



GILSON GUSTAVO LUCINDA MACHADO

**EVOLUÇÃO DO PERFIL PROTEOMETABOLÔMICO DE
PSEUDOFRUTOS DE *Hovenia dulcis*: DO
DESENVOLVIMENTO ONTOGENÉTICO À EXTRAÇÃO E
APLICAÇÃO *IN VIVO* DE POLISSACARÍDEOS**

**LAVRAS-MG
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Tese apresentada à Universidade Federal de
Lavras, como parte das exigências do Programa
de Pós-Graduação em Ciência dos Alimentos,
para a obtenção do título de Doutor.

Prof. Dr. Eduardo Valério de Barros Vilas Boas
Orientador

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DE *Hovenia dulcis*: DO DESENVOLVIMENTO ONTOGENÉTICO À
EXTRAÇÃO E APLICAÇÃO *IN VIVO* DE POLISSACARÍDEOS**

**EVOLUTION OF THE PROTEOMETABOLOMIC PROFILE OF *Hovenia dulcis*
PSEUDOFRUIT: FROM ONTOGENETIC DEVELOPMENT TO THE
EXTRACTION AND *IN VIVO* APPLICATION OF POLYSACCHARIDES**

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Doutor.

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RESUMO GERAL

Diante do limitado conhecimento sobre as bases moleculares que regem o desenvolvimento de *Hovenia dulcis* no Brasil e o potencial tecnológico de suas matrizes biológicas, este estudo teve como objetivo investigar, por abordagem multiômica integrada, o desenvolvimento de seus pseudofrutos e extrair, caracterizar e aplicar seus polissacarídeos (HDPs-BR) como revestimentos comestíveis na conservação pós-colheita de morangos. O desenvolvimento dos pseudofrutos foi caracterizado por análises físicas, físico-químicas, químicas e proteômica de alta resolução ao longo de sete estádios. Paralelamente, HDPs-BR extraídos de pseudofrutos maduros foram caracterizados e aplicados isoladamente ou combinados com alginato de sódio (SA) e eugenol (Eug) em morangos armazenados por 15 dias. O desenvolvimento de *Hovenia dulcis* estendeu-se por 180 dias, com amadurecimento efetivo aos 150 dias após a antese, marcado por intenso amolecimento, solubilização péctica e conversão de amido em açúcares. Quatrocentas e noventa e seis proteínas foram identificadas, por meio de análises proteômicas, das quais 222 foram diferencialmente expressas, evidenciando a participação coordenada do metabolismo energético, vias fotossintéticas, sinalização do etileno e defesas antioxidantes. A análise multivariada permitiu distinguir três fases de desenvolvimento: defesa química, transição metabólica e maturação sensorial, e revelar que a transição foi coordenada por alterações fisiológicas, físico-químicas e metabólicas. Os HDPs-BR apresentaram rendimento de extração de 5,31%, sendo constituídos majoritariamente por açúcares totais (62,68%) e ácidos urônicos (24,95%), além de apresentarem compostos fenólicos e atividade antioxidante, evidenciando seu caráter bioativo. Nos morangos, o desempenho dos revestimentos foi dependente da formulação e do período de armazenamento. HDPs-BR 0,5% favoreceram a manutenção da firmeza, enquanto HDPs-BR 1% contribuíram para a retenção de compostos fenólicos, antocianinas e coloração vermelha. A incorporação de SA e Eug proporcionou benefícios adicionais, destacando-se HDPs-BR 1% + Eug 0,1% pela manutenção da cor vermelha, elevado teor de antocianinas e atividade antioxidante. Os revestimentos também contribuíram para o controle microbiológico durante o armazenamento. Conclui-se que o desenvolvimento de *Hovenia dulcis* é sustentado por uma rede proteometabólica coordenada que redireciona recursos de mecanismos protetores para a aquisição de atributos associados à maturação, enquanto seus polissacarídeos constituem uma matriz bioativa promissora para o desenvolvimento de revestimentos comestíveis destinados à conservação sustentável de frutas frescas.

Palavras-chave: Amadurecimento; compostos bioativos; carboidrato, metabolismo energético, pós-colheita, uva-japonesa.

GENERAL ABSTRACT

Given the limited knowledge regarding the molecular mechanisms underlying the development of *Hovenia dulcis* in Brazil and the technological potential of its biological matrices, this study aimed to investigate pseudofruit development through an integrated multi-omics approach and to extract, characterize, and apply its polysaccharides (HDPs-BR) as edible coatings for the postharvest preservation of strawberries. Pseudofruit development was characterized through physical, physicochemical, chemical, and high-resolution proteomic analyses across seven developmental stages. In parallel, HDPs-BR extracted from mature pseudofruits were characterized and applied either alone or in combination with sodium alginate (SA) and eugenol (Eug) to strawberries stored for 15 days. The development of *Hovenia dulcis* extended over 180 days, with effective ripening occurring at 150 days after anthesis, characterized by pronounced softening, pectin solubilization, and the conversion of starch into sugars. Proteomic analyses identified 496 proteins, of which 222 were differentially expressed, revealing the coordinated involvement of energy metabolism, photosynthetic pathways, ethylene signaling, and antioxidant defense mechanisms. Multivariate analysis distinguished three developmental phases: chemical defense, metabolic transition, and sensory maturation and revealed that the transition was coordinated by physiological, physicochemical, and metabolic changes. HDPs-BR showed an extraction yield of 5.31% and were primarily composed of total sugars (62.68%) and uronic acids (24.95%). They also contained phenolic compounds and exhibited antioxidant activity, demonstrating their bioactive nature. In strawberries, coating performance depended on both formulation and storage period. HDPs-BR at 0.5% favored firmness retention, whereas HDPs-BR at 1% contributed to the retention of phenolic compounds, anthocyanins, and red coloration. The incorporation of SA and Eug provided additional benefits, with HDPs-BR 1% + Eug 0.1% standing out for maintaining red coloration, high anthocyanin content, and antioxidant activity. The coatings also contributed to microbiological control during storage. In conclusion, *Hovenia dulcis* development is supported by a coordinated proteometabolic network that redirects resources from protective mechanisms toward the acquisition of ripening-associated traits, while its polysaccharides represent a promising bioactive matrix for the development of edible coatings aimed at the sustainable preservation of fresh fruits.

Keywords: Ripening; bioactive compounds; carbohydrates; energy metabolism; postharvest; Japanese raisin tree.

INDICADORES DE IMPACTO

O presente estudo estabelece contribuições técnico-científicas, sociais e econômicas integradas, alinhando-se diretamente com os preceitos da Política Nacional de Assistência Técnica e Extensão Rural (PNATER) e com as metas globais dos Objetivos de Desenvolvimento Sustentável (ODS) da Agenda 2030 das Nações Unidas. Sob a dimensão socioambiental e de saúde pública, a elucidação das bases moleculares que regem o desenvolvimento de *Hovenia dulcis* valida cientificamente o perfil terapêutico e funcional de seus pseudofrutos, estimulando o consumo de alimentos promotores da saúde. Ao transpor esse conhecimento para o desenvolvimento de um revestimento biopolimérico natural e biodegradável ativado com eugenol, a pesquisa gera um impacto direto na redução do desperdício de alimentos de alta perecibilidade, como o morango, promovendo a segurança alimentar e oferecendo ao consumidor final uma alternativa livre de conservantes sintéticos. Essa transição para uma química verde mitiga os impactos ambientais causados pelo descarte de plásticos convencionais derivados de petróleo na cadeia de alimentos, impulsionando os conceitos de consumo e produção responsáveis (ODS 12), saúde e bem-estar (ODS 3) e combate à fome (ODS 2). Tecnicamente, o trabalho consolida um duplo ineditismo: estabelece o primeiro mapa proteometabólico do desenvolvimento da espécie no Brasil e propõe uma rota de bioeconomia circular ao transformar os polissacarídeos de uma matriz subutilizada em barreiras ativas eficientes contra a desidratação e o crescimento fúngico pós-colheita. No âmbito da inovação e propriedade intelectual, a comprovação da viabilidade técnica desses novos filmes abre precedentes para o depósito de patentes e transferência de tecnologia, preenchendo uma lacuna de mercado que hoje depende fortemente de insumos importados, o que fortalece a infraestrutura científica e a inovação industrial do país (ODS 9). Economicamente, a valorização biotecnológica da uva-japonesa abre novas vertentes para as indústrias química, farmacêutica e de embalagens, gerando oportunidades de mercado de alto valor agregado. No cenário agrícola e de extensão rural, essa abordagem impulsiona o desenvolvimento territorial e o fortalecimento de arranjos produtivos locais. Ao transformar uma espécie frequentemente encontrada de forma espontânea ou marginal em propriedades rurais das regiões Sul e Sudeste do país em uma matéria-prima de interesse industrial, o estudo estimula o extrativismo sustentável e o processamento local. Essa dinâmica descentralizada viabiliza a diversificação de renda na agricultura familiar, gerando impactos positivos no trabalho digno e no crescimento econômico regional (ODS 8), enquanto atua na valorização da sociobiodiversidade e no manejo sustentável de ecossistemas terrestres (ODS 15).

IMPACT INDICATORS

This study provides integrated technical-scientific, social, and economic contributions, aligning directly with the guidelines of the National Policy on Technical Assistance and Rural Extension (PNATER) and the global targets of the United Nations 2030 Agenda Sustainable Development Goals (SDGs). Under the socio-environmental and public health dimensions, elucidating the molecular bases governing the development of *Hovenia dulcis* scientifically validates the therapeutic and functional profile of its pseudofruits, thereby encouraging the consumption of health-promoting foods. By translating this knowledge into the development of a natural, biodegradable biopolymeric coating activated with eugenol, this research directly impacts the reduction of food waste in highly perishable commodities, such as strawberries, promoting food security and offering the final consumer an alternative free of synthetic preservatives. This transition toward green chemistry mitigates the environmental impacts caused by the disposal of conventional petroleum-derived plastics in the food supply chain, advancing the concepts of responsible consumption and production (SDG 12), good health and well-being (SDG 3), and zero hunger (SDG 2). Technologically, this work consolidates a dual novelty: it establishes the first proteometabolic map of the development of this species in Brazil and proposes a circular bioeconomy route by transforming the polysaccharides of an underutilized matrix into efficient active barriers against post-harvest dehydration and fungal growth. Within the scope of innovation and intellectual property, proving the technical viability of these new films sets precedents for patent filings and technology transfer, filling a market gap that currently relies heavily on imported inputs, which strengthens the country's scientific infrastructure and industrial innovation (SDG 9). Economically, the biotechnological valorization of the Japanese raisin tree opens new avenues for the chemical, pharmaceutical, and packaging industries, generating high-value-added market opportunities. In the agricultural and rural extension scenario, this approach drives territorial development and the strengthening of local productive arrangements. By transforming a species frequently found spontaneously or marginally on rural properties in the South and Southeast regions of the country into a raw material of industrial interest, the study stimulates sustainable extractivism and local processing. This decentralized dynamic enables income diversification in family farming, generating positive impacts on decent work and regional economic growth (SDG 8), while promoting the valorization of socio-biodiversity and the sustainable management of terrestrial ecosystems (SDG 15).

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Hovenia dulcis é uma espécie frutífera nativa de países asiáticos, conhecida popularmente como uva-japonesa. Sua introdução no Brasil remete à década de 1970, quando a Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) recebeu sementes provenientes da Academia Chinesa de Silvicultura. Inicialmente, a espécie foi empregada com intuito ornamental e de reflorestamento no Sul do país, região a partir da qual se dispersou pelas demais áreas de clima temperado e subtropical do território nacional.

A planta se destaca pelo porte alto, que facilmente ultrapassa os 10 metros de altura. Seus pseudofrutos, sementes, folhas, raízes e casca são empregados há séculos na medicina tradicional asiática. Seu florescimento no Brasil ocorre entre os meses de agosto e fevereiro, e seus pseudofrutos amadurecem de março a outubro. Morfologicamente, esses pseudofrutos são carnosos, desenvolvidos a partir de pedúnculos multifacetados que se ramificam em formato angular ou de "T", apresentando casca externa avermelhada, sabor adocicado e aroma forte. Do ponto de vista biológico, a espécie exibe comprovada atividade hepatoprotetora, anti-inflamatória, antioxidante, antirressaca e antidiabética (Thi Ngo et al., 2021). Esse perfil terapêutico é atribuído a uma ampla gama de compostos bioativos, incluindo flavonoides, esteróis, lignanas, fenilpropanoides, alcaloides, triterpenos e compostos fenólicos (como catequinas e taninos), os quais conferem alta propriedade funcional aos pedúnculos.

O desenvolvimento dos frutos e pseudofrutos compreende diferentes estádios: formação, crescimento, maturação e senescência. Durante essas etapas, ocorrem inúmeras alterações químicas e bioquímicas que culminam na modificação das características sensoriais aparência, sabor e odor. Apesar de os pseudofrutos de *Hovenia dulcis* serem amplamente estudados sob o aspecto farmacológico, o panorama de seu desenvolvimento fisiológico e amadurecimento ainda é escasso na literatura. Diante dessa lacuna, as tecnologias moleculares podem contribuir significativamente para a elucidação desses processos, auxiliando futuramente no manejo agrônomico e no melhoramento genético da espécie.

Nesse contexto, as ferramentas moleculares têm sido essenciais para entender as transformações provenientes do desenvolvimento de frutos e pseudofrutos. Ao correlacionar dados fenotípicos e fisiológicos, essas abordagens permitem avaliar de forma sistêmica as transformações celulares. Isso engloba o mapeamento de fluxos metabólicos em diferentes

organelas, a compreensão das alterações nos níveis de metabólitos e proteínas, bem como a identificação de modificações pós-traducionais que coordenam o amadurecimento.

