metabolites, metabolomic analysis by UPLC-QTOF-MS^E allowed the annotation of 64 non-volatile metabolites, including betalain and flavonoid derivatives, whose profiles were significantly altered by processing, such as pasteurization and addition of ascorbic acid. The chemical markers associated with the most accepted formulations reinforce the importance of the interaction between pitaya and cashew apple in the development of functional and stable juices, aligned with market demands for healthy and sustainable products.

In addition, the study demonstrated that the use of chemometric techniques, such as PCA and OPLS-DA, was effective in discriminating formulations and identifying key compounds responsible for metabolic and sensory differences between treatments. These pioneering results establish a solid scientific basis for the improvement of formulations and industrial processes.

Based on the sensory, chemical and physicochemical analyses performed, it is concluded that tropical red pitaya and cashew apple juice blends have high potential for industrial application, both as a sustainable alternative for the use of cashew apple peduncle and as an innovative product with added functional and sensory value. The findings of this study pave the way for new research aimed at process optimization and increasing the level of technological maturity (TRL), enabling their scalability for large-scale production and serving the global bioeconomy.

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APÊNDICE B - METABOLIC AND CHEMOMETRIC EFFECTS OF RED PITAYA JUICE PROCESSING: A SUSTAINABLE MEMBRANE CLARIFICATION APPROACH

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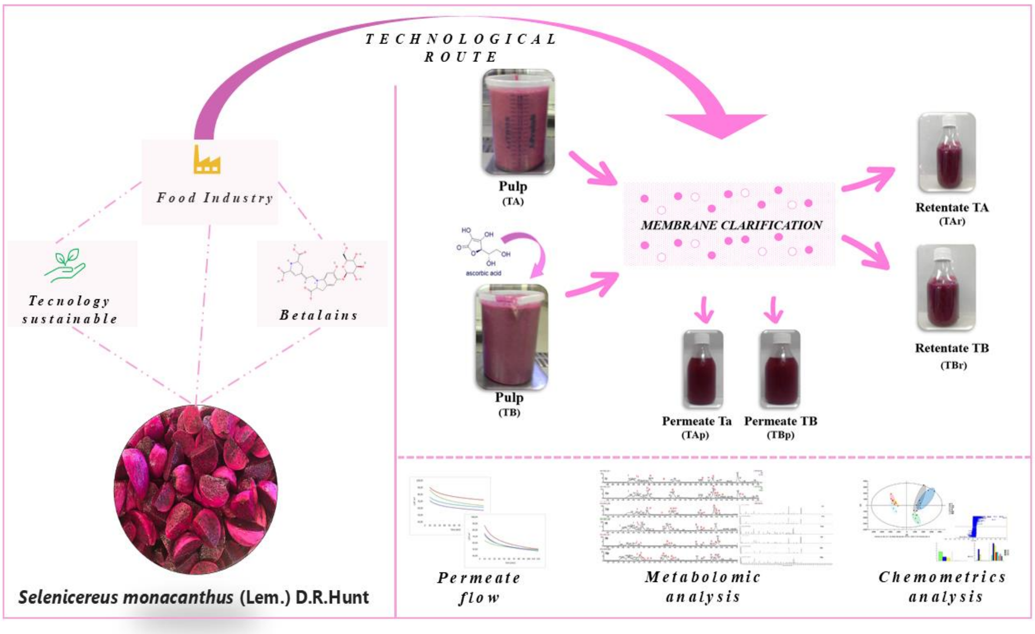
Graphical Abstract



Fonte: Author (2025)

Abstract

Red pitaya (*Selenicereus monacanthus* (Lem.) D.R.Hunt, *Cactaceae* family) is valued by the food industry for its color and potential health benefits. However, given post-harvest challenges, sustainable technologies like membrane clarification are being explored to valorize pitaya pulp. This study evaluates juices clarified by membranes in a closed system from two types of pulping in a pilot plant, and non-enzymatic treatment of red pitaya. Microfiltration proved viable: juice with pulping with peel and addition of ascorbic acid presents an interesting permeate flux (48.12 ± 1.61 L h^{-1} m^{-2}) than manually peeled pulp (64.65 ± 5.5 L h^{-1} m^{-2}) with a lower amount of dispersed particulates. In addition, significant differences were found between the turbidity values of the two clarified samples obtained in the two pulping processes, with better results for the sample originating from the manually peeled pulp process. Additionally, the differences in metabolic fingerprints were analyzed using ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry using elevated energy acquisition mode combined with multivariate statistical modeling for the simultaneous determination of 45 metabolites, including retained carbohydrates and novel betalain isomers. This work highlights membrane clarification as a sustainable route for producing natural colorants with functional potential.

Keywords: *microfiltered juices, red pitaya, clarification, multivariate analysis, permeate flow.*

1 Introduction

Red pitaya (*Selenicereus monacanthus* (Lem.) D.R.Hunt, *Cactaceae* family) is a fruit that has attracted the attention of the food industry due to its characteristics related to color, bioeconomy, potential health benefits, especially applications related to anxiolytic activity, cholesterol control and diabetes treatment (Holanda *et al.*, 2021; Lira *et al.*, 2023; Marques *et al.*, 2023). Red pitaya is rich in moisture and carbohydrates, containing prebiotic oligosaccharides and antioxidant betalains, and presenting significant prospects for the sustainable development of food products (Nabi *et al.*, 2023).

The application of technology after fruit harvesting has been a relevant topic in the global scenario. The FAO (*Food and Agriculture Organization of the United Nations*) reported a serious difficulty in the supply chain, in estimated at 13% of global food is post-harvest losses (FAO, 2022). In addition, enzymatic degradation during processing challenges its utilization, however, technological alternatives based on metabolic chemometrics can enhance chemical stability (Santos *et al.*, 2024).

Recent reports investigate the use of pitaya (*Hylocereus spp.*) pulp, peel, and seed in food technology, including as colorants in confectionery, yogurt, baked goods, and packaging (Huang *et al.*, 2021; Silva *et al.*, 2024). In this scenario, new sustainable technology routes are designed for juice manufacturing, among them clarification using membranes, which is an alternative to thermal methods, preserving heat-sensitive pigments while reducing turbidity (Vergara *et al.,* 2015; Urošević *et al.*, 2017; Servent *et al.*, 2020).

The use of enzymatic treatment combined with membrane clarification has been used in the manufacture of fruit juices (Satyannarayana, Kumar, 2023; Escobar *et al.*, 2024) A greater enzyme concentration, incubation time and temperature are reported to reduce turbidity, but may be related to color changes, including in the case of pitaya (Nur'aliaa *et al.*, 2011; Pui & Abdul Kalam Saleena, 2023, Kallel *et al.*, 2023). However, metabolic and chemometric insights into membrane-clarified cactus juices remain limited, particularly regarding non-enzymatic processing effects on betalain stability.

Therefore, this study will present the application of red pitaya microfiltration of different processes, highlighting potential markers in the analysis of non-volatile components using metabolomic and chemometric data, adding value to the raw material, aiming at sustainable development and filling an important gap in literature.

2 Material and methods

2.1 Raw material

Fresh red pitaya fruits (*Selenicereus monacanthus*) at commercial maturity (uniform size, 300–400 g, intact peel) were sourced from Jaguaribe Valley, Brazil (5° 53′ 27″ S, 38° 37′ 19″ W). Fruits were sanitized in 200 ppm sodium hypochlorite for 15 min, rinsed with distilled water, manually peeled (TA) or unpeeled (TB), and pulped using a pilot-scale (ITAMETAL/BONINA 0,25 DF). Pulp was stored at −18 °C and thawed at 5 °C for 24 h before use.

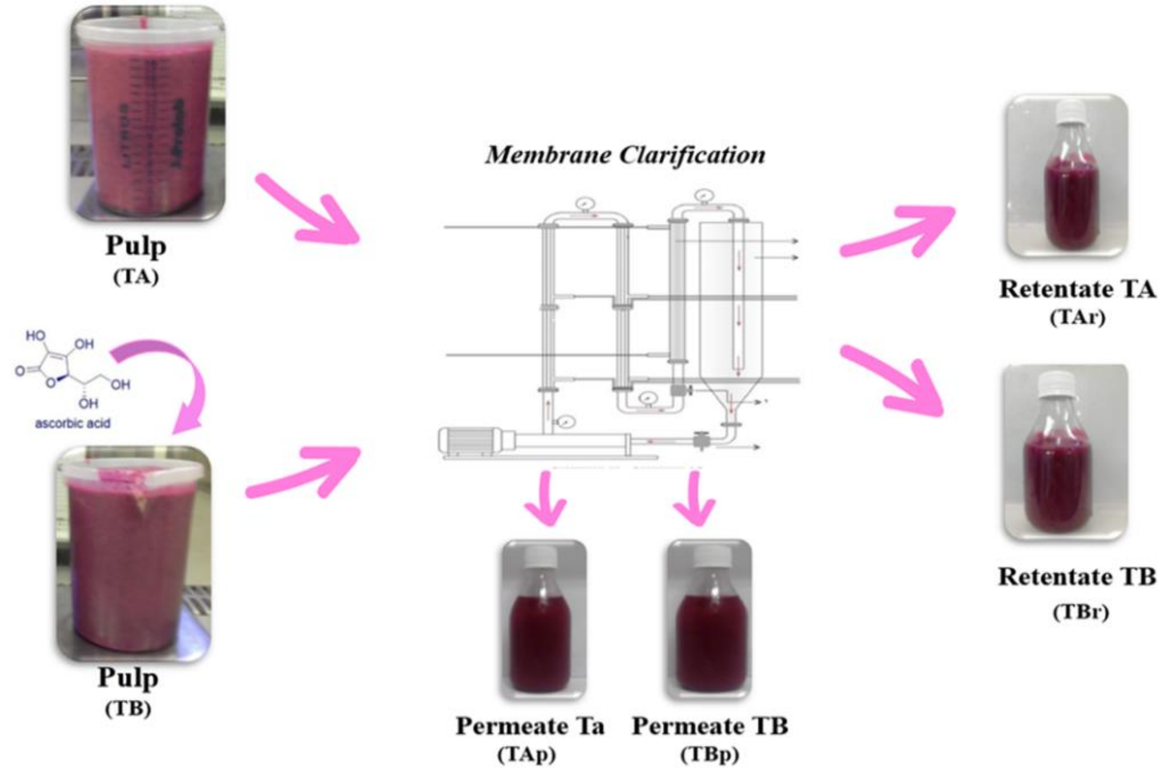
2.2 Membrane microfiltration process to obtain clarified juice

Microfiltration was performed using a pilot-scale unit (Pall Corporation, USA) with four tubular ceramic membranes (Membralox® T1-70, 0.2 µm pore size, 0.0055 m² area each). The system operated at 3.0 bar transmembrane pressure (TMP), 40 °C, and cross-flow velocity of 2 m/s for 120 min in total recycle mode. The definition of all stages of the system was carried out following the method of Silva *et al.* (2018) and Lima *et al.* (2020). Permeate and retentate were collected separately. Additionally, permeate flux was calculated as described by Ghosh *et al.* (2017):

Eq (1): $J = VP\ /\ t{\times}A$ where, J = permeate flow ($L.h^{-1}.m^{-2}$) VP = permeate volume (L) t = time (h) A = membrane area (m²).

For the pulping, the process used was described by Santos et al (2024). For this, two treatments were carried out, as follows: Treatment A (TA), in which the fruits were cut into quarters, the peels were removed manually before the pulping process and were strained through a 0.8 mm sieve generating the fractions after the clarification process: Retentate (TAr) and permeate (TAp); and Treatment B (TB), in which whole fruits were produced directly in the pulping process, at a dose of 500 mg kg^{-1} of ascorbic acid, to avoid oxidative processes, and the fruits were pulped on a 2.5 and 0.8 mm sieve generating the fractions after the clarification process (Retentate, TBr; and Permeate, TBp) (see Figure 1).

Figure 1. Red pitaya pulp microfiltration system*.



Fonte: Author (2025)

*Treatment A (TA), in which the fruit was cut into quarters, the skins were manually removed before the pulping process, and they were strained through a 0.8 mm sieve generating the fractions after the clarification process: retentate (TAr) and permeate (TAp). In treatment B (TB), in which whole fruits were produced directly in the pulping process, at a dose of 500 mg/kg of ascorbic acid, to avoid oxidative processes, and the fruits were pulping in a 2.5 and 0, 8 mm sieve, generating the fractions after the clarification process: retentate (TBr) and permeate (TBp).

2.3 Physical-chemical analysis

The physicochemical analysis was determined in the following samples: TA, TAr, TAp, TB, TBr and TBp. Measured using a calibrated pH meter (Mettler Toledo FE28, Switzerland) following AOAC 981.12." - Titratable Acidity: "Expressed as % malic acid, determined by titration with 0.1 N NaOH to pH 8.1 (AOAC 942.15). - Soluble Solids: Measured via digital refractometer (Atago PAL-3, Japan) calibrated with distilled water." - Color (CIE L*a*b*): "Analyzed using a colorimeter (Konica Minolta CR-400, Japan) with D65 illuminant and 10° observer. In addition, color differences (ΔE) were calculated using Eq. (2), according to Mokrzycki and Tatol (2011):

Eq (2): $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$

2.4 Analysis of the chemical profiles of the extracts by UPLC-QTOF-MS^E

2.4.1 Extraction procedure for analysis by UPLC-QTOF-MS^E and Chromatographic conditions

The samples obtained were freeze-dried (LIOTOP LP 510), stored in a freezer at -18°C and were extracted in biological quintuplets. The extracts were prepared from liquid-liquid extraction, following the modifications made by Chagas-Paula *et al.* (2015); Nehme *et al.* (2008); Santos *et al.* (2024). Freeze-dried samples (50 mg) were defatted with 4 mL hexane (95% purity) via vortexing (1 min) and sonication (20 min, 135 W). Polar compounds were extracted with 4 mL ethanol/water (70:30, v/v) under the same conditions. After centrifugation (3,000 × g, 10 min), the supernatant was filtered (0.22 µm PTFE) and stored at −80 °C until UPLC-QTOF-MS^E analysis.

The extracts were analyzed using the Acquity UPLC system (Waters, Milford, MA, USA) coupled to the Quadrupole/TOF system (QTOF, Waters, Milford, MA, USA). Samples were analyzed on a Waters Acquity UPLC BEH C18 column (150 × 2.1 mm, 1.7 µm) at 40 °C. Mobile phase: (A) 0.1% formic acid in water, (B) 0.1% formic acid in acetonitrile. Gradient: 2% B (0–1 min), 2–95% B (1–15 min), 95% B (15–17 min), 2% B (17.1–19 min). Flow rate: 0.4 $mL \cdot min^{-1}$; injection volume: 5 µL. The solvents employed were from the LiChrosolv® line of Sigma-Aldrich Chemical Company (Oakville, ON, Canada). For all analyses, high-purity Milli-Q water (Billerica, MA, USA) was used. Genistein purchased from Sigma Aldrich was used as an internal standard. The other reagents used (hexane, ethanol and acetonitrile) were from analytical grade.

The chemical profiles of the pulps (TA and TB), retentates (TAr and TBr) and permeates (TAp and TBp) for all treatments were determined by coupling the Waters ACQUITY UPLC system and a QTOF mass spectrometer (Waters, Milford, MA, USA) in positive ionization mode with the electrospray ionization (ESI) interface. ESI^+ mode; capillary voltage: 3 kV; source temperature: 120 °C; desolvation gas: 350 $L \cdot h^{-1}$ (N_2); Data acquired in MS^E mode (20–40 eV) over m/z 50–1180. The instrument was controlled by MassLynx 4.1 software (Waters Corporation, USA).

2.4.2 Metabolite identification with UPLC-QTOF-MS^E

To annotate the metabolites, the dataset obtained from UPLC-QTOF-MS^E was imported into the Mass Spectrometry - Data Independent AnaLysis (MS-DIAL 3.82) software. Therefore, spectral deconvolution, alignment, and peak filtering were performed (Lai *et al.*, 2018, Tsugawa *et al.*, 2015, Tsugawa *et al.*, 2019). Subsequently, the unknown metabolites can be shown by their elemental formulas and in silico mass spectral fragmentation with MS-FINDER 4.24 (Lai *et al.*, 2018, Tsugawa *et al.*, 2015). Structural elucidation and metabolite identification were based on molecular formula (MS) and fragmentation (MS/MS) with heuristic rules activated (Kind & Fiehn, 2007; Sumner *et al.*, 2007). Metabolite annotation was performed by comparing the data with information from databases such as the KNApSAcK Core System, PubChem, and ChemSpider databases. Following the parameters of the Metabolic Standards Initiative (MS) level 2.1 (Sumner *et al.*, 2007), presenting a presumed identification, including molecular formula and fragment ions. In addition, it is important to mention that the annotation of metabolites was based on chemotaxonomy (family, genus, and species).

2.5 Volatile metabolic composition

The volatile metabolic composition of each sample (TA, TAr, TAp, TB, TBr and TBp) was determined according to the method described by Santos *et al.* (2020) and Lima *et al.* (2020). For this, 1.24 g of NaCl were used added to 4g of each sample material. The volatile compounds were then isolated from the matrix by headspace solid phase microextraction (HS-

SPME) using DVB/CAR/PDMS 50 30µ fiber / µm^2 cm (Supelco, Bellefonte, PA, USA). Before analysis, the fiber was conditioned according to the temperature recommended by the manufacturer. GC-MS analysis was performed using chromatography (AGILENT TECHNOLOGIES, Inc., Palo Alto, CA, EUA) equipped with amass spectrometer (model 5977A). The chromatographic column used was the RTX 5 (size 30 m, internal diameter 0,25 mm, film thickness, 0,25 µm). The temperature program used was: 4°C/min ramp from 40 °C to 110 °C, 16 °C/min to 200 °C, remaining for 10 min and 7 °C/min at 230 °C. The interface temperature between the chromatograph and the mass selective detector was °C. ONE Ionization was carried out by electronic impact, 70 eV, with an ion source maintained at 150 °C.

2.6 Chemometric data analysis using UPLC-QTOF-MS^E

Raw UPLC-MS data were processed in MarkerLynx XS (Waters, v4.1) with noise threshold = 50, mass tolerance = 0.01 Da, and t_R window = 0.2 min. Peaks were aligned, normalized to total intensity, and Pareto-scaled. PCA and OPLS-DA models were built in SIMCA-P (v16.0, Umetrics) with 7-fold cross-validation. Model validity was assessed via permutation testing (n = 100). VIP scores >1.0 and $p < 0.05$ (ANOVA) defined significant markers.