Além dos fatos citados, vale ressaltar que os polissacarídeos dessa espécie têm recebido grande atenção de pesquisadores orientais, especialmente devido as enzimas envolvidas no metabolismo do álcool, como álcool desidrogenase (ADH) e aldeído desidrogenase (ALDH), as quais contribuem para a aceleração da depuração do etanol e redução dos danos hepáticos. (Lan et al., 2024). Essas características qualificam essa matriz biológica como uma matéria-prima promissora para o desenvolvimento de medicamentos e alimentos saudáveis, incluindo a possibilidade de ser utilizada na tecnologia de revestimentos comestíveis.

Biopolímeros são normalmente utilizados na elaboração de revestimentos comestíveis que podem ser usados na conservação de alimentos, preservando seu frescor e atributos de qualidade e, consequentemente, reduzindo o desperdício. Contudo, para potencializar a eficácia dessa barreira, torna-se imprescindível correlacionar as propriedades intrínsecas do polímero selecionado, as características fisiológicas do fruto a ser revestido e a dinâmica do método de aplicação.

Dentre os materiais mais empregados para o desenvolvimento desses filmes poliméricos estão os polissacarídeos, proteínas e lipídeos, embora nanomateriais e óleos essenciais possam ser incorporados como agentes ativos. Esses materiais devem ser seguros para o consumo, isentos de componentes tóxicos e alergênicos, possuir propriedades mecânicas adequadas, boa aderência à superfície do alimento, estabilidade durante o armazenamento, ação antifúngica, hidrofobicidade controlada, além de regular as trocas gasosas. Adicionalmente, devem apresentar baixo custo de obtenção e viabilidade de processamento industrial.

Esses fatores tornam os polissacarídeos de *Hovenia dulcis* um promissor alvo de estudo, embora até o presente momento, não haja relatos na literatura ou patentes depositadas empregando essa matriz polimérica com tal finalidade. Portanto, neste trabalho, propomos uma investigação multiômica integrada, combinando análises físicas, físico-químicas e químicas à proteômica de alta resolução, com a finalidade de mapear as transformações biológicas desde a antese até o amadurecimento dos pseudofrutos, elucidando como a coordenação entre o metabolismo central e as vias metabólicas especializadas define o potencial tecnológico desta espécie, etapa que originou o primeiro artigo da presente tese. O segundo artigo teve por finalidade extrair os polissacarídeos de *Hovenia dulcis*, desenvolver um revestimento comestível a partir desses compostos e avaliar a sua aplicação na conservação pós-colheita de morangos. Deste modo, este estudo é pioneiro por analisar os pseudofrutos do ponto de vista molecular e por aplicar seus polissacarídeos na pós-colheita de frutas.

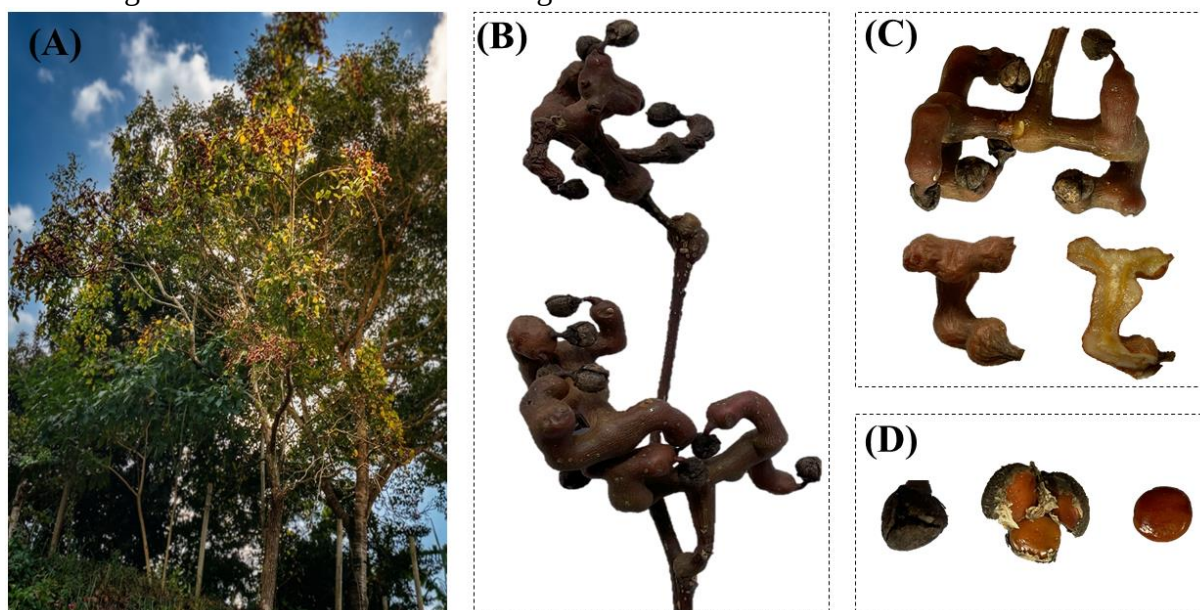
2 REFERENCIAL TEÓRICO

2.1 *Hovenia dulcis*

Hovenia dulcis Thunb. é uma espécie arbórea pertencente à família Rhamnaceae. Caracterizada como uma árvore decídua, ela apresenta troncos e galhos lisos com pequenas saliências respiratórias, podendo atingir até 10 metros de altura. Suas folhas possuem uma textura fina e flexível e são sustentadas por pecíolos de até 6 cm. Visualmente, apresentam formato largamente ovalado medindo de 8 a 15 cm de comprimento por 6 a 12 cm de largura, com a base arredondada ou levemente em formato de coração, pontas curtas e afinadas, e bordas suavemente denteadas (Figura 1A) (Hyun et al., 2010). Conhecida popularmente como uva-japonesa, a espécie carrega um histórico tradicional de uso medicinal e alimentar no Japão, China e Coreia (Morales et al., 2017), onde praticamente todas as suas partes incluindo pseudofrutos, sementes, folhas, cascas e raízes são aproveitadas em preparações terapêuticas (Song et al., 2020).

Os pseudofrutos são as partes mais consumidas de *Hovenia dulcis* (Peng et al., 2018; Song et al., 2020). Botanicamente, o fruto é o produto do desenvolvimento do ovário das angiospermas. Ao produto do desenvolvimento de tecidos extracarpelares dá-se o nome de pseudofruto. Enquanto, em alguns casos, o fruto verdadeiro é suculento e sensorialmente agradável e, portanto, a porção comestível, em outros, essas características são atribuídas ao pseudofruto, como acontece em *Hovenia dulcis*. O fruto verdadeiro de *Hovenia dulcis* é uma cápsula trilocular seca e indeiscente que contém as sementes. Já os pseudofrutos são formados a partir dos eixos da inflorescência (pedúnculos) que sofrem acentuada hipertrofia durante o amadurecimento. Assim, os caules retorcidos da inflorescência, quando maduros, tornam-se inchados, irregulares (3-7 mm de diâmetro), carnosos, de coloração marrom - avermelhado e doces devido ao seu alto teor de sacarose (Yang et al., 2019; De Biaggi et al., 2020) (Figura 1 B - D). Esses pseudofrutos, também conhecidos como uva-japonesa, emanam aroma semelhante à pêra madura, apresentando boa aceitabilidade para consumo humano, em parte, pelos elevados teores de açúcares (Maieves et al., 2015).

Figura 1 – Características morfológicas dos frutos e sementes de *Hovenia dulcis*



(A) Planta adulta em ambiente natural. (B) Pedúnculos maduros de *Hovenia dulcis*. (C) Pseudofrutos destacados do pedúnculo, contendo semente, sem semente e em secção transversal (C) Sementes inteiras, abertas e isolada.

Fonte: Do autor (2026).

Os pseudofrutos de *Hovenia dulcis* Thumb. têm sido considerados veículos não desprezáveis de flavonoides, lignana, sesquiterpenóides, triterpenóides e ácido benzoico, compostos que lhes conferem capacidade antioxidante, antimicrobiana, antidiabética anti-inflamatória, anti-obesidade e atividade laxativa (Choi et al., 2017; Morales et al., 2017; Peng et al., 2018; Yang et al., 2019; De Biaggi et al., 2020; Oh et al., 2020; Song et al., 2020; De Godoi et al., 2021; Sferrazza et al., 2021; Thi Ngo et al., 2021). As sementes também são consideradas fontes de compostos bioativos e apresentam propriedades diuréticas (Xu et al., 2020; Sferrazza et al., 2021). A presença de biopolímeros e compostos fenólicos nessa matriz sugere um alto potencial para a extração de ingredientes funcionais, como polissacarídeos para o desenvolvimento de coberturas ativas.

Seu uso como medicamento fitoterápico tem sido relatado para o tratamento de diversas doenças, sobretudo alcoolismo e doenças hepáticas (Choi et al., 2017; Kim et al., 2017; Wang et al., 2017; Qiu et al., 2019, He et al., 2024; Kim et al., 2025; Kwon et al., 2025), sendo comprovado a capacidade do extrato de *Hovenia dulcis* de estimular a atividade metabólica das enzimas álcool desidrogenase (ADH) e aldeído desidrogenase (ALDH), elevando a taxa do metabolismo do álcool, mitigando em certa medida os dados induzidos pelo álcool no corpo. Além de regular o desequilíbrio intestinal e modular vias biossintéticas de fenilalanina, tirosina e triptofano, mitigando assim o estresse oxidativo induzido pelo consumo de álcool e

potencialmente auxiliando na prevenção do alcoolismo agudo e das lesões hepáticas (Lan et al., 2024; Paik et al., 2024).

Os pseudofrutos podem ser consumidos *in natura*, ou processados na forma de vinhos, bebidas, compotas, geleias, passas, e utilizados como veículo de antioxidante em embutidos cárneos (Pinto et al., 2017; Xu et al., 2020; Machado & Gonçalves, 2021; Schaefer et al., 2022). Pouco se sabe sobre o pseudofruto em países ocidentais como o Brasil (Sferrazza et al., 2021), onde a espécie ocorre principalmente de forma naturalizada e não cultivada, apresentando uma variabilidade metabólica que constitui um promissor elemento de estudo para abordagens ômicas. Tais ferramentas permitem o mapeamento sistemático de proteínas e metabólitos responsáveis pela qualidade sensorial e funcional, fornecendo o embasamento necessário para a valorização desta matriz como alimento detentor de compostos benéficos à saúde e como promissora fonte de biopolímeros estruturais para a ciência dos alimentos.

2.2 Proteômica no estudo de frutos

O sequenciamento do DNA de alto rendimento na década de 90 foi seguido por inovações tecnológicas que fornecem medições em escala de genoma para muitas das espécies moleculares existentes no interior da célula (Joyce & Palson, 2006). A compreensão da função genética pode ser abordada por meio de diversas técnicas integradas, comumente denominadas ômicas: genômica, transcriptômica, proteômica e metabolômica (Zyvi et al., 2015). Essas técnicas, aliadas à bioinformática, popularmente denominada como biologia computacional, podem desempenhar um papel importante na segurança alimentar. Novos métodos e softwares podem ser usados como ferramentas para processamento, análise e interpretação de dados biológicos originados a partir de diversas abordagens bioquímicas e ômicas (genômica, transcriptômica, proteômica e metabolômica) para decifrar a investigação mecanística do processo biológico que conecta a cascata biológica: célula-cromossomo-DNA-RNA-proteína-metabólito (Prakash et al., 2020).

A proteômica é um dos campos mais ativos de pesquisa biológica, dada a sua ampla aplicação (Goez et al., 2018), sendo uma ferramenta que simplifica o estudo da expressão global de proteínas e fornece uma gama de informações sobre as proteínas individuais envolvidas em respostas biológicas específicas (Chen et al., 2020). Estudos de proteômica abordam a comparação qualitativa e/ou quantitativa de proteomas expressos em um sistema biológico em diferentes condições ambientais em dado momento (Prakash et al., 2020). Sendo uma área de

pesquisa que cresce em ritmo acelerado, está se tornando cada dia mais importante, oferecendo uma nova abordagem para se identificar e estudar as interações proteína-proteína na matriz alimentar, em alimentos *in natura* e processados, bem como a interação entre proteínas e outros componentes dos alimentos, como os polissacarídeos estruturais.

A proteômica tem sido utilizada como uma importante ferramenta na exploração sistemática dos mecanismos de crescimento, desenvolvimento e estudo do metabolismo secundário em plantas (Tang et al., 2020; Martínez-Esteso et al., 2015). Cabe destacar que o perfil do proteoma representa uma espécie de impressão digital capaz de confirmar a origem natural e avaliar a genuinidade de frutas e bebidas comerciais, protegendo o consumidor de qualquer tipo de adulteração (Fasoli & Righetti, 2015).