2.7 Statistical Analysis

Data were tested for normality (Shapiro-Wilk) and homoscedasticity (Levene's test) before applying for Tukey's HSD test ($p < 0.05$) in AgroEstat 1.0 (Barbosa and Maldonado, 2010). Non-normal data were log-transformed. Outliers (>3 SD) were excluded. In addition, the peak areas of volatile compounds obtained from their chromatograms were normalized by the treated sum and scaled accordingly with autoscaling.

3 Results and discussion

3.1 Membrane microfiltration processes

The pitaya pulp from treatments A (TA) and B (TB) subjected to the microfiltration process, in a closed system for 120 min, presented the following averages of the duplicates of the four permeate flux membranes: 64.65 ± 5.5 L h^{-1} m^{-2} and 48.12 ± 1.61 L h^{-1} m^{-2}, respectively (see Figure 2). Some characteristic phenomena of microfiltration are reported in juice production by Urošević *et al.* (2017) Bevilacqua *et al.* (2018), Lu, Bao & Huang, (2021), Satyannarayana, Kumar, (2023) and observed, in Figure 2, that Initially temporal polarization, in which there is a sharp decline in permeate flux, in both red pitaya fruit treatments (Treatment A and Treatment B). This decline can be directly related to the concentration of solids accumulated in the membrane during clarification, due to the difference in concentration between the permeate and the retentate. Then, fouling is observed through precipitation processes, in which the retained solutes accumulate near the membrane surface, creating a high concentration layer that increases the resistance to tangential flow. Finally, the pseudostationary stage was not completely reached in the period of the evaluation because there was no total obstruction of the membrane filtration capacity.

The TBp sample, which is obtained using pitaya with peel added with ascorbic acid during pulping, presents a higher yield in the pulping process and an interesting microfiltration profile compared to TAp, since no combined sample clarification process was performed. In addition, a greater decrease in the permeate flux was observed, since there is a higher concentration of macromolecules on the membrane surface originating from the fruit cell wall, phenolic compounds, insoluble pectins, cellulose and hemicelluloses and particulate matter. This membrane fouling profile by macromolecules were also reported by Ismail *et al.* (2012), Bevilacqua *et al.* (2018), Escobar *et al.* (2024) and Silva *et al.* (2024).

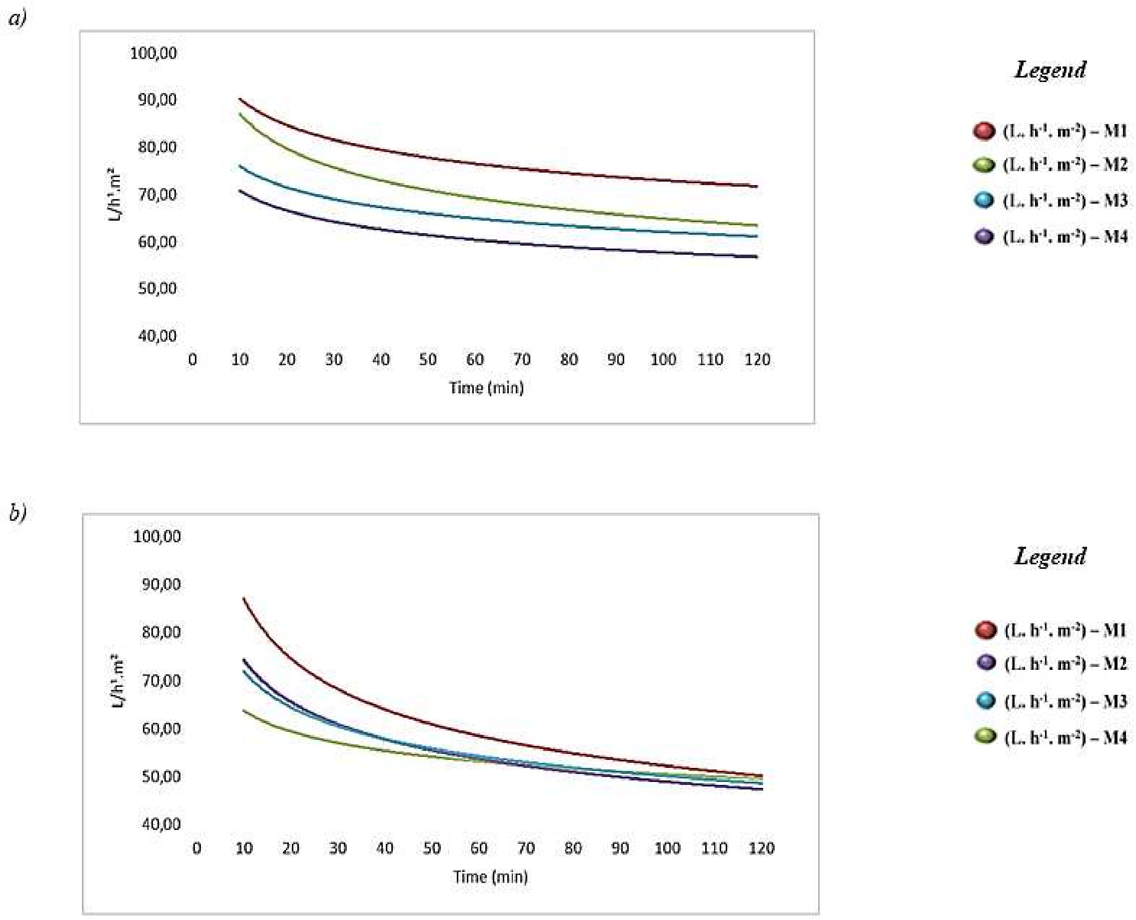
Data in the literature on microfiltration of pitaya are limited. Our research group report a permeate membrane flux of 57.6 ± 1.9 L h^{-1} m^{-2} for pitaya clarification, using a process which consists in applied enzymatic treatment (Pectinex® Ultra AFP, 2000 mg L^{-1}, treated at 40 °C, for 45 min at 2,5 Hz) prior the microfiltration, and using pitaya without the peel after its manual separation. Meanwhile, Silva *et al.* (2024) evaluated two pulping processes to elucidate the permeate flux and fouling in an aluminum oxide membrane. It was observed that traditional processing (manual peel separation) with enzymatic treatment (Pectinex Ultra AFP (1000 mg

L, treated at 40°C for 45 min at 2,5 Hz) obtained a better permeate flux performance (27% lower permeate flux compared to the treatments 'pitaya pulp without peel' and 'pitaya pulp with peel').

Although enzymatic treatment is recognized as important for optimizing the microfiltration process as it generates greater reproducibility in the process of obtaining extracts through the breaking of cellulose, pectin or polysaccharide bonds and the consequent release of compounds present in plant cells (Servent *et al.*, 2020, Escobar *et al.*, 2024), some chemical changes can occur (Pui & Abdul Kalam Saleena, 2023, Kallel *et al*., 2023). Particularly for pitaya, Nur‘aliaa *et al.* (2008) observed a negative effect on the luminosity value (L*) in white pitaya juice (*Hylocereus undatus*) microfiltered, previously treated with Pectinex Ultra SP-L enzyme, varying for 20–100 min, 30–50°C and enzyme concentrations (0.01–0.1%). The authors show a negative and significant ($p < 0.01$) effect for the temperature used. In a later report, Nur‘aliaa *et al.* (2011), the effects of Pectinex Ultra SPL and Pectinex CLEAR enzymes were compared, and different amounts of carbohydrates and polyphenols were found in red pitaya fruit juice. This may be related to the action of pectinases on the walls cells and the pectin degradation mechanism depends on the composition of the substrate.

In addition, Schweiggert et al. (2009) also reported changes in the pattern of chemical compounds responsible for coloring, during enzymatic treatment combined with liquefaction (7.9 °C, 3 days, 0.92% (w/w) ascorbic acid, 1% (v/w) pectinase 62 L) for the production of coloring food from pitaya (Hylocereus sp.), as several pigments were hydrolyzed. This reinforces the importance of understanding in the following sections the physicochemical, chemical, metabolic and chemometric characteristics of microfiltration samples non-enzymatic processing effects on betalain stability.

Figure 2. Average permeates flux density curves, 40°C, Average pressure: 3 bar, at the four transmembrane pressures studies referring to each membrane, (Ptm_1, Ptm_2, Ptm_3 and Ptm_4): (a) TAp – Treatment A and (b)TBp – Treatment B.



Fonte: Author (2025).

3.2 Physical-chemical and chemical results

The different chemical and physical-chemical compositions resulting from the different 6 samples from the pitaya red fruit clarification process are presented in Table 1. The data were submitted to statistics to assess whether there is a significant difference between the samples with 5% significance for the Tukey test.

The analyses of the soluble solids indicated significant differences between the TA and TB pulps, TAp and TBp permeates and TAr and TBr retentates. In addition, the final products from treatments A and B presented low pH and acidity values. In previous research, treatments applied with the addition of acidulants in the pitaya pulping stage were related to low pH values, acidity and greater stability of betacyanins (Leong *et al.*, 2018). Our research group in a previous report also found that the addition of ascorbic acid to pitaya pulp with peel samples provided greater chemical and enzymatic stability associated with low pH and acidity values (Santos *et al.*, 2020) and similar values were also presented of pitaya juice and microfiltered by Wong & Siow, (2014) and Lima *et al.* (2020), respectively.

Previously, the literature reported that different types of pulping processes produce different color profiles, since the inner part of the peel also contains organic compounds derived from betalains (Martins *et al.*, 2024, Santos *et al.*, 2024). The whole pitaya juice has high turbidity and viscosity, but consumers prefer clarified products (Nur'aliaa *et al.*, 2008; Nur'aliaa *et al.*, 2011), in table 1, the addition of the membrane clarification process in the different pulping processes were effective in reducing the turbidity of the permeate samples, TAp and TBp.

In addition, high L* values, which was confirmed in this study, in which the Luminosity values of the permeate samples (TAp and TBP) were higher than those of the pulp samples (TA and TB) and retentate samples (TAr and TBr), confirming the efficiency of the clarification process. However, there was a significant difference between the permeate fractions (TAp and TBP) in relation to the b* parameter, yellow/blue coordinate. Meanwhile, the retentate samples (TAr and TBr) in the membranes showed a significant difference in the L* a* b* parameters, confirming the different fouling profiles in the membranes. The color distinction (ΔE) was calculated by numerically comparing the TA and TB fractions and the retained and permeate fractions of each process, respectively. The total difference in color in the permeate samples exceeded “five”, with similar values being obtained by Lima *et al.* (2020) in the pitaya red food colorant, indicating that the color difference was noticeable to the human eye (Mokrzycki & Tatol, 2011).

However, when evaluating the retained samples (TAr and TBr), values lower than “five” were obtained. These results reaffirmed the importance of understanding the metabolic profile to understand the main organic compounds present in the samples responsible for the characteristic coloration. This information is described in 3.3 item.

Table 1. Effects of processing on the physical-chemical and chemical characteristics of pitaya pulps (TA and TB), retentate (TAr and TBr) and permeates (Tap and TBp) relative to samples from each treatment subjected to the microfiltration process.

Análises*	***TA***	***TAr***	***TAp***	***TB***	***TBr***	***TBp***
Soluble solids (°brix)	9.96 ± 0.06[b]	9.83 ± 0.00[c]	9.97 ± 0.06[b]	8.87 ± 0.06[d]	8.5 ± 0.00[e]	10.9 ± 0.00[a]
pH	4.88 ± 0.02[a]	4.54 ± 0.02[b]	4.55 ± 0.13[b]	4.47 ± 0.01[b]	4.3 ± 0.01[c]	4.38 ± 0.01[bc]
Acidity (%acid malic)	0.33 ± 0.03[b]	0.35 ± 0.03[ab]	0.26 ± 0.05[bc]	0.34 ± 0.00[ab]	0.35 ± 0.04[a]	0.24 ± 0.01[c]
Turbidez	>1000 ± 0.00[a]	>1000 ± 0.00[a]	10.5 ± 0.20[b]	>1000 ± 0.0[a]	>1000 ± 0.00[a]	14.47 ± 0.32[c]
Color	L* 28.7 ± 0.04[c]	L* 29.43 ± 0.07[b]	L* 38.96 ± 0.25[a]	L* 27.73 ± 0.02[d]	L* 28.44 ± 0.05[c]	L* 38.56 ± 0.45[a]
	a* 23.8 ± 0.03[bc]	a* 24.97 ± 0.02[b]	a* 50.58 ± 0.53[a]	a* 21.37 ± 0.09[c]	a* 17.78 ± 0.08[d]	a* 50.8 ± 0.93[a]
	b* 4.19 ± 0.00[c]	b* 4.69 ± 0.03[c]	b* 24.26 ± 0.68[a]	b* 3.26 ± 0.06[d]	b* 2.74 ± 0.02[d]	b* 23.03 ± 0.13[b]
	-	ΔE 1.47	ΔE 35.00	-	ΔE 3.70	ΔE 37.07

*Treatment A (TA), in which the fruits were cut into quarters, the skins were manually removed before the pulping process, and they were strained through a 0.8 mm sieve generating the fractions after the clarification process: retentate (TAr) and permeate (TAp). In treatment B (TB), in which whole fruits were produced directly in the pulping process, at a dose of 500 mg/kg of ascorbic acid, to avoid oxidative processes, and the fruits were cooked in a 2.5 and 0 sieve, 8 mm generating the fractions after the clarification process: retentate (TBr) and permeate (TBp). Means with the same letters, on the same line, do not vary at the 5% significance level for the Tukey test.

Fonte: Author (2025)

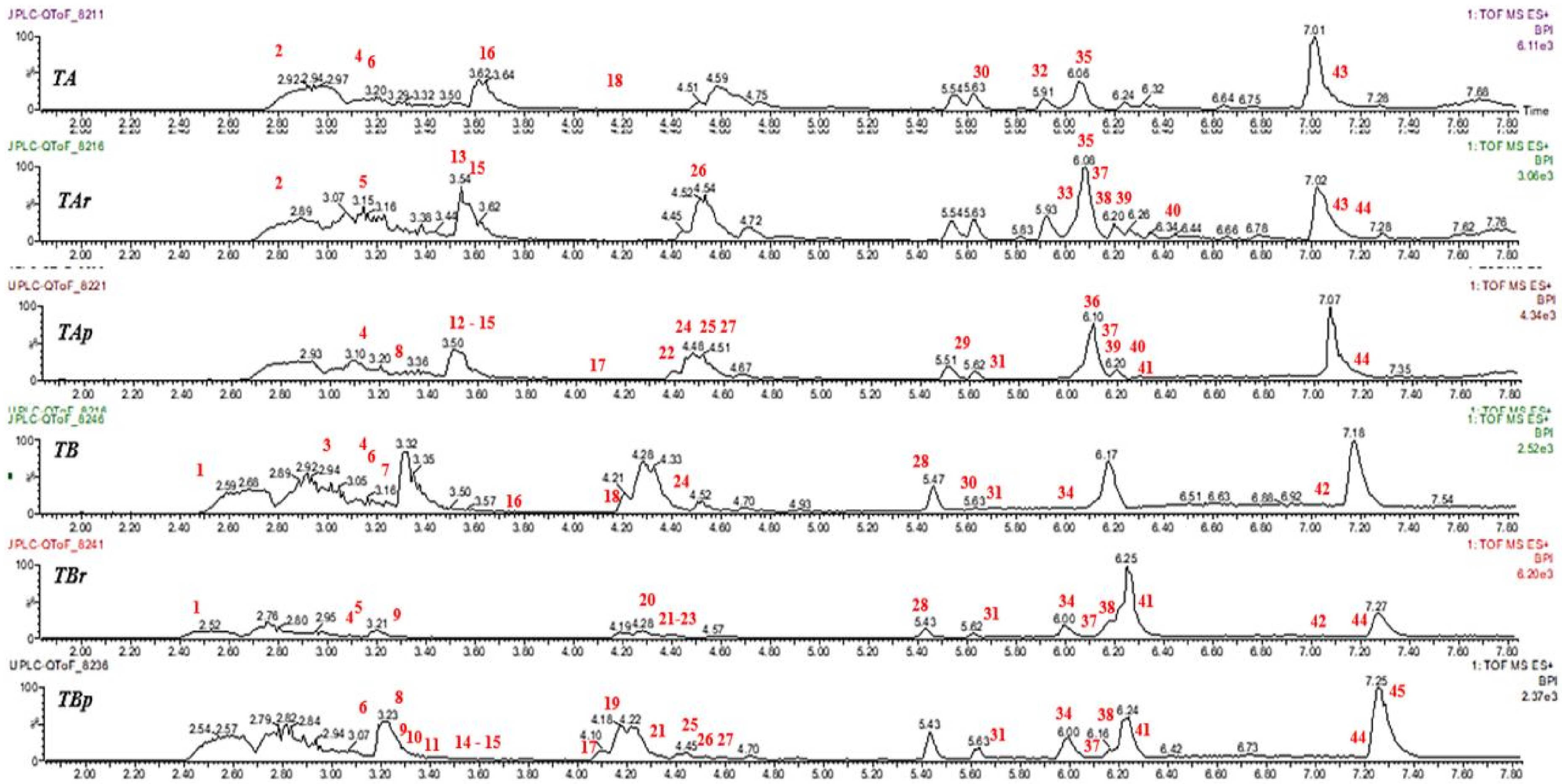
3.3 Metabolites annotation in the extracts

Hydroethanolic extracts of pitaya samples were obtained from the pulp of 6 samples, permeate and retentate from each pitaya treatment. The extractions were performed in biological quintuplicates. Subsequently, the extracts were analyzed by UPLC-QTOF-MS^E, with electrospray ionization operating in positive mode, ESI^+. In Figure 3, it is possible to observe the chromatogram representing the extract analyses. The metabolite annotation was designed through studies of fragmentation mechanisms and consultation of the SciFinder, ChemSpider and PubChem databases. The detected peaks and the metabolite annotation are summarized in Table 2. In general, it is important to mention that in recent years there have been significant advances in the identification and characterization of pitaya, as highlighted by Santos *et al.* (2024), Lira *et al.* (2020) and Lima *et al.* (2020).

These publications were developed to facilitate the identification of the compounds present in this fruit. However, even with the accumulated knowledge, the research still required an exploratory approach to identify the mass compounds (MS^2) to identify the compounds present in the retentate (TAr and TBr) and in the permeate fraction of pitaya (TAp and TBp), a novelty that is being presented for the first time. For accurate identification, several parameters were considered, including the molecular ion in positive mode, isotopic pattern, mass accuracy below 5 ppm and the fragmentation patterns known and documented in the literature. Table 2 provides a concise summary of the UPLC-QTOF-MS^E chromatographic and MS data, which includes information such as retention time, theoretical mass, molecular formula, observed mass (MS), as well as fragments ions (MS/MS).