As proteínas, ao contrário dos transcritos de genes, são os efetores diretos das respostas ao estresse das plantas. A investigação nas mudanças do proteoma das plantas é de grande relevância para se entender os mecanismos moleculares subjacentes à tolerância ao estresse da planta. Logo, o estudo proteômico comparativo pode identificar proteínas que mudam em abundância, o que pode ser empregado para se relatar a resiliência da planta ao estresse (Hao et al., 2021). Além disso, o emprego da proteômica, no estudo de frutos, pode auxiliar na revelação de uma série de genes/proteínas que podem estar envolvidos no seu desenvolvimento (Wu et al., 2014) e na construção de sua qualidade (Cai et al., 2018). Por exemplo, notou-se alteração no perfil de proteínas responsáveis pelos processos fisiológicos durante o desenvolvimento de tomates (Szymanski et al., 2017). Em pêssogo, as proteínas expressas diferencialmente (DEPs), identificadas durante o seu amadurecimento e senescência, foram principalmente associadas ao metabolismo de carboidratos, energia, degradação da parede e membrana celular, transdução de sinal e resposta ao estresse (Huan et al., 2019). Segundo Jiang et al. (2020), as proteínas mais importantes associadas ao amadurecimento do pêssogo são a poligalacturonase (PG), pectina liase (PL), pectina esterase (PE), proteínas de choque térmico (HSP), peptidil-prolil isomerase (PPIase), UDP-glicosiltransferase (UGT) e 9-cis-epoxicarotenoide dioxigenase (NCED). A identificação dessas enzimas com papéis funcionais críticos, especialmente as relacionadas à despolimerização de pectinas, fornece o embasamento necessário para se compreender a funcionalidade de polissacarídeos extraídos dessa matriz para fins tecnológicos.

O emprego de análises combinadas (metabolômica, proteômica, transcriptômica) permitiram compreender a via biossintética dos carotenoides e informações abrangentes sobre os eventos biológicos relevantes no amadurecimento da banana (Heng et al., 2019; Li et al., 2019), como a rápida degradação de componentes da parede celular, que culmina com o amolecimento da fruta. Das proteínas diferencialmente expressas, as enzimas de ativação de

carboidratos (CAZmes) desempenham importante papel no metabolismo da parede celular em especial na degradação de seus polissacarídeos. Além disso, proteínas relacionadas ao estresse, defesa, sinalização e metabolismo foram implicadas no amadurecimento da banana (Xiao et al., 2019), sendo relatadas diferenças significativas nos teores de proteínas envolvidas na formação e degradação do amido, metabolismo de açúcar e redução de oxidação de polifenóis (Bhuiyan et al., 2020).

Chin et al. (2019), ao realizarem a análise proteômica comparativa sobre o processo de amadurecimento em duas variedades de manga tropical, verificaram que as variedades em estudo produziram diversos tipos de proteínas expressas de formas diferentes nos estádios verde-maturo e maduro. Essas proteínas foram associadas, principalmente, à síntese de etileno, produção de voláteis, degradação da parede celular e estresse, com um grupo adicional de proteínas relacionadas ao metabolismo de carboidratos. Similarmente, ao estudarem a resposta proteômica de kiwis de diferentes cultivares ao etileno exógeno, Shin et al. (2020) verificaram que 18% e 10% das proteínas foram associadas à resposta de defesa em kiwis “Hayward” e “Garmrok”, respectivamente. As demais proteínas estavam relacionadas à biossíntese proteica e ao ciclo de Calvin durante o amadurecimento das frutas.

Ao investigar o perfil proteômico e metabolômico de uva em função do estágio de desenvolvimento e alta temperatura, Lecourieux et al. (2020) verificaram que as categorias de ontologia genética ‘estresse’, ‘proteína’, ‘metabolismo secundário’ e ‘parede celular’ foram predominantemente alteradas sob altas temperaturas. As temperaturas elevadas prejudicaram fortemente o metabolismo de carboidratos, de forma dependente do estágio de desenvolvimento e da duração do tratamento. Ressalta-se que altas temperaturas alteram as principais proteínas que impulsionam o desenvolvimento e o amadurecimento das frutas. Adicionalmente, estudos recentes têm expandido o uso de análises multiômicas para a caracterização de frutos nativos brasileiros e espécies negligenciadas. O estudo multiômico do pequi (*Caryocar brasiliense*) confirmou seu padrão não climatérico, demonstrando que o metabolismo da metionina é voltado preferencialmente à síntese de compostos secundários, e não de etileno (Da Costa et al., 2024). Segundo esses autores, o amadurecimento é regulado por fatores de transcrição da família MADS-box, proteínas reguladoras responsáveis pelo controle da expressão de genes envolvidos no desenvolvimento e na maturação dos frutos, sendo esse processo marcado pelo aumento da atividade da álcool desidrogenase, que eleva a produção de hexanoato de etila, o composto volátil majoritário (50,68%). Além disso, as enzimas monodesidroascorbato redutase, ascorbato peroxidase, piruvato quinase, frutose-1,6-bifosfato aldolase, fitoeno 15eleifi, fenilalanina amônia-liase, corismato 15eleifi e chalcona-flavonona isomerase foram

identificadas como biomarcadores do acúmulo de vitamina C, carotenoides e fenólicos, definindo a qualidade funcional do fruto maduro (Da Costa et al., 2024).

De forma semelhante, o emprego dessas ferramentas em frutos de *Annona crassiflora* (marolo) permitiu revelar que o estágio imaturo é caracterizado por um intenso investimento na maquinaria de síntese proteica, impulsionado por biomarcadores como o fator de iniciação da tradução eucariótica 5^a, o fator de iniciação eucariótica 4^a-9 e a espermidina 16eleifi 1 (Da Costa et al., 2025). Esse desenvolvimento é finamente regulado por fatores de transcrição das famílias GATA, MADS-Box, MYB, F-box e HD-Zip, que coordenam processos críticos como o sistema antioxidante e a degradação da parede celular. Ao atingir a maturação, o fruto consolida-se como uma matriz rica em compostos bioativos, incluindo o ácido ascórbico, carotenoides, alcaloides e compostos fenólicos, com um perfil aromático predominantemente composto por ésteres voláteis (Da Costa et al., 2025).

Já na gabirola (*Campomanesia pubescens*), a análise multiômica permitiu revelar que o amadurecimento é marcado pela transição metabólica da fotossíntese para a produção de energia e defesa (Machado et al., 2025). A redução da enzima ribulose-1,5-bifosfato carboxilase/16eleifican (RuBisCO) sinaliza a conversão de cloroplastos em cromoplastos, enquanto o aumento da fosfoglicerato quinase e da frutose-bifosfato aldolase intensifica a glicólise. O acúmulo de nutrientes e compostos de defesa é impulsionado pela ativação da fenilalanina amônia-liase e da *o*-metiltransferase, resultando em altos teores de trigonelina e ácido gálico. Além disso, a regulação da S-adenosilmetionina 16eleifi confirma a importância da biossíntese de etileno na coordenação fisiológica desse fruto (Machado et al., 2025).

O estudo proteômico fornece abundantes recursos para um estudo mais aprofundado sobre o desenvolvimento e qualidade de frutos (Zhang et al., 2021). Além de fornecer informações para se entender as redes regulatórias que controlam o amadurecimento (Wang et al., 2021), tal abordagem é de grande interesse para aumentar o conhecimento acadêmico e para o melhoramento de estratégias agrícolas e econômicas. O emprego da análise citada tem contribuído para se compreender as transições dos frutos do estágio imaturo ao maduro; contudo, devido às características inerentes a cada fruta, mais pesquisas devem ser realizadas, pois cada espécie cultivada ou silvestre exibe eventos diferentes que lhes atribuem características próprias. Assim, ao se investigar o tráfego de proteínas, bem como a transdução de sinal e a sinalização molecular, é possível uma melhor compreensão da imagem metabólica sistêmica completa exibida ao longo do desenvolvimento e amadurecimento dos frutos (Palma; Carpas & Río, 2011).

Pode-se dizer, então, que uma compreensão mais detalhada do perfil proteico como um parâmetro de qualidade dos alimentos está se tornando cada vez mais importante para impulsionar a transformação do setor alimentício rumo ao aumento da sustentabilidade, biodiversidade e disponibilidade de alimentos saudáveis. Com todos os diferentes aspectos da qualidade alimentar, incluindo segurança, autenticidade, aspectos sensoriais e requisitos nutricionais, a proteômica ajudará a otimizar as tecnologias de produção e processamento (Geisslitz & Scherf, 2021). Desse modo, o emprego de análises multiômicas em pseudofrutos de *Hovenia dulcis* ao longo do desenvolvimento pode auxiliar no entendimento de sua fisiologia e da forma como os metabólitos secundários são sintetizados e acumulados durante o seu desenvolvimento.

2.3 Polissacarídeos de fontes vegetais

Nos últimos anos, os polissacarídeos de origem vegetal têm sido amplamente estudados por seus efeitos antioxidantes, bacteriostáticos, antitumorais, antivirais, imunomoduladores, anti-inflamatórios, antirradiação, probióticos e antidiabéticos. Esses biopolímeros apresentam amplas aplicações em alimentos funcionais, biomedicina e produtos farmacêuticos, devido a propriedades funcionais como elevada hidrofiliabilidade (ou hidrofobicidade, dependendo do seu contexto), características reológicas ideais e a capacidade de atuar como agentes 17eleificante, espumantes, emulsificantes e espessantes (Yang et al., 2020).

Dentre essas fontes vegetais, os polissacarídeos de *Hovenia dulcis* têm recebido grande atenção da comunidade científica, especialmente no Oriente, devido ao seu potencial em interferir na atividade das enzimas etanol e lactato desidrogenase (Zhang et al., 2024). Essa bioatividade desempenha um papel substancial na atenuação dos sintomas da ressaca, além de auxiliar na prevenção e tratamento da hepatite, esteatose hepática, cirrose e distúrbios neurológicos relacionados ao álcool (Liu et al., 2024). Tais benefícios caracterizam essa espécie como uma matéria-prima promissora para o desenvolvimento de medicamentos e alimentos saudáveis (Liu et al., 2024; Zhang et al., 2024; Zhang et al., 2026), incluindo a possibilidade de seu uso na elaboração de embalagens comestíveis.

Contudo, para sua aplicação industrial, o isolamento adequado de suas frações é uma etapa crucial. Historicamente, a extração de polissacarídeos de *Hovenia dulcis* baseia-se no método clássico por água quente seguida de precipitação alcoólica, embora técnicas modernas e ecoeficientes, como a extração assistida por ultrassom, micro-ondas e enzimas, venham ganhando espaço para otimizar o rendimento (Yang et al., 2019; Yang et al., 2020; Yang et al.,

2022; Zhang et al., 2024; Zhu et al., 2025). Ressalta-se, entretanto, que o sucesso desse processo depende rigorosamente do controle de parâmetros operacionais, tais como a temperatura, o tempo de reação e a proporção entre o solvente e a matéria-prima (Liu et al., 2020). Além disso, etapas subsequentes de desproteinização e centrifugação são indispensáveis para isolar as frações purificadas de interesse biológico (Zhang et al., 2024).

Uma vez isoladas essas macromoléculas, a compreensão de suas propriedades físicas é fundamental para a sua viabilidade industrial. A massa molecular dos polissacarídeos de *Hovenia dulcis* varia amplamente de 3,1 kDa a 5.794 kDa. Essa ampla variação pode estar relacionada ao tratamento de secagem pré-extração das matérias-primas, aos métodos de extração, às técnicas de purificação e aos métodos analíticos. Geralmente, maior massa molecular em polissacarídeos vegetais corresponde a uma proporção menor de polissacarídeos de baixa massa molecular em seu produto hidrolisado, potencialmente levando a uma maior biodisponibilidade (Zhang et al., 2024).

Além da massa molecular, as propriedades reológicas são determinantes para o comportamento desses compostos. Apesar de os polissacarídeos do pseudofruto de *Hovenia dulcis* apresentarem uma viscosidade aparente com comportamento de afinamento por cisalhamento, ainda são necessários estudos adicionais para discernir se o seu comportamento é estritamente não newtoniano ou próximo do newtoniano (Zhang et al., 2024). Contudo, sabe-se que diferentes métodos de extração produzem diferentes componentes polissacarídicos, o que leva a variações na viscosidade aparente dos polissacarídeos extraídos (Yang et al., 2019).

Essas variações físicas e reológicas ocorrem porque a atividade biológica dos polissacarídeos está intrinsicamente ligada às suas estruturas, especialmente à composição e proporção de vários monossacarídeos (Zhang et al., 2024). Os polissacarídeos de *Hovenia dulcis* são compostos por Manose (Man), Ramnose (Rha), Ácido galacturônico (GalA), Glicose (Glc), Galactose (Gal), Arabinose (Ara) e Fucose (Fuc), esses açúcares apresentaram proporções molares de, respectivamente 2,94:16,03:4,45:21,62:24,81:26,97:3,18 na fração extraída por solvente acelerado (ASE-HDPs) e de 4,52:19,05:21,28:5,09:22,09:22,61:5,36, naquela obtida por água quente (HWE-HDPs). Em termos de razão molar, os principais monossacarídeos foram Rha, Glc, Gal, Ara na fração ASE-HDPs, e Rha, GalA, Gal, Ara na fração HWE-HDPs (Yang et al., 2019). A literatura também relata a presença de um polissacarídeo complexo do tipo pectina, constituído por homogalacturonana (HG) e rhamnogalacturonana-I (RG-I). O HG caracteriza-se como um polímero de resíduos de α -1,4-D-GalpA que pode carregar grupos éster metílicos e também pode ser acetilado no esqueleto de galacturonana. Por sua vez, o esqueleto de RG-I contém o dissacarídeo médio de $[\rightarrow 4-\alpha$ -

GalpA – (1→2) – α - Rhap- (1 →]n com regiões ramificadas, que podem estar ligadas na Rha na posição O-4 (Wang et al., 2017). Contudo, discrepâncias na composição dos monossacarídeos e nas proporções molares entre esses polissacarídeos podem ser observadas em decorrência dos diferentes métodos de secagem, técnicas de separação e purificação, e metodologias analíticas (Zhang et al., 2024).