Therefore, the annotation of the compounds was conceived through the correlation of the precursor ions (protonated ions) observed in the MS spectra with their respective product ions (fragment ions) observed in the MS/MS spectra.

Figure 3. Representative chromatograms of the six different materials from pitaya: pulps (TA and TB), retentate (TAr and TBr) and permeates (Tap and TBp) in positive ionization mode *(*ESI$^+$*).*



Fonte: Author (2025)

Table 2. Metabolites annotation UPLC-Q-TOF-MS^E chromatographic and mass spectrometry data of pitaya microfiltrate in positive mode ion*.

n°	t_R *(min)*	*Adduct*	*m/z (MS)* Observed	*m/z (MS)* Calculated	*Mass Error (ppm)*	*Fragment ions (MS/MS)*	*Empirical Formula*	*Metabolite annotation*	*pitaya microfiltrate* TA	TAr	TAp	TB	TBr	TBp	*Referênces*
1	2.54	$[M+H]^+$	752.8668	-	-	640.7951 524.4376 410.5431 296.5539	-	unknown	-	-	-	✓	✓	-	-
2	2.83	$[M+H]^+$	752.8668	-	-	640.8001 524.4383 410.5444 296.5501	-	unknown	✓	✓	-	-	-	-	-
3	3.06	$[M+H]^+$	683.1470	683.1460	1.50	551.1429 389.1000	$C_{29}H_{31}N_2O_{19}^+$	2'-O-Apiosyl-betanin	-	-	-	✓	-	-	(Wybraniec *et al.*, 2009; Szot, 2015; Santos *et al.*, 2024)

4	3.14	[M+(K +H_2O) +H]$^+$	399.0892	399.0899	1.75	219.0275	$C_{12}H_{22}O_{11}$	Sucarose	✓	-	✓	✓	✓	-	(Hua *et al.*, 2018; Wei *et al.*, 2019; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)
5	3.15	[M+K]$^+$	219.0267	219.0270	1.37	-	$C_6H_{12}O_6$	Glucose	-	✓	-	-	✓	-	Lima *et al.*, 2020
6	3.19	[M+K+ H]$^+$	705.1872	705.1850	-1.84	543.1473 203.0533	$C_{19}H_{34}O_{16}$	Maltotetraose	✓	-	-	✓	-	-	(Matros *et al.*, 2015; Dasaesamoh *et al.*, 2016; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)
7	3.23	M$^+$	769.1956	769.1956	0.00	683.1955 543.2001 525.2345 381.1988	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers de philocactin	-	-	-	✓	-	-	(Wybraniec *et al.*, 2007, 2010; Fathordoobady *et al.*, 2016;;

															Lira *et al.*, 2020; Santos *et al.*, 2024)
8	3.28	M^+	637.1486	637.1517	-4.87	389.1153	$C_{27}H_{29}N_2O_{16}^+$	isomers 15S/15R phyllocactin (O-malonyl-betanin)	-	-	✓	-	-	✓	(Santos *et al.*, 2024)
9	3.28	$[M+H]^+$	483.2023	483.2037	-2.89	-	$C_{15}H_{35}N_2O_{15}$	2'- apiosyl-15R/15S-isomers de phyllocactin	-	-	-	-	✓	✓	-
10	3.32	M^+	769.1960	769.1956	0.52	683.1955 637.1955 593.1598 551.1500 389.1001	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers de phyllocactin	-	-	-	-	-	✓	(Wybraniec *et al.*, 2007, 2010; Fathordoobady *et al.*, 2016; Lira *et al.*, 2020; Santos *et al.*, 2024)

11	3.39	$[M+H]^+$	705.1926	705.1913	1.80	683.1955 543.2001 525.2345 381.1988	$C_{47}H_{29}O_7$	unknown	-	-	-	-	-	✓	-
12	3.50	M^+	769.1966	769.1956	1.30	683.1955 637.1559 593.1598 552.1500 389.1001	$C_{32}H_{37}N_2O_{20}^+$	2’- apiosyl-15R/15S-isomers de phyllocactin	-	-	✓	-	-	-	(Wybraniec *et al.*, 2007, 2010; Fathordoobady *et al.*, 2016; Lira *et al.*, 2020; Santos *et al.*, 2024)
13	3.54	M^+	769.1949	769.1956	-0.91	683.1955 637.1564 593.1600 551.1486 389.1006	$C_{32}H_{37}N_2O_{20}^+$	2’- apiosyl-15R/15S-isomers de phyllocactin	-	✓	✓	-	-	-	(Wybraniec *et al.*, 2007, 2010; Fathordoobady *et al.*, 2016; Santos *et al.*, 2024 Lira *et al.*, 2020)
14	3.54	$[M+H]^+$	483.2012	483.2037	-5.14	-	$C_{15}H_{35}N_2O_{15}$	unknown	-	-	✓	-	-	✓	-
15	3.55	M^+	637.1495	637.1515	-3.14	389.1031	$C_{27}H_{29}N_2O_{16}^+$	Isomers 15S/15Rphyllocactin (O-	-	✓	✓	-	-	✓	(Santos *et al.*, 2024)

								malonyl-betanin)							
16	3.62	M^+	769.1969	769.1956	1.69	683.1956 637.1570 593.1600 551.1499 389.1009	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers de phyllocactin	✓	-	-	✓	-	-	(Wybraniec *et al.*, 2007, 2010; Lira *et al.*, 2020; Santos *et al.*, 2024)
17	4.10	M^+	551.1500	551.1508	-1.47	389.0999	$C_{24}H_{26}N_2O_{13}$	betanin	-	-	-	✓	-	✓	(Stintzing *et al.*, 2002; Belhadj Slimen *et al.*, 2017; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)
18	4.18	M^+	769.1961	769.1956	1.30	683.1955 637.1559 593.1598 551.1500 389.1001	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers de phyllocactin	✓	-	-	✓	-	-	(Wybraniec *et al.*, 2007, 2010; Lira *et al.*, 2020; Santos *et al.*, 2024)

19	4.25	M^+	637.1486	637.1517	-4.87	389.0934	$C_{27}H_{29}N_2O_{16}^+$	isomers 15S/15R-phyllocactin (O-malonil-betanin)	-	-	-	-	-	✓	(Santos *et al.*, 2024)
20	4.28	M^+	593.1613	593.1618	-0.84	549.1838 389.1005	$C_{26}H_{29}N_2O_{14}^+$	6′-malonyl-2′-descarboxi-betanin	-	-	-	-	✓	-	(Lima *et al.*, 2020)
21	4.29	$[M+H]^+$	483.2017	483.2037	-4.13	-	$C_{15}H_{35}N_2O_{15}$	unknown	-	-	-	-	✓	✓	-
22	4.39	M^+	551.1500	551.1508	-1.47	389.0981	$C_{24}H_{26}N_2O_{13}$	isobetanin	-	-	✓	-	-	-	(Belhadj Slimen *et al.*, 2017; Stintzing *et al.*, 2002)
23	4.40	M^+	551.1500	551.1508	-1.47	389.1011	$C_{24}H_{26}N_2O_{13}$	betanin isomers	-	-	-	-	✓	-	(Stintzing *et al.*, 2002; Lira *et al.*, 2020; Lima *et al.*, 2020; Santos *et al.*, 2024)

24	4.48	M^+	769.1918	769.1956	-4.94	683.2001 637.1604 593.1578 551.1480 389.1013	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers phyllocactin	-	-	✓	✓	-	-	(Wybraniec *et al.*, 2007, 2010; Lira *et al.*, 2020; Santos *et al.*, 2024)
25	4.49	M^+	637.1502	637.1517		389.0899	$C_{27}H_{29}N_2O_{16}^+$	isomers 15S/15R-phyllocactin (O-malonyl-betanin)			✓			✓	(Santos *et al.*, 2024)
26	4.51	M^+	769.1975	769.1956	2.47	683.1960 637.1589 593.1634 551.1467 389.1013	$C_{32}H_{37}N_2O_{20}^+$	2'- apiosyl-15R/15S-isomers de phyllocactin	-	✓	-	-	-	✓	(Wybraniec *et al.*, 2007, 2010; Lira *et al.*, 2020; Santos *et al.*, 2024)
27	4.52	$[M+H]^+$	483.2011	483.2037	5.38	-	$C_{15}H_{35}N_2O_{15}$	unknown	-	-	✓	-	-	✓	-
28	5.47	$[M+H]^+$	427.2696	427.2675	-4.90	271.2433 215.2956 153.5630	$C_{23}H_{39}O_7$	unknown	-	-	-	✓	✓	-	-

29	5.62	$[M+H]^+$	637.1502	637.1502	-2.40	-	$C_{27}H_{29}N_2O_{16}^+$	isomers 15S/15Rphyll ocactin (O-malonyl-betanin)	-	✓	-	-	-	-	(Suh *et al.*, 2014; Wybraniec *et al.*, 2007, 2010; Lira *et al.*, 2020; Santos *et al.*, 2024)
30	5.63	$[M+H]^+$	599.4952	599.4940	2.00	575.5068 274.2797 256.2672	$C_{27}H_{28}N_2O_{16}$	unknown	✓	-	-	✓	-	-	-
31	5.64	$[M+H]^+$	575.5026	575.5039	-2.25	425.2421 318.2078 274.1056	$C_{37}H_{67}O_4$	unknown	-	-	✓	✓	✓	✓	-
32	5.91	$[M+H]^+$	318.2977	318. 2981	-1.30	300.0012 282.1543 270.1491	$C_{14}H_{36}N_7O$	unknown	✓	-	-	-	-	-	-
33	5.92	$[M+H]^+$	489.3362	489.3369	4.70	300.0002 282.1543 270.1491	$C_{33}H_{45}O_3$	unknown	-	✓	-	-	-	-	-

34	6.00	$[M+H]^+$	318.2977	318.2981	-1.30	300.0012 282.1580 270.1499	$C_{14}H_{36}N_7O$	unknown	-	-	-	✓	✓	✓	-
35	6.07	$[M+H]^+$	505.3893	505.3893	0.00	504.2425 459.3022	$C_{31}H_{53}O_5$	unknown	✓	✓	-	-	-	-	-
36	6.10	$[M+H]^+$	459.3266	459.3263	0.7	-	$C_{32}H_{43}O_2$	unknown	-	-	✓	-	-	-	-
37	6.14	$[M+H]^+$	459.3251	459.3263	-2.61	-	$C_{32}H_{43}O_2$	unknown		✓	✓		✓	✓	-
38	6.17	$[M+H]^+$	575.5026	575.5039	-2.25	425.2430 318.1290 274.1234	$C_{37}H_{67}O_4$	unknown	-	✓	-	-	✓	✓	-
39	6.20	$[M+H]^+$	599.3039	599.3039	0.20	459.2409 375.1219	$C_{39}H_{67}O_4$	unknown	-	✓	✓	-	-	-	-
40	6.24	$[M+H]^+$	575.5020	575.5039	-3.30	460.0009 375.1211	$C_{37}H_{67}O_4$	unknown	-	✓	✓	-	-	-	-
41	6.30	$[M+H]^+$	459.3240	459.3263	5.00	-	$C_{32}H_{43}O_2$	unknown			✓		✓	✓	
42	7.01	$[M+H]^+$	473.2552	473.2539	1.30	457.2509 317.2498	$C_{27}H_{37}O_7$	unknown	-	-	-	✓	✓	-	-
43	7.18	$[M+H]^+$	599.3039	599.3039	4.30	459. 2564	$C_{39}H_{67}O_4$	unknown	-	✓	✓	-	-	-	-

44	7.20	$[M+H]^+$	457.2745	457.2761	-3.50	375.2397 -	$C_{19}H_{41}N_2O_{10}$	unknown		✓	✓		✓	✓	-
45	7.25	$[M+H]^+$	577.5197	577.5196	0.20	473.2455 435.2500 457.2511 317.2501	$C_{37}H_{69}O_4$	unknown	-	-	-	-	-	✓	-

* The symbols (✓) or (–), which represent the confirmation or denial of the existence of that compound, were assigned in the Table 2 to each metabolite juice process clarification.

Fonte: Author (2025)

Considering the lack of a discernible correlation between the ions detected in the MS spectra and the ion fragments presented in the MS/MS spectra, it was impossible to identify any of these compounds based on the metabolites described in the various databases consulted in the attempt to identify the following compounds: **1** (t_R = 2.54 min), **2** (t_R = 2.83 min), **9** (t_R = 3.28 min), **11** (t_R = 3.39 min), **14** (t_R = 3.54 min), **21** (t_R = 4.29 min), **27** (t_R = 4.52 min), **28** (t_R = 5.47 min), **31** (t_R = 5.64 min), **32** (t_R = 5.91 min), **33** (t_R = 5.92 min), **34** (t_R = 6.0 min), **35** (t_R = 6.07 min), **36** (t_R = 6.10 min), **37** (t_R = 6.14 min), **38** (t_R = 6.17 min), **39** (t_R = 6.20 min), **40** (t_R = 6.24 min), **41** (t_R = 6.30 min), **42** (t_R = 7.01 min), **43** (t_R = 7.18 min), **44** (t_R = 7.20 min), **45** (t_R = 7.25 min) based on the metabolites described in several consulted databases proved to be unviable.

In Peak **4** (t_R = 3.14 min), the adduct ion $[M + K + (H_2O)]^+$ was observed at *m/z* 399.0892, ($C_{12}H_{22}O_{11}$), which has been tentatively identified as sucrose. In the MS^2 spectrum, typical fragment ions were observed at *m/z* 219.0275 Da $[glucose + K]^+$. Also, this disaccharide was identified in red pitaya extracts, in line with previous studies Hua *et al.* (2018), Wei *et al.* (2019), Lira *et al.* (2020), Lima *et al.* (2020) and Santos *et al.* (2024).

The MS^1 spectrum of compound **5** (t_R = 3.15 min) showed an ion at *m/z* 219.0267 Da, which was tentatively identified as glucose ($C_6H_{12}O_6$, $[M + K]^+$), indicating the formation of a potassium adduct. Moreover, representative MS^1 spectra showing the structure of the base peaks of maltotetraose (peak **6**) with the ion $[M + K + H]^+$ at *m/z* 705.1872. The fragmentation patterns in the MS^2 spectrum at *m/z* 543.1473 and *m/z* 203.0533 resulted from the loss of the *m/z* [705 – 162] and [543 – (2 × 162)], respectively (Matros *et al.*, 2015, Dasaesamoh *et al.*, 2016, Lira *et al.*, 2020, Lima *et al.*, 2020 and Santos *et al.*, 2024).

In addition to the carbohydrate derivatives mentioned earlier, betalain isomers were tentatively identified. In the recent literature, Silva *et al.* (2024) using 2D NMR analysis in DMSO-d_6 to detect betanin ($C_{18}H_{16}N_2O_8$) in microfiltered pitaya red concentrate dye, observed low signal intensity, suggesting the occurrence of a previously reported phenomenon known as degradation and regeneration of betalain. The literature has described and demonstrated that the chemical structure of betalain derivatives significantly influences their stability and the products generated after oxidation (Wybraniec *et al.,* 2013). Meanwhile, Schweiggert (2018), when describing alternative natural pigments in foods and beverages, reported that isomerized forms of betanin were observed after application of acid treatment. This chemical behavior was also observed in the TB (pulp), TBr (retentate) and TBp (permeate) samples, added with ascorbic acid, as reported in the observation of the next peaks.

Peak **3** (t_R = 3.06 min) was observed in the chromatogram, with the ion $[M + H]^+$ at *m/z* 683.1460 and molecular formula $C_{29}H_{31}N_2O_{19}^+$. The MS^2 spectrum revealed fragment ions at 551 Da and 389 Da. These compounds were identified in extracts from the *Cactaceae* and *Amaranthaceae* families, in accordance with previous studies on betalain isomers, which showed a fragmentation pattern consistent with those obtained by ESI-MS/MS (Wybraniec *et al.*, 2009, Szot *et al.*, 2015, Henarejos-Escudero *et al.*, 2018 and Santos *et al.* 2024).

Compounds **10** (t_R = 3.32 min), **12** (t_R = 3.50 min), **13** (t_R = 3.54 min) **16** (t_R = 3.32 min), **18** (t_R = 4.18 min), **24** (t_R = 4.48 min) and **26** (t_R = 4.51 min) were tentatively identified as isomers of the 2'-apiosyl-15*R*/15*S*-phyllocactin, with a molecular formula of $C_{32}H_{37}N_2O_{20}^+$. They were observed in the MS^1 spectrum with the ions $[M^+]$ at *m/z* 769.1960, 769.1966, 769.1949, 769.1969, 769.1961, 769.1918 and 769.1975. The analysis of the fragmentation observed in the MS/MS spectrum revealed fragment ions at 683 Da, 637 Da, 593 Da, 551 Da and 389 Da. Additionally, compound **7** (t_R = 3.23 min), which has the molecular formula $C_{32}H_{37}N_2O_{20}^+$ appeared at 769.1956 Da in the MS^1 spectrum. The MS^2 spectra showed different fragmentation processes for the compounds, which may indicate various routes. These similar fragmentation profiles at 683.1955 Da, 543.2001 Da, 525.2345 Da, 381.1988 Da were previously reported by Wybraniec *et al.* (2007, 2010); Fathordoobady *et al.* (2016), Lira *et al.* (2020), Lima *et al.* (2020) and Santos *et al.* (2024).