Para elucidar a organização tridimensional dessas estruturas, técnicas avançadas de microscopia são empregadas. A análise por Microscopia de Força Atômica (MFA) revelou que, em água, os polissacarídeos de *Hovenia dulcis* apresentavam uma morfologia irregular das partículas poliméricas, sem apresentar conformação linear de cadeia. Isso sugere a ocorrência de agregação entre as moléculas da cadeia de açúcar dos polissacarídeos, formando partículas poliméricas por meio da reticulação de polissacarídeos. Além disso, as análises de MFA indicaram que o polissacarídeo em estudo apresentou uma altura molecular de 4,3 nm, valor que supera significativamente a faixa usual de 0,1 nm a 1,0 nm característica de polissacarídeos de fita simples. Essa divergência sugere a atuação de forças de van der Waals entre as cadeias macromoleculares, promovendo a agregação de filamentos únicos em estruturas poliméricas maiores. Como consequência, o diâmetro mensurado torna-se superior ao de uma molécula isolada (Zhang et al., 2024).

Como consequência direta dessa complexidade estrutural, a gama de aplicações funcionais torna-se bastante vasta. Dentre as atividades biológicas dos polissacarídeos de *Hovenia dulcis*, destacam-se as ações antiglicosilante, hipoglicêmica, antioxidante, antifadiga, a regulação imunológica e a proteção contra a doença hepática alcoólica. Esse panorama tem contribuído para seu emprego na área alimentícia, como no desenvolvimento de cápsulas, no aumento da funcionalidade de matrizes alimentares e como realçador de cor em vinhos. Além disso, vale destacar que *Hovenia dulcis* também contém pectinas que, quando incorporadas a produtos panificados, elevam o volume da massa, reduzem a necessidade de farinha, prolongam o tempo de maturação e conferem elasticidade e sabor agradável. Os polissacarídeos de pectina de *Hovenia Dulcis* não apenas manifestam os efeitos típicos das pectinas, mas também possuem uma notável capacidade de retenção de líquido (Zhang et al., 2024).

Diante das propriedades estruturais e biológicas descritas, aliadas à escassez de investigações sobre o uso dessa matriz como biopolímero na conservação pós-colheita de frutos, evidencia-se o potencial de *Hovenia dulcis*, passível de ser explorado cientificamente na área de tecnologia de alimentos, especialmente no desenvolvimento de barreiras protetoras biológicas, como os revestimentos comestíveis.

2.4 Revestimentos comestíveis na conservação de frutos

Revestimentos comestíveis à base de biopolímeros têm sido propostos para a conservação de alimentos e produtos alimentícios, visto que auxiliam no prolongamento da vida útil e funcionalidade, mantendo os atributos de qualidade, sensoriais e nutricionais dos produtos, preservando o frescor, reduzindo o desperdício e contribuindo para a eficiência econômica dos materiais de embalagem (Petkoska et al., 2021; Kumar et al., 2023). Contudo, é necessário considerar o material a partir do qual o filme será elaborado, o tipo de alimento sobre o qual o filme será aplicado e o método de aplicação para se potencializarem os efeitos dos filmes (Iversen et al., 2022).

Os plásticos convencionais, usados normalmente na produção de embalagens, são derivados do petróleo e apresentam baixa degradabilidade, o que coloca em xeque o equilíbrio do meio ambiente e a sustentabilidade. Logo, urge a necessidade de se encontrar e colocar em uso alternativas aos plásticos convencionais. Com efeito, a tecnologia que envolve os revestimentos comestíveis é considerada uma alternativa sustentável e biodegradável no campo das embalagens ativas de alimentos quando comparada às embalagens convencionais. Deste modo, as áreas de desenvolvimento e aplicação de filmes comestíveis estão em ascensão, devido à sua versatilidade, ao seu potencial de produção a partir de uma grande diversidade de materiais e como fonte de diferentes substâncias ativas, como agentes antioxidantes e antimicrobianos (Petkoska et al., 2021; Teixeira-Costa & Andrade, 2022; Pillai et al., 2024).

Os materiais empregados na produção desses revestimentos são polímeros naturais, oriundos de fontes comestíveis. Esses materiais podem ser modificados e dar origem a filmes e revestimentos sem diferença específica em sua composição material, mas com diferentes espessuras. Os filmes são usualmente empregados na elaboração de envoltórios, bolsas, sacos, cápsulas e invólucros, enquanto os revestimentos são empregados diretamente sobre a superfície do alimento (Petkoska et al., 2021). Contudo, tanto os filmes como os revestimentos possuem a finalidade de melhorar as barreiras aos gases e à umidade, além de proteger os produtos de danos e contaminação mecânica, química e microbiológica (Hamed; Jakobsen & Lerfall, 2022).

Os materiais empregados na elaboração de embalagens comestíveis são polissacarídeos, proteínas, lipídios, compósitos (Carina et al., 2021; Daniloski et al., 2021; Hamed; Jakobsen & Lerfall, 2022; Petraru & Amariei, 2023), nanomateriais (como nanopartículas e nanocompósitos) e óleos essenciais (Jafarzadeh et al., 2021; Petkoska et al., 2021; Perez-Vazquez et al., 2023). Esses materiais devem apresentar algumas características, a saber: ser

seguros para consumo, isentos de componentes tóxicos e alergênicos; ter propriedades mecânicas adequadas para evitar danos na superfície do alimento durante a manipulação; aderir à superfície do alimento; ser insípidos ou possuir sabor agradável; apresentar estabilidade no tempo e evitar o desenvolvimento de mofo; ter característica higroscópica controlada; manter a troca gasosa adequada de oxigênio e dióxido de carbono; promover a melhoria das propriedades estruturais; e apresentar baixo custo e fabricação fácil e economicamente viável (Avramescu et al., 2020).

A aplicação mais evidente dos revestimentos comestíveis se dá em frutas e hortaliças. Sabe-se que as perdas mais evidentes da qualidade de frutas e hortaliças *in natura* ocorrem, sobretudo, ao longo do armazenamento e transporte. Geralmente, essas perdas são caracterizadas pela perda de umidade, perda de massa, alterações microbianas e bioquímicas, danos mecânicos e alterações sensoriais que devem ser minimizadas ou evitadas. Deste modo, tendo como premissa que o processo respiratório destes alimentos continua após a colheita, a aplicação de um revestimento comestível em frutas e hortaliças frescas poderá afetar a permeabilidade aos gases, sobretudo a difusão de oxigênio, inibindo, assim, o processo de amadurecimento (Petkoska et al., 2021). Em contraposição às vantagens, a limitação das trocas gasosas por meio do revestimento comestível pode promover o desenvolvimento de condições anaeróbicas que levam à fermentação alcoólica, culminando em alterações negativas nas características sensoriais dos alimentos embalados (Petkoska et al., 2021). Logo, cuidados devem ser assumidos para se evitar que isso aconteça.

As pesquisas empregando revestimentos comestíveis em frutos nos últimos anos têm se elevado, com relatos de sua aplicação, por exemplo, em ameixa, banana, morango, nectarina e tangerina (Ansarifar & Moradinezhad, 2021; Sowmyashree et al., 2021; Bhan et al., 2022; Hu et al., 2022; Avci et al., 2023). Nos trabalhos citados, verificou-se que o uso do revestimento melhorou a qualidade e estendeu a vida pós-colheita dos frutos.

Apesar dos benefícios da utilização de polissacarídeos na formulação de embalagens alimentícias sustentáveis, ainda existem gargalos importantes a serem superados para sua incorporação em larga escala. Dentre os principais desafios, destacam-se a presença de sabores e cores residuais pouco atraentes provenientes de suas fontes naturais e a dificuldade na padronização da espessura do filme. Além disso, a seleção criteriosa do polímero ideal e do método de aplicação adequado para revestimentos de base vegetal continua sendo um obstáculo técnico complexo (Gupta et al., 2024).

Diante do exposto, embora existam limitações técnicas a serem superadas, a utilização de polissacarídeos de *Hovenia dulcis* no desenvolvimento de revestimentos biodegradáveis

representa um avanço estratégico para mitigar o desperdício alimentar. Além de promover a sustentabilidade na cadeia de suprimentos de frutas, a caracterização e aplicação dessa matriz vegetal consolidam a inserção de um novo ingrediente funcional com propriedades tecnológicas comprovadas e de grande interesse para o setor de embalagens alimentícias principalmente para a aplicação em morangos.

2.5 Resvestimentos comestíveis em morango

O morango (*Fragaria* × *ananassa*) é uma espécie nativa de regiões de clima subtropical, atualmente cultivada em praticamente todo o mundo, desde regiões subtropicais até tropicais (Farida et al., 2023). Trata-se de uma das frutas mais consumidas globalmente, devido às suas características sensoriais atrativas coloração e sabor, além de seu elevado valor nutricional (Almenar et al., 2007). Embora sejam classificados como frutos não climatéricos, os morangos apresentam vida pós-colheita extremamente curta em razão de sua elevada atividade metabólica, alta suscetibilidade à decomposição fúngica e rápida deterioração, fatores que comprometem suas características sensoriais, nutricionais e químicas (Pinzon et al., 2020; Nunes et al., 2021).

A elevada perecibilidade constitui um dos principais desafios da cadeia produtiva de frutas e hortaliças. Os morangos são particularmente suscetíveis à perda de qualidade durante o armazenamento em decorrência de danos mecânicos, elevada taxa respiratória, perda de água, deterioração microbiológica e processos naturais de senescência (De Bruno et al., 2023). Dessa forma, reduzir a velocidade de deterioração desses frutos representa um desafio de grande relevância sob os pontos de vista tecnológico, econômico e ambiental. Além disso, o aumento da demanda por frutos frescos, associado às elevadas perdas qualitativas e quantitativas observadas após a colheita, tem impulsionado o desenvolvimento de estratégias capazes de prolongar a vida útil, preservar a qualidade e minimizar perdas e desperdícios ao longo da cadeia produtiva (De Bruno et al., 2023).

Tradicionalmente, a principal estratégia utilizada para prolongar a vida útil dos morangos consiste no armazenamento sob refrigeração. Entretanto, embora o armazenamento em baixas temperaturas reduza a taxa respiratória e retarde os processos de deterioração, essa prática, quando empregada isoladamente, não é suficiente para preservar a qualidade dos frutos durante toda a cadeia de comercialização. Isso ocorre porque os morangos frequentemente são submetidos ao transporte e à comercialização em temperatura ambiente, condição que favorece a rápida perda de qualidade devido à elevada perecibilidade da espécie (Saleem et al., 2021).

Nesse contexto, os revestimentos comestíveis têm se destacado como uma alternativa promissora para prolongar a vida útil de frutas frescas e minimamente processadas. Esses revestimentos atuam como barreiras semipermeáveis à transferência de gases e vapor de água, reduzindo a taxa respiratória, retardando a perda de umidade e preservando as características físico-químicas e sensoriais durante o armazenamento (Yousuf, Qadri & Srivastava, 2018). Além disso, podem ser produzidos a partir de diferentes biomateriais, incluindo carboidratos, proteínas, lipídios ou formulações multicomponentes, permitindo o desenvolvimento de sistemas com propriedades específicas para diferentes aplicações (Pinzon et al., 2020).

Entre os materiais empregados na formulação de revestimentos comestíveis, os polissacarídeos destacam-se devido à sua biodegradabilidade, biocompatibilidade e capacidade de formação de filmes. Esses materiais incluem diversas gomas naturais, amidos, dextrinas, pectinas, celulose e seus derivados, quitosana, alginato e carragenanas, entre outros (Yousuf, Qadri & Srivastava, 2018). Dentre eles, o alginato de sódio é um dos biopolímeros mais amplamente estudados. Trata-se de um polissacarídeo aniônico naturalmente obtido e refinado a partir de diferentes espécies de algas pardas, considerado seguro para consumo pela *Food and Drug Administration* (FDA) dos Estados Unidos e aprovado como aditivo alimentar pela Comissão Europeia (CE) (Parreidt; Müller, & Schmid, 2018; Alharaty & Ramaswamy, 2020). Em solução aquosa, o alginato de sódio apresenta elevada capacidade de formação de filmes e revestimentos; entretanto, para reduzir sua solubilidade em água e aumentar sua resistência mecânica, torna-se necessária a adição de íons divalentes, como o cálcio. Esses íons promovem ligações cruzadas entre as cadeias poliméricas, formando uma estrutura tridimensional conhecida como modelo *egg-box*, responsável pela formação de géis densos e mecanicamente estáveis (Parreidt; Müller, & Schmid, 2018).

Além dos biopolímeros, compostos bioativos naturais vêm sendo incorporados aos revestimentos comestíveis com o objetivo de conferir propriedades antioxidantes e antimicrobianas. Entre esses compostos destacam-se os óleos essenciais, que são líquidos aromáticos e voláteis extraídos de diferentes partes das plantas, como flores, botões florais, sementes, folhas, cascas, caules, frutos e raízes. Estudo demonstra que os óleos essenciais e seus constituintes apresentam atividade antimicrobiana contra microrganismos deteriorantes e patógenos de origem alimentar, embora sua eficácia possa variar de acordo com a matriz alimentícia. De modo geral, compostos fenólicos presentes nesses óleos atuam promovendo alterações na permeabilidade das membranas celulares dos microrganismos, sendo bactérias Gram-positivas normalmente mais sensíveis que Gram-negativas. Contudo, fatores como possíveis interações entre os constituintes dos óleos essenciais e os componentes dos alimentos,

além de efeitos sinérgicos ou antagônicos entre seus compostos, ainda demandam investigações adicionais para ampliar sua aplicação comercial (Burt, 2004).