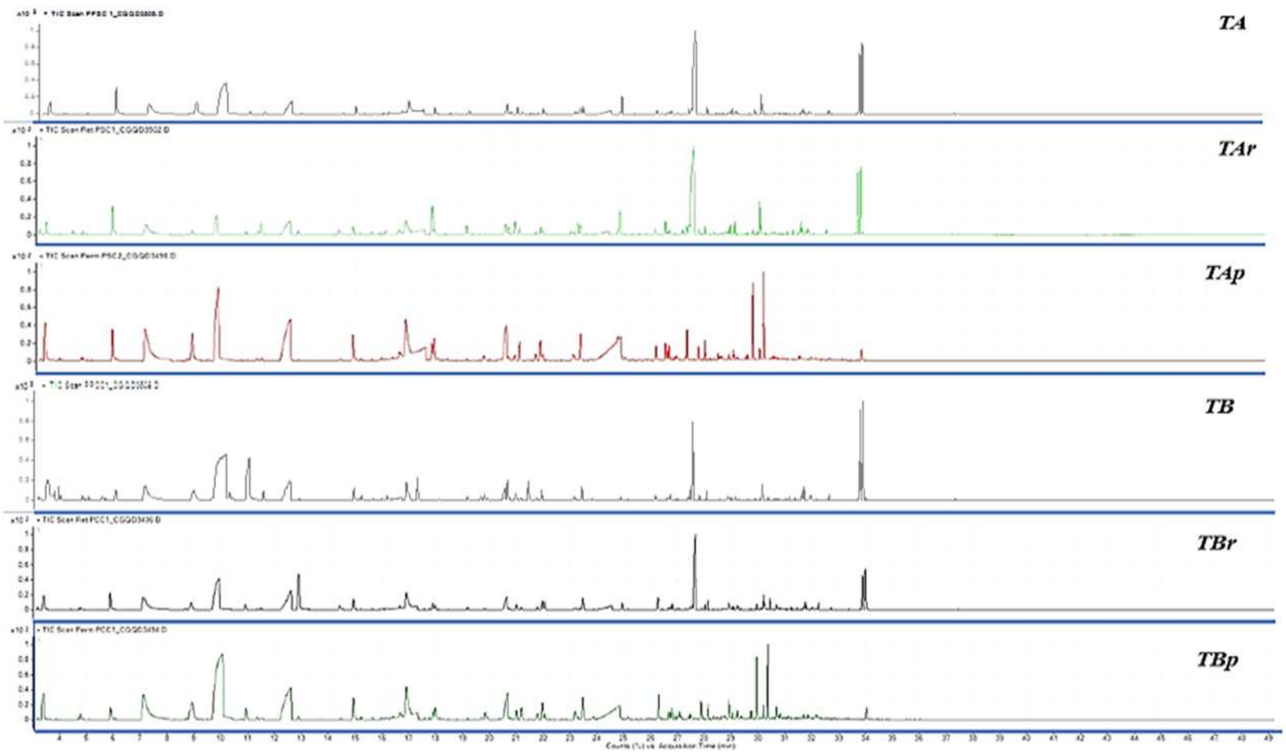
Peaks **8** (t_R = 3.28 min, MS^1 637.1486 Da), **15** (t_R = 3.55 min, MS^1 637.1495 Da), **19** (t_R = 4.25 min, MS^1 637.1486 Da), **25** (t_R = 4.49 min, MS^1 637.1502 Da), **29** (t_R = 5.62 min, MS^1 637.1502 Da) were tentatively identified as isomers of 15*S*/15*R*-phyllocactin (*O*-malonyl-betanin) - $C_{27}H_{28}N_2O_{16}^+$). In addition, the fragments ions observed at *m/z* 389 (betanidin) agreed with those reported in the literature by Suh *et al.*, 2014; Wybraniec *et al.* (2007, 2010); Lira *et al.* (2020) and Santos *et al.* (2024).

The three isomeric forms of 15*R*/15*S*-betanin (**17**, **22** and **23**) were observed at different retention times (t_R: 4.10 min, 4.39, and 4.40 at *m/z*: 551.0000, 551.0000 and 551.0000, respectively). Additionally, in the MS/MS spectrum was observed common peak known as betanidin at 389 Da (formed due to loss of sugar moiety) (Stintzing *et al.*, 2002, Wybraniec *et al.,* 2007, 2010; Belhadj Slimen *et al.*, 2017; Lira *et al.*, 2020; Lima *et al.*, 2020 and Santos *et al.*, 2024). Compound **20** (t_R = 4.28 min) was tentatively identified as 6´malonyl-2´-decarboxy-betanin presents as ion adducts at 593.1613 Da. In the MS^2 spectrum, the following fragments were observed at 551 Da, 549 Da and 389 Da. The fragmentation pattern is similar to that previously presented by Lima *et al.* (2020) in MS^1 and MS^2.

Betalain isomers are composed of betalamic acid, whose the structure is responsible for the coloring of pitaya pigments, the main one being the chromophore (Strack, Vogt and Schliemann 2003, Khan, 2016, Polturak & Aharoni, 2018). Previously, section **3.2** reported the difference in color profile between samples obtained in different pulping processes and subjected to membrane clarification, retentate and permeate. This metabolic difference may be chemically related to the different membrane fouling profiles presented in section **3.1**. In addition, previous on samples of multifunctional red pitaya concentrates obtained by microfiltration with the presence of betalain derivatives described non-toxic products with potential functional benefits based on tests on zebrafish related to the hypoglycemic effect and good sensory acceptance with untrained consumers (Lima *et al.*, 2020, Gengatharan *et al.*, 2021, and Silva *et al.*, 2024).

Furthermore, to gain a better understanding of metabolism, the profile of volatile compounds from pulp formulations (TA and TB), retentate (TAr and TBr), and permeates fractions (TAp and TBp) were determined. The chromatogram analysis also showed a difference between the volatile components of the samples, see Figure 4. The differences in volatile components between pitaya pulps have already been reported by Santos *et al.* (2020), and Lima *et al.* (2020) has already described the volatile profile of red pitaya permeate concentrate in yogurt, in the present work an apparent difference can also be observed between the permeate and retentate samples. Therefore, this reinforces the importance of delving deeper into the main chemical markers that will be presented in the next section.

Figure 4. Representative chromatograms of the volatile compounds containing six different samples pulps (TA and TB), retentate (TAr and TBr) and permeates (TAp and TBp).



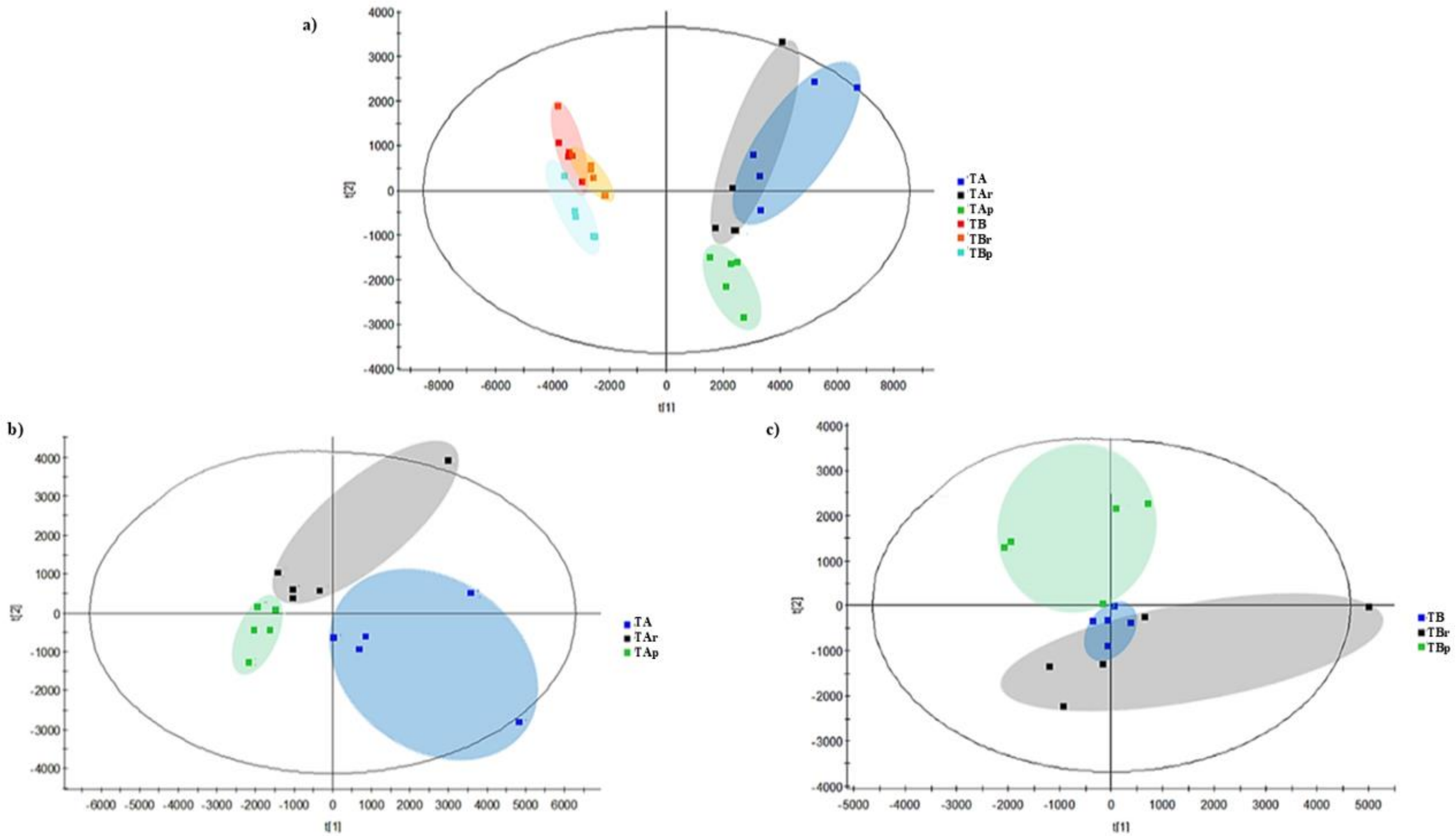
Fonte: Author (2025)

3.4 Multivariate analysis

Exploratory analysis of main compounds used the relationship of the identified compounds with the evidenced samples, in order to verify possible groupings due to the complexity and high dimensionality of the data set found by UPLC-QTOF-MS^E (total of 45 compounds x 6 chromatograms = 270 variables) in the composition of the organic components of the pulps subjected to treatments during pulping (TA and TB) and the fractions obtained from membrane clarification, the retentates (TAr and TBr) and the permeates (TAp and TBp) of red pitaya. Figure 5 illustrates the two-dimensional results (PC1 × PC2) of the samples. According to the results graphs (see Figure 5a), the variability of the model of the pulp samples, retentates and permeates obtained 70% of the total variance. The TA and TB pulp is in positive PC1 and negative PC1, presenting results already presented in Santos *et al.* (2024).