Entre os compostos bioativos mais empregados destaca-se o eugenol, um composto fenólico aromático presente principalmente em espécies pertencentes às famílias *Lamiaceae*, *Lauraceae*, *Myrtaceae* e *Myristicaceae*, sendo o principal constituinte do óleo essencial de cravo (*Syzygium aromaticum*) (Ulanowska & Olas, 2021). Trata-se de um líquido oleoso de coloração incolor a amarelo-claro, pouco solúvel em água e altamente solúvel em solventes orgânicos, caracterizado por aroma intenso e picante. O eugenol apresenta reconhecidas propriedades antioxidantes, antimicrobianas, anti-inflamatórias e analgésicas, características que despertam crescente interesse para sua incorporação em revestimentos comestíveis destinados à conservação pós-colheita de frutas. Sua associação com matrizes poliméricas, como o alginato de sódio, possibilita o desenvolvimento de sistemas de revestimento capazes de atuar simultaneamente como barreira física e como veículo para liberação controlada de compostos bioativos, contribuindo para a redução da deterioração microbiológica e oxidativa e, conseqüentemente, para a manutenção da qualidade e o prolongamento da vida útil dos morangos (Burt, 2004; Ulanowska & Olas, 2021).

Portanto, considerando a eficácia dos revestimentos comestíveis à base de alginato de sódio, as propriedades antioxidantes e antimicrobianas do eugenol e o potencial tecnológico dos polissacarídeos extraídos de *Hovenia dulcis*, a combinação desses materiais constitui uma estratégia inovadora e promissora para o desenvolvimento de revestimentos comestíveis destinados à conservação pós-colheita de frutas e hortaliças. Apesar dos avanços obtidos com o emprego de biopolímeros convencionais, ainda são escassos os estudos que exploram o uso de polissacarídeos provenientes de *Hovenia dulcis* em sistemas de revestimento, evidenciando uma lacuna científica que justifica o desenvolvimento de pesquisas voltadas à avaliação de sua aplicação, especialmente na conservação de morangos, uma das frutas mais suscetíveis às perdas pós-colheita.

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**ARTICLE 1 - BUILDING *Hovenia dulcis* PSEUDOFRUIT QUALITY
FROM PROTEOMIC AND METABOLOMIC PERSPECTIVES**

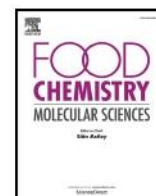
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 Sugars. Oxidative stress

ABSTRACT

Hovenia dulcis Thunb. (Japanese grape tree) pseudofruit exhibits a period of growth of 180 days, with maturation initiated between 120 and 150 days after anthesis (DAA). Effective ripening occurs 150 DAA, characterized by intense softening, pectic solubilization and starch-sugar conversion. Multi-omic analysis identified 496 proteins, with 222 being differentially expressed. Significant highlights included energy metabolism (Malate Dehydrogenases, Glyceraldehyde-3-phosphate Dehydrogenase, Pyruvate Kinase) and photosynthetic pathways (Ribulose-1,5-bisphosphate Carboxylase/Oxygenase, Photosystems I and II), indicating that ripening demands a high energy supply. Multivariate analysis stratified the development into three phases: chemical defense (S1-S2), metabolic transition (S3-S6), and sensory ripening (S7). The Variable Importance in Projection (VIP) score and Principal Component 1 (PC1) loadings confirmed that the transition is governed by changes in volatile compounds (2-heptanol), respiratory physiology, density, and Hue angle, with a central role for malate dehydrogenase. Pearson correlations revealed a coordinated system in which central metabolism (Glyceraldehyde-3-phosphate Dehydrogenase, Triose Phosphate Isomerase) is coupled with ethylene signaling (S-adenosylmethionine Synthetase) and antioxidant defenses (Superoxide Dismutase, Catalase, and Peroxiredoxin 2). It is concluded that *H. dulcis* development is sustained by a strategic proteometabolic network that redirects the investment from protective biomolecules toward the specialization of sensory attributes, defining the final quality of the pseudofruit.

1. Introduction

Hovenia dulcis Thunb. is a deciduous perennial tree belonging to the Rhamnaceae family, widely distributed across Asia, particularly in China, Japan, and Korea (Peng et al., 2018; Thi Ngo et al., 2021; Xu et al., 2020). The species is noted for its ethnobotanical importance, having been used for centuries in Asian culinary and medicine (Morales et al., 2017). Its various parts, including fruits, pseudofruits, seeds, leaves, roots, and bark, are extensively employed in traditional Oriental medicine due to their therapeutic properties (Song et al., 2020).

The pseudofruits of this species, popularly known as the Japanese grape tree, are the most consumed parts (Peng et al., 2018; Song et al., 2020). However, the seeds also stand out as a significant source of natural antioxidants, offering protective effects against oxidative stress-related conditions, such as alcoholic liver disease (Meng et al., 2020). Similarly to cashew apple (*Anacardium occidentale*), the floral peduncle develops into a flavorful and succulent pseudofruit. During maturation, the twisted peduncles of the Japanese grape tree become swollen, irregular, and fleshy (3–7 mm in diameter), acquiring a reddish-brown coloration and a sweet flavor due to high sucrose content

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(De Biaggi et al., 2020; Yang et al., 2019). Beyond *in natura* consumption, these pseudofruits can be processed into wines, juices, fermented alcoholic beverages, jams, jellies, and raisins, or used as a source of antioxidants in processed meat products (Machado & Gonçalves, 2021; Pinto et al., 2017; Schaefer et al., 2022; Xu et al., 2020).

Changes in physical properties and nutritional composition during maturation of *H. dulcis* pseudofruits have been reported in the literature (Maievas et al., 2015; Maievas et al., 2017). The chemical composition of these pseudofruits is characterized by high sugar levels and presence of bioactive compounds such as flavonoids, lignans, sesquiterpenoids, triterpenoids, and benzoic acid. These substances provide the pseudofruits with diverse biological properties, including antioxidant, antimicrobial, antidiabetic, anti-inflammatory, anti-obesity, and laxative activities (Maievas et al., 2015; Choi et al., 2017; Morales et al., 2017; Peng et al., 2018; Yang et al., 2019; De Biaggi et al., 2020; Oh et al., 2020; Song et al., 2020; De Godoi et al., 2021; Sferazza et al., 2021; Thi Ngo et al., 2021). Furthermore, studies indicate that both the seeds and pseudofruits of *H. dulcis* possess phytotherapeutic potential in treating various diseases, particularly alcoholism and liver disorders (Choi et al., 2017; Kim et al., 2017; Kim et al., 2023; Qiu et al., 2019; Wang et al., 2017).

Despite the extensive knowledge regarding the pharmacological, nutritional, and functional properties of the species, the molecular processes regulating the synthesis and accumulation of nutrients and bioactive compounds throughout pseudofruit development remain unknown. Therefore, investigating these mechanisms could significantly contribute to the valorization of the species and expand its application potential in the food and pharmaceutical industries. Moreover, understanding the molecular mechanisms underlying the construction of quality in fruits and pseudofruits is fundamental to supporting genetic improvement and domestication efforts, as well as guiding best production practices, with emphasis on mineral fertilization, irrigation, and thinning.

In this context, proteomic analysis emerges as an essential tool for understanding the molecular mechanisms involved in fruit and pseudofruit development. This approach allows for the global identification and quantification of proteins expressed at different developmental stages, providing detailed information on the biochemical processes underlying maturation and the production of secondary metabolites (Chen et al., 2020). Deciphering the molecular determinants of quality is vital for subsidizing domestication and breeding programs, as well as directing precision agronomic practices, such as mineral fertilization management and harvest point optimization. Consequently, the present study proposes an integrated multi-omic investigation, combining physical, physicochemical, and chemical analyses with high-resolution proteomics. The objective is to map biological transformations from anthesis to ripening, elucidating how the coordination between central metabolism and specialized metabolite pathways defines the technological potential of this species.

2. Materials and methods

2.1. Experimental design

The experiment followed a completely randomized design (CRD), consisting of seven treatments (developmental stages, from S1 to S7) and three replicates. Three distinct trees were used as biological replicates for each treatment. All laboratory analyses were performed in technical triplicate.

2.2. Plant material

H. dulcis pseudofruits were harvested from trees located at the Federal University of Lavras (UFLA), Minas Gerais, Brazil (latitude 21° 14' 43" S, longitude 44° 59' 59" W, and altitude of 919 m). The selected trees were situated in the same experimental area under uniform

edaphoclimatic conditions, ensuring that all biological replicates were exposed to the same environmental factors. Harvest dates were defined based on the peak flowering period, which occurred on October 25, 2022. Sampling intervals of 30 days were established, considering the long developmental cycle of the pseudofruit, as previously described (Maievas et al., 2015). For each developmental stage, approximately 500 g of pseudofruits were collected from different quadrants of the canopy of each of the three independent trees, to ensure the representativeness of the biological material and minimize environmental variations. The complete developmental cycle of the pseudofruits spanned 210 days, resulting in seven sample collections designated as S1, S2, S3, S4, S5, S6, and S7, corresponding to 30, 60, 90, 120, 150, 180, and 210 days after anthesis (DAA), respectively (Fig. 1).

Pseudofruits were collected from November 2022 to May 2023 and transported in insulated boxes to the Fruit and Vegetable Postharvest Laboratory at the Federal University of Lavras. In the laboratory, the *H. dulcis* pseudofruits consisting of the fleshy peduncle and the seeds attached to their external surface were separated. A portion of the pseudofruits was pulverized using liquid nitrogen and stored at -80°C (Coddlab, model CL374-86 V, Piracicaba, Brazil) for subsequent molecular and physicochemical analyses; the seeds were discarded.

Texture, respiratory activity, color, density, and mass analyses were performed on the fresh peduncles.

2.3. Analyses

2.3.1. Mass

The mass of 10 pseudofruits per replicate was evaluated using a semi-analytical balance (Mettler Toledo, model PC 2000, Greifensee, Switzerland), and the results were expressed in grams (g).

2.3.2. Respiratory rate

Approximately three grams of pseudofruits were placed in 50 mL glass flasks, hermetically sealed, and maintained at a controlled temperature of 25°C to ensure metabolic homogeneity. The CO_2 concentration was determined using a PBI Dansensor gas analyzer (CheckPoint model, Ringster, Denmark), after 1 h 30 min of rest. Results, expressed as % of CO_2 , were converted to $\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$.

2.3.3. Density

Density was measured using an analytical balance (Shimadzu, model AUX 320, Kyoto, Japan) equipped with a specific density measurement kit, model SMK 401.

2.3.4. Coloration

Thirty pseudofruits per replicate were analyzed for skin color using a colorimeter (Konica Minolta, model CR-400, Osaka, Japan) in the L^* , a^* , b^* , h° , C^* color space of the International Commission on Illumination (CIE).

2.3.5. Total chlorophyll

Total chlorophyll content was determined spectrophotometrically



Fig. 1. *H. dulcis* pseudofruits at different stages of development.

(Biochrom, model EZ Read 2000, Cambridge, United Kingdom) in accordance to [Paradiso et al. \(2018\)](#), with results expressed in mg 100 g⁻¹ of sample.

2.3.6. Total carotenoids

Carotenoids were extracted and quantified using the spectrophotometric method (Biochrom, model EZ Read 2000, Cambridge, United Kingdom) as described by [Rodríguez-Amaya \(2001\)](#), with adaptations. Results were expressed in mg 100 g⁻¹ of sample.

2.3.7. Anthocyanins

Total anthocyanin content was estimated spectrophotometrically according to [Lee and Francis \(1972\)](#), adapted by [Barcia et al. \(2012\)](#). Results were expressed as milligrams of cyanidin-3-glucoside 100 g⁻¹ of sample.

2.3.8. Starch

Extraction was performed according to [Zanandrea et al. \(2009\)](#). Glucose resulting from starch hydrolysis was determined as described by [Dische \(1962\)](#), using anthrone reagent (Sigma-Aldrich, St. Louis, MO, USA). Results were expressed as grams of glucose 100 g⁻¹ of sample.

2.3.9. Firmness

Determined in 30 pseudofruits per replicate at three different points using a texture analyzer (Stable Micro Systems, model TA.XT2i, Godalming, United Kingdom) with a TA39 cylindrical probe (2 mm diameter). Test parameters were: pre-test speed 1 mm/s, test speed 2.0 mm/s, post-test speed 10.00 mm/s, and a trigger force of 5 g. Results were expressed in Newtons (N).

2.3.10. Soluble and Total pectin

Total and soluble pectins were extracted by precipitation in a hydroethanolic solution (95%) (Sigma-Aldrich, St. Louis, MO, USA) according to [McCreaddy and McComb \(1952\)](#). Quantification was performed via colorimetric method (Biochrom, model EZ Read 2000, Cambridge, United Kingdom) at 530 nm using carbazol (Sigma-Aldrich), as the chromogenic agent ([Bitter & Muir, 1962](#)). Results were expressed in mg of galacturonic acid 100 g⁻¹ of sample.

2.3.11. pH, titratable acidity (TA), and soluble solids (SS)

Samples were ground in water at a 1:3 (w/v) ratio and filtered through organza. The filtrate was used for pH, TA, and SS determinations. pH was measured using a digital pH meter (TECNAL, TEC-7, Piracicaba, Brasil), calibrated with buffer solutions (pH 4.0 and 7.0). TA was determined by titration with 0.01 N sodium hydroxide (NaOH; Sigma-Aldrich, St. Louis, MO, USA) using phenolphthalein (Sigma-Aldrich) as an indicator, according to [Association of Official Analytical Chemistry \(2019\)](#). Results were expressed in mg of malic acid 100 g⁻¹ of sample. SS were determined using a digital refractometer (ATAGO, model PR-100, Tokyo, Japan) with automatic temperature compensation, and the results were expressed as %, as described by [Association of Official Analytical Chemistry \(2019\)](#).