Then, describing a complementary view with the application of microfiltration as fractions: retentates (TAr and TBr) and permeates (TAp and TBp) present a similar behavior in relation to the evident separation between the fractions in positive PC1 and negative PC1. These results confirm that the application of different treatments during pulping and the application of the clarification process result in different organic compositions of red pitaya samples. Furthermore, in agreement with the data presented, they were demonstrated as retentate and permeate samples within the same processing in Figures 5(b) and 5(c) and a variability of 77% and 73% of total variation was observed, respectively. S-Plot and graphs of the discriminants of the samples to obtain confirmation on the behavior of the retentate and the permeate juice in relation to the permeability in the membrane of the chemical compounds.

Figure 5. Analysis of main compounds of red pitaya fractions subjected to clarification: (a) TA x TAr x TAp x TB x TBr x TBp, (b) TA x TAr x TAp, (c) TB x TBr x TBp.



Fonte: Author (2025)

3.4.1 Potential chemical markers

The different metabolic profiles separated and disclosed in the PCA were confirmed from the OPLS-DA evaluation, where it was confirmed in Figures 6 (a1) and 6 (b1) in which it was possible to verify the clear separation of the two groups of discriminating metabolite samples, closer to the ends of the graph axis and distances from the center. The chemical marker stands out in red. In addition, it contains the same comparative numbering in the bar graph. The samples presented variability of the retentate and permeate samples model with values of 76% and 85% of total variation, respectively.

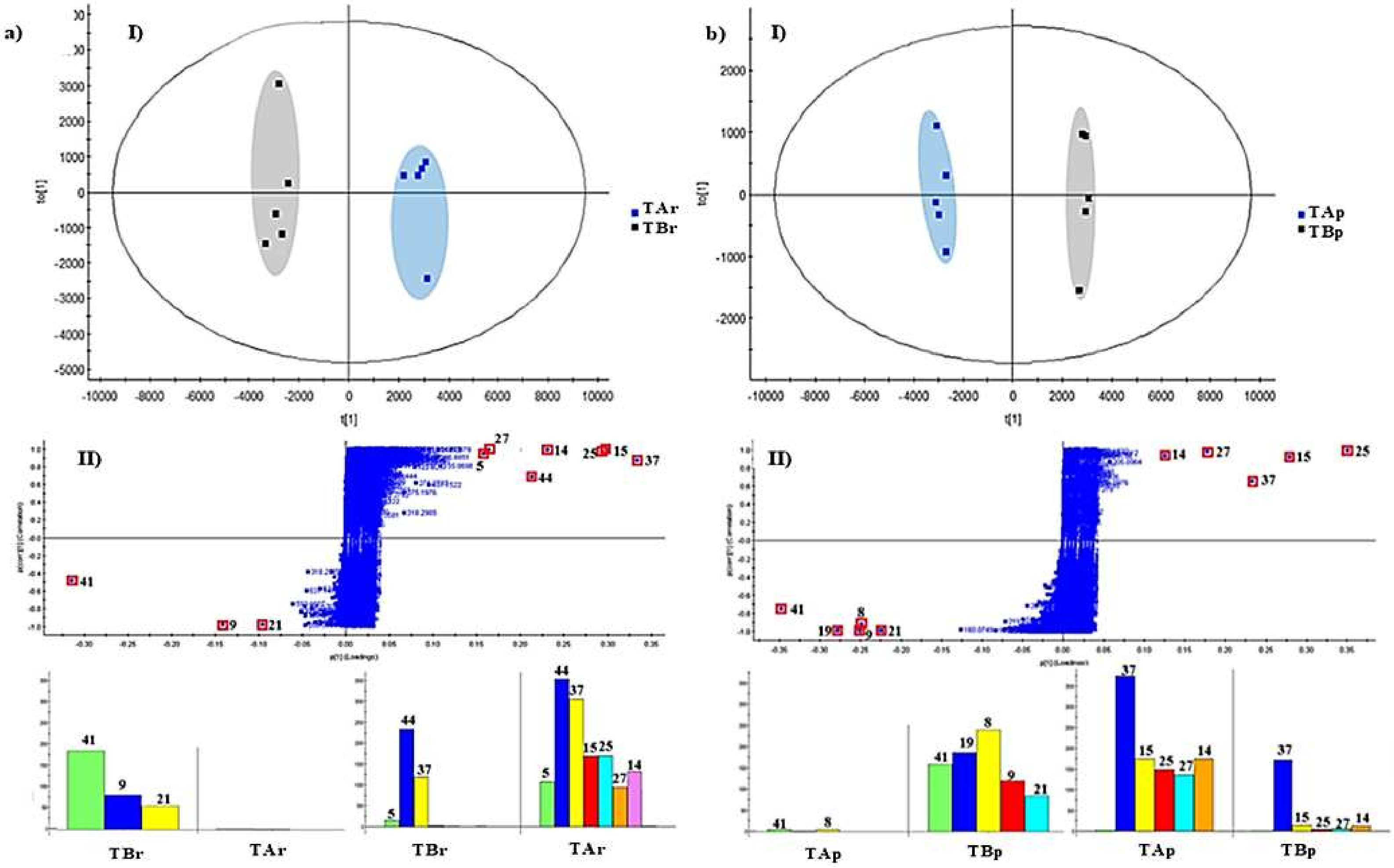
Therefore, you can see that these data are plausible indicative of a statistically significant difference between the metabolic compositions of the retentate and permeate samples. Furthermore, it can be inferred that the different pulping processes alter the interaction of the juices with the membrane permeability, as already described in section 5.1, through the understanding of the specificities of the polarization through time polarization and Foulling, where through the OPLS-DA model the metabolic difference of the two clusters in PC1 becomes evident, see Figure 6 (a1) and 6 (b1). The chemical markers of the TBr and TAr treatments can be observed in Figure 6 (aII). Compounds **41** (unknown), **9** (phyllocactin isomers) and **21** (unknown) are responsible for the differentiation of TBr from TAr. Meanwhile, components **5** (Glucose), **14** (unknown), **15** (*O*-malonyl-betanin), **25** (*O*-malonyl-betanin), **27** (unknown) and **44** (unknown) were responsible for the distinction of TAr in relation to TBr.

Other markers are found in the samples permeated tangentially by the pores of the TAp and TBp membranes, see Figure 6 (bII), and are responsible for the classification of TAp from TBp, they are: **41** (unknown), **19** (*O*-malonyl-betanin), **8** (*O*-malonyl-betanin), **9** (phyllocactin isomers) and **21** (unknown). On the other hand, when evaluating the markers responsible for differentiating TBp from TAp, the following compounds stand out: **14** (unknown), **15** (*O*-malonyl-betanin), **25** (*O*-malonyl-betanin), **27** (unknown), and **37** (unknown). This change in the marker profile may be related to the influence of the pulping step, which directly influences the microfiltration process of the samples.

In general, we can infer that the fractions resulting from microfiltration reflect the profile of the chemical markers in all formulations influenced by the two pulping processes, corroborating the report by Santos *et al.* (2024) and may be related to the behavior of organic compounds during the clarification process, as they may become more embedded in the membrane, favoring the achievement of the membrane pore clogging stage in a shorter time, thus providing permeate flux. In addition, the presence of betalain isomers as chemical markers of retentate and permeate samples reinforces the importance of a sustainable approach for processing juices with functional potential, since the authors Holanda *et al.* (2021), Lira *et al.* (2020, 2023) and Marques *et al.* (2023) previously reported potential benefits related to dyslipidemia, hypoglycemic and anxiolytic when studying the pulp, peel and seed of red pitaya based on tests in mice and zebrafish model.

Thus, considering all the data collected (permeate flow, physical-chemical, metabolomic and multivariate analyses), it can be seen that microfiltration is an interesting technological alternative, since the permeate pitaya juice with pulping with peel and addition of ascorbic acid presents a promising metabolic profile, due to conservation or generation of organic components.

Figure 6. Graphs of orthogonal partial least squares analyzes - OPLS and chemical markers - SPLOT of the formulations: (a) TAr x TBr, (b) TAp x TBp.



Fonte: Author (2025)

4 Conclusion

This is the first study to present microfiltration of red pitaya non-enzymatic treatment and chemical profile data. The microfiltration process applied in two different pitaya tretaments (TA or TB) was effective in reducing the turbidity of the juices. The permeate flow of the clarified samples showed characteristic behavior profiles, with better performance for TAp. In the permeate juice, 45 metabolites, such as carbohydrates and betalain isomers, were detected by UPLC-QTOF-MS^E. In addition, chemometric methods (PCA, OPLS-DA and S-Plot) were essential to identify the inherent differences in the metabolic profiles of the pulp, permeate and retentate samples from the TA or TB treatment. It should be acknowledged that the experiments were carried out at a pilot plant scale, which may not fully reflect the complexity and variability inherent in food industry environments.

Therefore, the results of this study are promising and will be valuable for future optimizations aimed at scaling up industrial production in the food sector. Specifically, pitaya juice clarified as a natural colorant rich in compounds with functional potential. Additionally, it will support the development of eco-friendly industrial practices in the technological process, thereby adding value to red pitaya.

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