2.3.12. Total sugars (TS)

Total sugars were extracted in 95% ethanol and determined spectrophotometrically (Biochrom, model EZ Read 2000, Cambridge, United Kingdom) at 620 nm after alcohol evaporation, using anthrone (Sigma-Aldrich, St. Louis, MO, USA) as the chromogenic agent ([Dische, 1962](#)). The results were expressed as grams of glucose 100 g⁻¹.

2.3.13. Vitamin C

Vitamin C content was determined by a colorimetric method using 2,4-dinitrophenylhydrazine (Sigma-Aldrich, St. Louis, MO, USA), and the results were expressed as mg of ascorbic acid 100 g⁻¹ ([Strohecker and Henning, 1967](#)).

2.3.14. Scanning Electron microscopy (SEM)

For the cell wall morphological analysis, samples of pseudofruits at different developmental stages were cut with a scalpel and placed into microtubes containing modified Karnovsky fixative, where they were kept until sample preparation. For specimen preparation, the samples were fractured in liquid nitrogen and dehydrated through an acetone gradient, followed by critical-point drying (Bal-Tec, model CPD 030, Balzers, Liechtenstein). Subsequently, the specimens were mounted on stubs, coated with gold using a sputter coater (Bal-Tec, model CPD 030, Balzers, Liechtenstein), and observed under a field-emission scanning electron microscope (Tescan, model MIRA3, Brno, Czech Republic) at an accelerating voltage of 10 keV.

2.3.15. Histochemistry

2.3.15.1. Dehydration. Samples fixed in Karnovsky solution were dehydrated through a graded ethanol series (25%, 50%, 75%, 90%, and two changes of 100%), with vacuum applied during the first four steps. In the final steps, the tubes were left open. After dehydration, infiltration was carried out with a mixture of activated resin and ethanol (1:1, v/v), followed by vacuum for 15 min, and the samples were stored at 4 °C for 48 h. Subsequently, samples were immersed in pure resin, subjected to vacuum, and stored for 94 h.

2.3.15.2. Embedding. For embedding, the inclusion resin was prepared by mixing activated resin and hardener (7.5:0.5 mL) on ice. The molds were partially filled and placed in an oven at 60 °C for 10 min. The samples were added before the second polymerization step, which lasted 24 h. After curing, the blocks were mounted on wooden cubes and stored with silica gel for 24 h. Sections were obtained using an ultramicrotome (Reichert-jung, Ultracut model, Vienna, Austria). The sections were then mounted on slides, stained with Lugol's solution, and observed under a stereoscopic microscope with epifluorescence (Nikon, model SMZ 1500, Tokyo, Japan).

2.3.16. Phenolic profile

Phenolic compounds were analyzed as described by [Machado et al. \(2024b\)](#), using high-performance liquid chromatography (HPLC-DAD-UV-Vis). Identification was performed based on retention times, which were compared with standards of catechin (CAT), vanillin (VAN), resveratrol (RES), gallic (GA), chlorogenic (CGA), caffeic (CA), syringic (SA), *p*-coumaric (*p*-CA), *o*-coumaric (*o*-CuA), *m*-coumaric (*m*-CuA), ferulic (FA), rosmarinic (RA), and *trans*-cinnamic (*t*-CA) acids in methanol. The results were expressed in mg per 100 g⁻¹ of fresh sample.

2.3.17. Volatile compounds

Volatile compounds of *H. dulcis* were analyzed using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS), according to [Da Costa et al. \(2024\)](#). Samples (5 g) were placed in 20 mL vials and equilibrated at 40 °C for 30 min, followed by extraction using a DVB/CAR/PDMS fiber (50/30 µm). The determination was performed on a GC-MS system (Shimadzu) equipped with an SLB-5MS column (30 m × 0.25 mm × 0.25 µm), using helium (1.0 mL min⁻¹) as the carrier gas. The temperature ramp started at 40 °C (held for 30 min), with an increase of 3 °C min⁻¹ up to 220 °C. The interface and ion source temperatures were set at 240 °C and 220 °C, respectively. Identification was conducted by comparing mass spectra with the Wiley 8 and NIST libraries.

2.3.18. Proteomics

2.3.18.1. Freeze-drying. The samples were frozen at -75 °C (Coldlab, model CL 120-86 V, Piracicaba, Brazil) for 24 h and then freeze-dried (Edwards, model L4KR, São Bernardo do Campo, Brazil) at -30 °C under a vacuum pressure of 0.998 mbar for 72 h in the dark (Meira et al.,

2023). Once freeze-dried, the samples were transported to the Vale Technological Institute, located in the city of Belém (1° 27' 18" S, 48° 30' 9" W), the capital of the state of Pará, northern Brazil, where they underwent proteomic analyses.

2.3.18.2. Protein extraction and quantification. Proteomic analysis was performed according to the protocol described by Do Nascimento et al. (2022). The fruits were ground in liquid nitrogen until they reached a fine powder consistency. To each sample, 10 mL of extraction buffer containing sucrose (1.5 M), Tris-HCl (1.5 M, pH 8), 10% sodium dodecyl sulfate (SDS), 100 mM phenylmethylsulfonyl fluoride (PMSF), polyvinylpyrrolidone (PVPP), and ultrapure water were added, along with 100 µL of protease inhibitor cocktail (P8340 Sigma-Aldrich) and 500 µL of β-mercaptoethanol. After five 30-s sonication cycles (Bandelin Electronic, Berlin, Germany), the extracts were divided into ten microtubes, each with the addition of 700 µL of phenol per tube. The samples were vortexed (MixMate, Eppendorf AG, Hamburg, Germany) and centrifuged (5810 R, Eppendorf, Hamburg, Germany) twice at 20,800 x g for 8 min to separate the phenolic phase and remove any aqueous phase or SDS residues. Then, 1300 µL of ammonium acetate in methanol was added for protein precipitation for approximately 24 h at −80 °C (Innova U570, Eppendorf, Hamburg, Germany). Another centrifugation was performed at 20,800 xg for 8 min, and the supernatant was discarded. The precipitate was transferred to a new microtube and washed four times with 80% acetone. A final wash with 70% ethanol was performed, and the precipitate was air-dried in a vacuum concentrator (miVac DNA, Genevac Ltd., Ipswich, UK) for approximately 7 min. The extracts were solubilized in 200 µL of 0.2% RapiGest (Waters, Milford, MA, USA) and stored for further analysis.

2.3.18.3. Protein digestion. The digestion preparation was initially performed by reducing proteins with dithiothreitol (DTT, 5 mM, Sigma-Aldrich, St. Louis, MO, USA), incubating for 25 min at 56 °C, followed by alkylation with iodoacetamide (IAA, 14 mM; Sigma-Aldrich) for 30 min. Residual IAA was removed by adding DTT (5 mM) again, with a 15-min incubation. Samples were diluted at a 1:5 ratio with ammonium bicarbonate (50 mM), followed by the addition of CaCl₂ (1 mM). The enzymatic digestion was carried out sequencing-grade modified trypsin (20 ng/µL; Promega, Madison, WI, USA) for 16 h at 37 °C. Subsequently, trifluoroacetic acid (TFA; Sigma-Aldrich) was added at a final concentration of 0.4% of the sample volume to stop the enzymatic reaction.

2.3.18.4. Protein identification and data analysis. Five micrograms of peptides were analyzed using a NanoACQUITY UPLC ultraperformance liquid chromatography system (Waters, Milford, MA, USA), configured for two-dimensional fractionation. The proteomic profile was determined through a label-free quantification (LFQ) approach using data-independent acquisition (MS^E). For the first dimension, a 5 µm XBridge™ BEH130 C18 analytical column (300 µm × 50 mm) was used at a flow rate of 2 µL min^{−1}. The second dimension comprised a trap C18 column, 5 µm (180 µm × 20 mm), and an analytical 1.7 µm BEH130™ C18 column (100 µm × 100 mm) at a flow rate of 400 nL min^{−1}. Samples were separated into 5 fractions using acetonitrile gradients of 10.8, 14.0, 16.7, 20.4, and 65.0%. The chromatograph was directly coupled to an ESI-Q-ToF Synapt G2S mass spectrometer (Waters) operating in positive mode with continuous fragmentation (MSE), with collision energy oscillating between 5 and 40 eV. Mass spectra were acquired within a range of 50 to 1200 Da, with a scan time of 0.5 s and an interscan interval of 0.1 s. Peak width and mass spectrum resolution were set to automatic mode. The peptide (lockmass) [Glu-1]-fibrinopeptide, with a mass of 785.4827 Da and + 2 charges state, was used as reference and read every 30 s (according to lockmass spray settings).

Proteomic data were processed using Progenesis QI v4.2 software (Waters) for identification and quantification, using the UniProt TrEMBL database restricted to the Viridiplantae taxonomy (UniProtKB/Swiss-

Prot, uniprot.org). Peptide identification was accepted for probabilities above 90% and proteins above 95%. Significant differences in protein abundance were determined by one-way ANOVA ($p < 0.05$). To minimize false positives, a False Discovery Rate (FDR) correction was applied, and only proteins with a q-value <0.1 were considered significant. Functional annotation of proteins was performed using Blast2GO version 4.0 (Biobam, Valencia, Spain). PCA and the heatmap were generated with the ClustVis web tool (University of Tartu, Tartu, Estonia, <https://biit.cs.ut.ee/clustvis/>); using Euclidean distance for the heatmap clustering. Protein-protein interaction networks and functional enrichments were inferred based on functional analysis with STRING version 12.0 (STRING Consortium, <http://string-db.org/>, accessed on March 23, 2025), using *Arabidopsis thaliana* homologous proteins as the reference species.

2.3.19. Statistical analysis

Statistical analyses were conducted using the SISVAR software (Ferreira, 2010), and the selection of regression models was refined in GraphPad Prism 8.0.1. The corrected Akaike Information Criterion (AICc) was adopted to ensure parsimony and avoid anomalous fluctuations. For each variable, first, second, and in specific cases of clear biological trends third-order models were compared. Higher-order models were only maintained when the ΔAIC and the correction probability demonstrated unequivocal superiority over simpler models, thereby preventing overfitting.

Statistical analysis and integration of proteomics, metabolomics, and physicochemical data were performed using the MetaboAnalyst 6.0 platform. Data were organized into abundance matrices and subjected to pre-processing for missing value imputation through the LOD (Limit of Detection) method, replacing non-detected values with 1/5 of the lowest positive value for the variable.

To reduce technical variability and normalize data distribution, Log10 transformation was applied, followed by Auto-scaling (mean-centering divided by the standard deviation). Correlation analysis was performed using the Pearson coefficient (r) to evaluate linear associations between proteins and metabolites. Hierarchical clustering was conducted based on Euclidean distance and Ward's algorithm to identify co-regulation blocks during ripening. Additionally, multivariate analyses such as PCA and PLS-DA (including VIP score calculation) were employed to identify the primary biomarkers for the different maturation stages.

3. Results and discussion

3.1. Physical, physicochemical, and chemical changes associated with the quality formation of *H. dulcis* pseudofruits during development

The mass of the *H. dulcis* pseudofruits analyzed increased 14-fold, from 0.202 g to 3.052 g, 14-fold on 30 and 180 days after anthesis (DAA), followed by a reduction up to 210 DAA (Fig. 2a, $p < 0.05$). Therefore, the first 180 days of pseudofruit development are characterized by growth driven by cell division and expansion. The reduction observed after 180 DAA can be associated with water loss resulting from respiration and transpiration, as well as the intensification of catabolism, which is common during ripening and senescence. According to Saltveit (2019), water loss in fruits as observed in the present experiment is common during ripening and may stem from the respiratory process as well as physiological and biochemical changes that increase with transpiration.

In short, the aerobic respiratory process consists of the oxidation of glucose, derived from organic compounds present in cells, and its transformation into water, carbon dioxide, and chemical energy. A reduction of 93.76% in the respiratory rate of the analyzed pseudofruits was observed throughout the studied developmental period, following a pattern typical of non-climacteric fruits (Fig. 2b, $p < 0.05$). Climacteric fruits exhibit a peak in CO₂ production before, during, or after ripening,

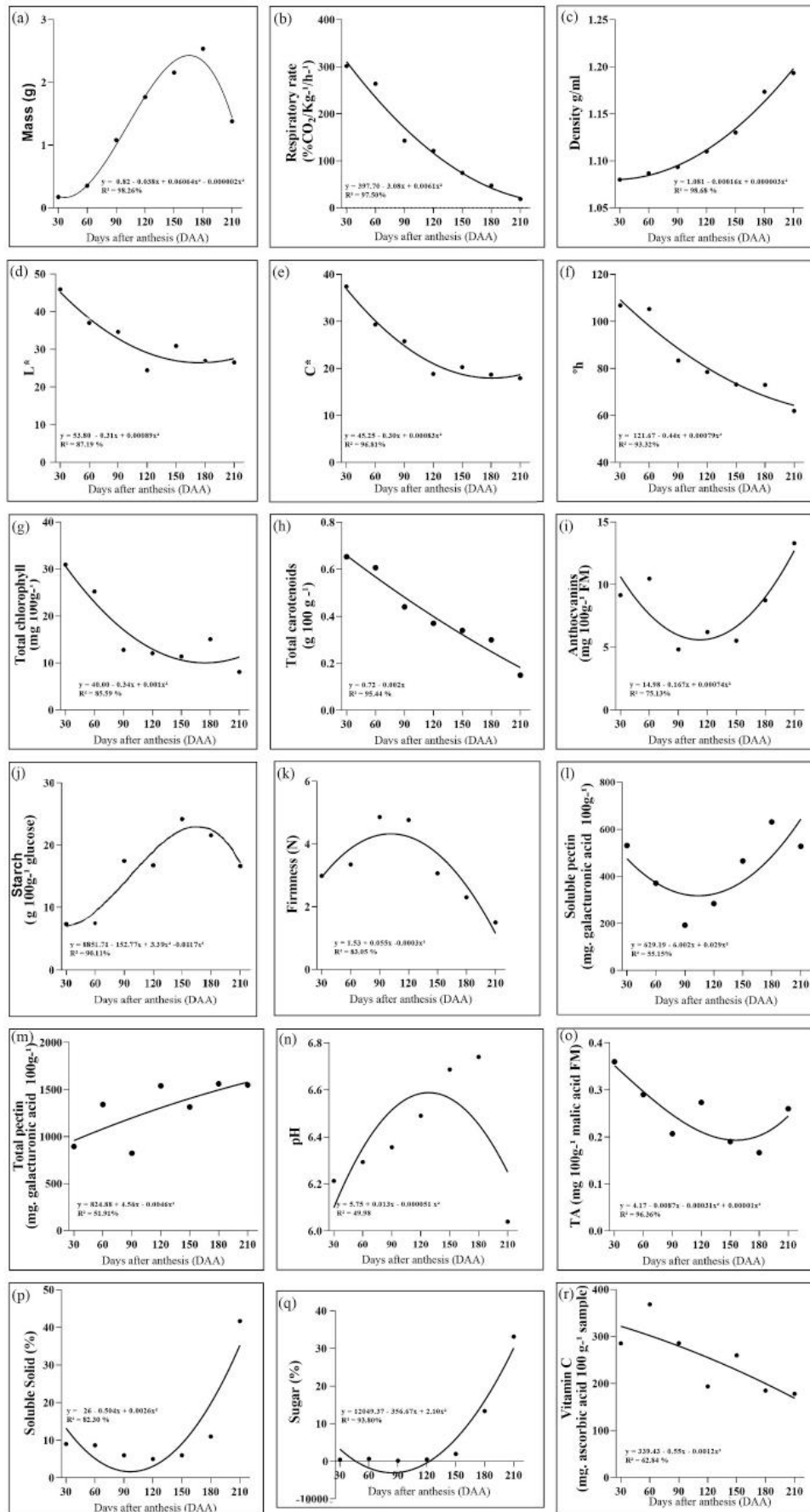


Fig. 2. Physical, physicochemical, and chemical changes during the development of *H. dulcis* pseudofruits.

which does not return to original levels even after the decline. Since transient increases in respiratory activity were not identified, especially during the ripening and senescence of the pseudofruits, it is suggested that they exhibit a typical non-climacteric behavior. While the current data suggests a non-climacteric pattern, the observed starch variations justify future high-resolution monitoring to provide a definitive physiological characterization.

The pseudofruits exhibited a quadratic behavior regarding density (from 1.06 to 1.18 g mL⁻¹) from 30 to 210 days of development (Fig. 2c, $p < 0.05$). This behavior can be explained by the translocation of photoassimilates from the leaves to the fruits and their subsequent transformation into various organic compounds responsible for the construction of quality, with little air accumulation in the intra- and extracellular spaces. Lower densities suggest a greater accumulation of gases, such as O₂ and CO₂, among other volatile compounds, particularly within the parenchymatous tissue, which reduces mass accumulation relative to the volume of the plant organs.

Despite the decarboxylation of organic matter associated with increased catabolism, water loss leads to the concentration of dry matter, which may be linked to the increase in density. The release of gases from inside the fruits, whether due to cellular decompartmentalization or the intensification of volatile emanation, both common during ripening and senescence, can contribute to the rise in density. Indeed, volatile compounds are characterized by low molecular mass, and typically, the higher their concentration, the lower the density, as they occupy the space of heavier compounds. Furthermore, water loss tends to reduce fruit volume through shriveling, which consequently leads to an increase in density, since volume and density are inversely proportional quantities.

Significant changes in the skin color of *H. dulcis* pseudofruits were confirmed throughout their development (Fig. 2 d, e, f, $p < 0.05$). A decreasing quadratic behavior was observed for the variables L*, C*, and h°, respectively. The reduction in L* highlights the darkening of the pseudofruits during development. The decrease in C* values indicates lower color vividness or saturation, suggesting that the pseudofruits lost the intense coloration present in the early stages. Meanwhile, the reduction in h° points to a shift in hue from green to yellowish-red, directly associated with the color transition characteristic of ripening. By the end of the developmental process, the pseudofruits of the Japanese grape tree exhibited a yellowish-red coloration (Fig. 1), typical of the mature pseudofruits under study (Machado et al., 2022). A reduction in L*, C*, and h° variables was also reported by Sehn et al. (2021) when evaluating the same pseudofruit at three developmental stages, indicating a consistent pattern in skin color changes across different regions.

A quadratic behavior was observed for the total chlorophyll content throughout the development of the pseudofruits of the Japanese grape tree (Fig. 2g, $p < 0.05$). Intense degradation of the pigment was noted during the first 90 DAA, followed by a stabilizing trend. Chlorophylls are pigments responsible for photosynthesis, especially in leaves, and for the greenish coloration of plants. The intense degradation of chlorophylls in the first 90 days of development coincides with the shift in h° from the yellow/green quadrant (90 to 180°) to the red/yellow quadrant (0 to 90°) (Fig. 2f). Therefore, as the concentration decreases from 30 to approximately 10 mg 100 g⁻¹, chlorophylls no longer impact the greenish skin coloration of the pseudofruits of the Japanese grape tree (Fig. 1 and Fig. 2f). It is noteworthy that chlorophyll degradation is a process manifested at various stages of fruit development, particularly during ripening (Kapoor et al., 2022).

As observed for chlorophyll, a reduction in the total carotenoids of the pseudofruits of the Japanese grape tree was noted, although it occurred in an intense and linear manner throughout the entire development (Fig. 2h, $p < 0.05$). The presence of carotenoids in young fruits indicates that the yellowish-red coloration during ripening is due to the unmasking of these pigments as a result of chlorophyll degradation, rather than their synthesis. Since carotenoids are essential components of the pigment-protein complexes in thylakoids, the regulation of

carotenogenesis in green tissues appears to be linked to the formation of chlorophylls, proteins, lipids, and chloroplast development. This highly regulated process is not yet fully understood; however, it is known that light and its intensity play a crucial role in regulating carotenoid formation within the chloroplast (Bramley, 2002).

Anthocyanins, pigments that provide colors typically ranging from blue to red, were quantified in pseudofruits of the Japanese grape tree (Fig. 2i). Anthocyanin levels dropped from approximately 10 mg 100 g⁻¹ at 60 DAA to about 5 mg 100 g⁻¹ at 90 DAA. An increase was observed starting from 150 DAA, with approximately 12 mg 100 g⁻¹ quantified at 210 DAA. In a previous study, Machado et al. (2022) were unable to identify anthocyanins in the pseudofruits of the Japanese grape tree. Failures in the extraction process, as well as in the determination method, may be the causes for the lack of anthocyanin identification in the pseudofruit. However, the influence of genetic and edafoclimatic factors on the synthesis and degradation of this pigment cannot be ruled out. The pigment analyses (Figures g, h, i) indicate that the color changes in the pseudofruit of Japanese grape tree (Fig. 1 and Figs. 2 d, e, f) are associated with shifts in chlorophylls, carotenoids, and anthocyanins.

The levels of SS and TS kept stable during first 150 days of development, while starch content increased by more than 190% (Fig. 2 j, p, q). From then on, SS and TS increased and the starch content dropped. Sugars seem to be the principal SS of pseudofruit from 150 days of development, but not before that. In fact, the sugars are the majoritary compounds of SS in the most of fruit, although oligosaccharides, soluble pectins, and organic acids also contribute to that fraction. The observed increase in sugars and, consequently, in SS, can be related to the translocation of photoassimilates and, mainly, starch degradation (Fig. 2 j, p, q and Fig. 3 a and b). It is noteworthy that sucrose is the main translocation sugar from leaves to fruits, and its accumulation and interconversion into glucose and fructose determine the increase in TS. Furthermore, fruits that accumulate starch typically have this polysaccharide enzymatically hydrolyzed during ripening, resulting in an increase in sugars and SS (Cordenunsi-Lysenko et al., 2019).

Scanning electron microscopy (SEM) and histochemical analyses (Fig. 3 a, b) demonstrate changes in cellular structure and starch distribution during ripening, corroborating the previous statement.

The firmness of the pseudofruits exhibited a quadratic pattern, increasing during the first 90 DAA and decreasing from 120 DAA onward (Fig. 2k, $p < 0.05$). Fluctuations in total pectin content (Fig. 2m) were observed throughout development, although an increase from 894 to 1549 mg 100 g⁻¹ was recorded between the first and last day of evaluation. Soluble pectin content (Fig. 2l) decreased from 531 to 192 mg 100 g⁻¹ between 30 and 90 DAA, then increased to reach 631 mg 100 g⁻¹ at 180 DAA and decreased again to 527 mg 100 g⁻¹ at 210 DAA.

Titrate acidity (TA) and pH are inversely related variables. A decrease in TA of the Japanese raisin tree pseudofruits was observed alongside an increase in pH from 30 to 180 DAA, followed by a reversal of these trends up to 210 DAA (Fig. 2n and o). The rise in pH generally occurs due to a reduction in the concentration of hydrogen ions released by organic acids, which are consumed during the respiratory process (Barragán-Iglesias et al., 2018). Thus, the consumption of organic acids was prominent at 180 DAA. In the subsequent 30 days, the increase in TA can be associated with the concentration of organic acids resulting from transpiration losses, rather than their synthesis, as the final stage of development is typically dominated by catabolic processes.

Fruit texture is influenced by water and polysaccharides, with firmness being one of the most important aspects of this quality attribute in fruits. During growth, fruits tend to be firmer due to the synthesis of structural compounds such as starch and cell wall components (Machado et al., 2024), like the pectin observed in the Japanese grape tree pseudofruits. On the other hand, during ripening, softening tends to be conspicuous in most fruits (Machado et al., 2024), as also observed here. Therefore, the presence of starch and pectins (Fig. 2, j, l, and m) can be associated with the firmness of the Japanese grape tree

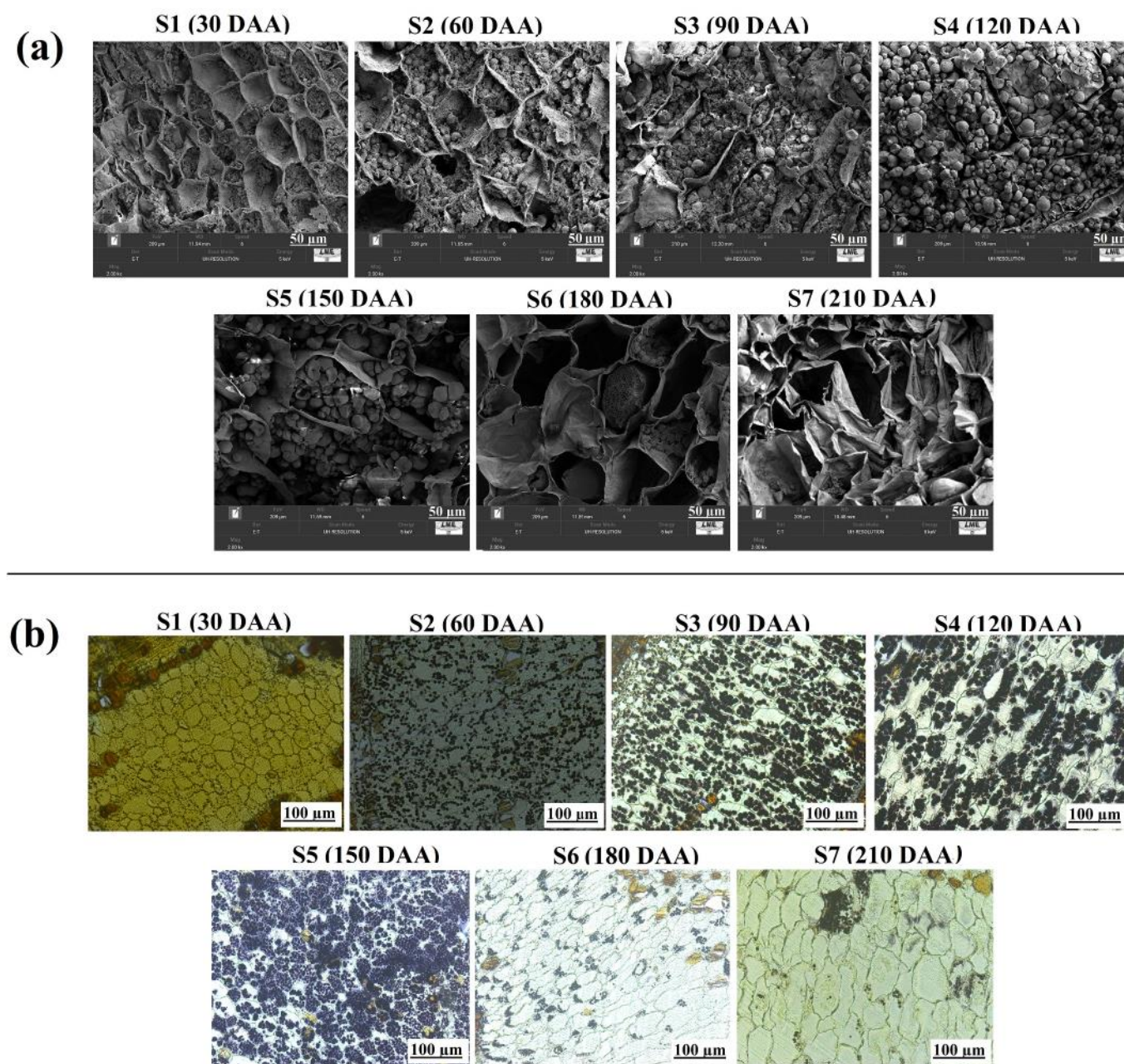


Fig. 3. (a) Scanning electron microscopy (SEM) and (b) histochemistry of the *Hovenia dulcis* pseudofruits throughout development.

pseudofruits. The drop in firmness noted from 120 DAA (Fig. 2 k) can be related to the increase in pectic solubilization (Fig. 2 l, m), resulting from its depolymerization starting at 90 DAA, and to starch hydrolysis (Fig. 2 j) starting at 180 DAA. Furthermore, depolymerization, with the consequent solubilization of pectins, results in the release of oligosaccharides, contributing to the rise in SS content (Fig. 2 p) (Beckles, 2012).

The vitamin C content of the Japanese grape tree pseudofruits increased from 285 to 368 mg 100 g⁻¹ between 30 and 60 DAA, followed by a downward trend reaching approximately 184 and 178 mg 100 g⁻¹ at 180 and 210 DAA, respectively (Fig. 2 r). This reduction can be attributed to the activity of enzymes such as ascorbate oxidase, peroxidases, catalases, and superoxide dismutase, which participate in ascorbic acid metabolism (Mellidou et al., 2012). The vitamin C content found in mature Japanese grape tree pseudofruits (178 mg 100 g⁻¹) is higher than that reported by Machado et al. (2022), who observed values ranging from 90.88 to 117.06 mg 100 g⁻¹ for the same mature pseudofruit. This indicates that the same pseudofruit can exhibit

variations in vitamin C synthesis and degradation, possibly influenced by factors such as cultivation conditions, variety, and developmental stage. It is noteworthy that approximately 50 g of the mature produce would be sufficient to meet the daily requirements of adults for this vitamin. On the other hand, pseudofruits in the early stages of development could be used as raw material for vitamin C extraction by the pharmaceutical or cosmetic industries.

Based on the physical, physicochemical, and chemical analysis results, it can be inferred that *H. dulcis* pseudofruits extended their growth over 180 DAA. The drop in firmness, stabilization of chlorophyll degradation, and the onset of pectic solubilization between 120 and 150 days suggest that maturation began during this period. On the other hand, ripening, the final stage of maturation in which fruits and pseudofruits become attractive to seed dispersal agents and suitable for consumption, started from 150 DAA, considering the intense softening accompanied by pectic solubilization, a consistent increase in sugars and soluble solids, and a rise in anthocyanins, which are primarily

responsible for the typical coloration of mature pseudofruits (Figs. 1 and 2). Furthermore, the photomicrographs and histochemical results support the finding that ripening began at 150 DAA, given the structural breakdown of amyloplasts and cell walls, as well as starch degradation

phenomena typical of late development. In summary, *H. dulcis* pseudofruit development comprised 180 days of growth (stages S1-S6). Maturation overlapped this period from 120 DAA (stages S4-S7), and ripening began at 150 DAA (stages S5-S7).

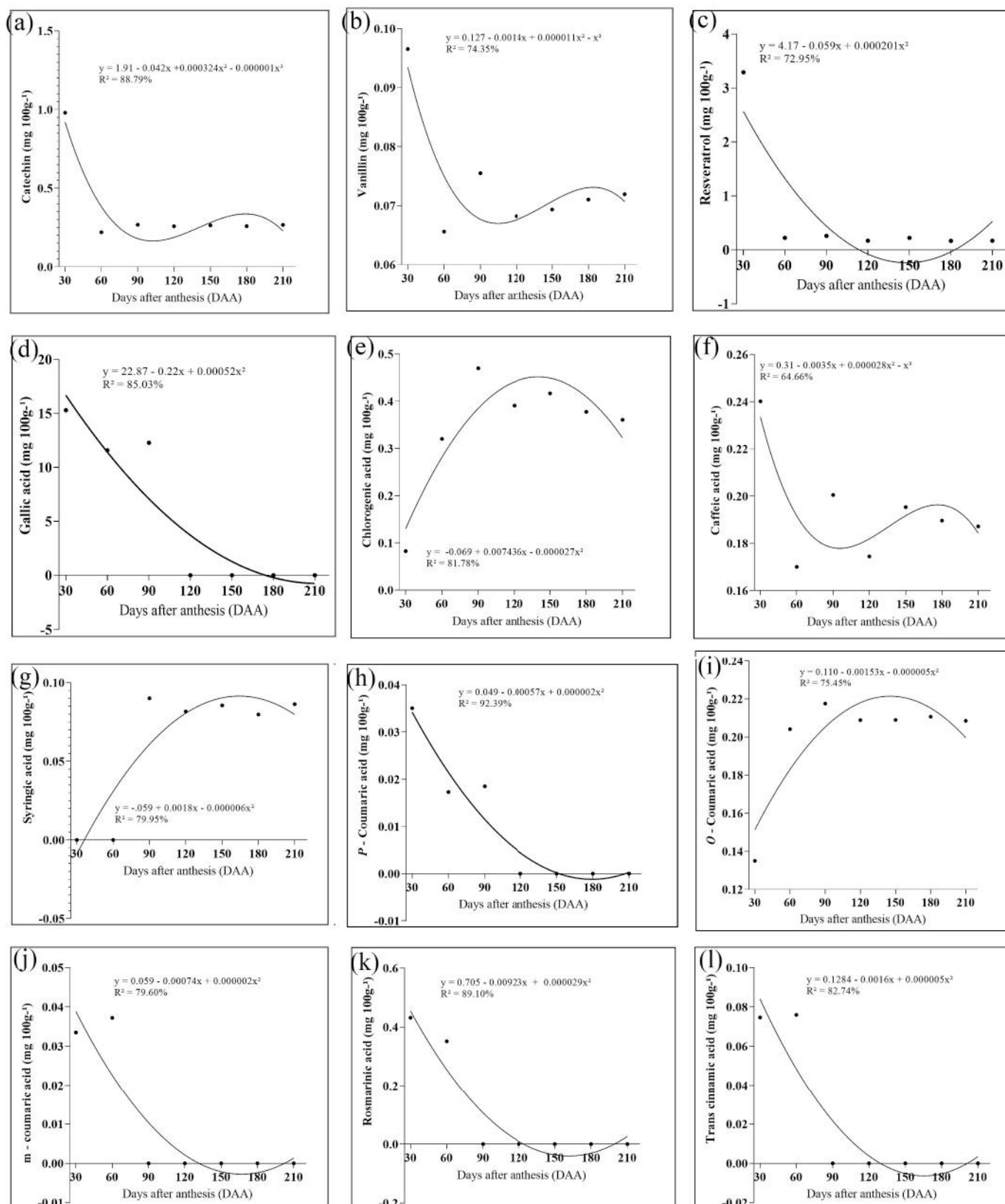


Fig. 4. Phenolic profile of *H. dulcis* pseudofruits during development.

3.2. Phenolic profile

In *H. dulcis* pseudofruits, twelve phenolic compounds were identified, comprising nine acids [gallic (GA), chlorogenic (CGA), caffeic (CaA), syringic (SA), *p*-, *m*-, and *o*-coumaric (*p*-, *m*-, and *o*-CuA), rosmarinic (RA), and *trans*-cinnamic (*t*-CiA)], one flavonoid (catechin), one stilbene (resveratrol), and one aldehyde (vanillin) (Fig. 4). CAT, VAN, RES, CGA, CaA, and *o*-CuA were identified throughout the entire developmental period, unlike the other compounds. SA was identified only from 120 and 90 DAA, respectively, while the presence of GA and *p*-CuA was limited to 30, 60, and 90 DAA, and *m*-CuA, RA, and *t*-CiA were limited to 30 and 60 DAA.

Among the identified phenolic compounds, GA was the most abundant component, although present only in the early stages of development, with concentrations ranging from 11.60 to 15.30 mg 100 g⁻¹ (Fig. 4d). The high concentration of GA at 30 DAA, followed by its complete disappearance after 90 DAA, suggests a strong metabolic flux through the phenylpropanoid pathway and its derivatives in young tissues, likely acting as a chemical defense against herbivory during early development (Singh et al., 2017). The remaining phenolics were quantified at concentrations below 1 mg 100 g⁻¹, except for RES at 30 DAA (3.3 mg 100 g⁻¹) (Fig. 4). The presence of these compounds, even at low concentrations, reinforces the metabolic complexity and functional profile of the pseudofruits, in permanent change throughout development.

The sharp reduction in the concentrations of CAT, VAN, RES, and CaA observed between 30 and 60 DAA chronologically coincides with the progressive increase in pseudofruit firmness, which reaches its peak at 90 DAA (Fig. 2k). From a biochemical perspective, this simultaneous behavior suggests that the reduction of these free phenolic compounds did not result from degradation, but the rerouting of their metabolic precursors in the phenylpropanoid pathway toward the synthesis of structural polymers, such as lignin (Vogt, 2010). This intense initial lignification acts by cementing the cellulose and hemicellulose matrix, which justifies the hardening and firmness gain of the young tissue. In contrast, the stabilization of these phenolics at basal levels after 60 DAA and the subsequent decline in firmness, starting from 120 DAA, mark the end of this structural deposition phase and the beginning of the hydrolytic softening processes typical of fruit ripening. On the other hand, an increase in the concentrations of CGA and *o*-CuA were observed from 30 to 90 DAA, also followed by relative stabilization. The temporary accumulation of CGA may be associated with a physiological response of protection against oxidative stress (Rashidi et al., 2022), generated by the high metabolic rates typical of the transition period between cell division and expansion.

The SA, which was identified only starting at 90 DAA, and *p*-CuA, *m*-CuA, RA, and *t*-CiA, identified only in the early stages of development, remained relatively stable at low concentrations, fluctuating around 0.09, 0.02, 0.037, 0.35, and 0.076 mg 100 g⁻¹, respectively. Although present in small amounts, these metabolites may act as biosynthetic intermediates or in maintaining tissue redox balance. The restricted detection of compounds such as *p*-CuA and *t*-CiA to the early phases reinforces their role as active intermediates of the phenylpropanoid pathway, which are rapidly consumed and incorporated into matrices of greater macromolecular complexity of the cell wall (Vanholme et al., 2019) as the pseudofruit advances in its ontogeny, besides undergoing a natural physical dilution effect due to the volumetric expansion of the tissue.

The levels of CAT and RES dropped from 0.97 and 3.3 mg 100 g⁻¹ to 0.26 and 0.17 mg 100 g⁻¹ in immature green (30 DAA) and ripe (210 DAA) pseudofruits, respectively. The average CAT observed in ripe pseudofruits was lower than those reported for ripe fruits of *P. edulis* (0.6 mg 100 g⁻¹), *A. crassiflora* (7.99 mg 100 g⁻¹), and *C. brasiliense* (4.2 mg 100 g⁻¹) (Da Costa et al., 2023; Da Costa et al., 2024; Da Costa, Silva, et al., 2025), as was the RES content compared to that reported for ripe fruits of *C. brasiliense* (0.66 mg 100 g⁻¹) (Da Costa et al., 2024). Both

compounds, CAT and RES, are synthesized from the fusion of *p*-coumaroyl-CoA (derived from the phenylpropanoid pathway) with three molecules of malonyl-CoA, through the action of chalcone synthase (CHS) and stilbene synthase (STS), respectively (Hasan & Bae, 2017; Yao et al., 2025). These differences reflect specificities in enzymatic regulation. For example, the highly branched nature of the catechin biosynthetic pathway (Zheng et al., 2014) may justify the variability observed in the ontogeny of *H. dulcis*. As the enzymes CHS and STS compete for the same precursor (*p*-coumaroyl-CoA), the drastic reduction of both compounds toward ripening suggests a downregulation of these specific branches or a redirection of the carbon flux toward the synthesis of cell wall polymers. On the other hand, the reduction of resveratrol throughout ripening suggests metabolic redirection or its consumption in defense mechanisms and redox protection during the early stages (Hasan & Bae, 2017). Besides its physiological function, resveratrol is recognized for its therapeutic potential in combating inflammation, diabetes, and cardiovascular and neurodegenerative pathologies (Berretta et al., 2020). Catechins, in turn, offer various health benefits, including the ability to prevent and reduce skin damage, high antioxidant activity, UV protection, as well as antimicrobial, anti-allergic, anti-inflammatory, antiviral, and anticancer properties (Bae et al., 2020).

The maximum CGA content was observed at 90 DAA (0.46 mg 100 g⁻¹), declining to 0.36 mg 100 g⁻¹ in the ripe pseudofruit (Fig. 4e). This behavior resembles that observed by Wang et al. (2023) when studying apple (*Malus domestica*) cultivars throughout development. According to these authors, CGA peaks occurred in the early stages of development, although concentrations subsequently decreased to basal levels. The intermittent action of key enzymes, such as hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) and *p*-coumarate 3-hydroxylase (C3H), coordinates CGA metabolism, responding to biotic and abiotic stresses, including light radiation, thermal variations, and phytohormone signaling (Soviguidi et al., 2022). Therefore, these factors may have influenced the changes in CGA levels observed during the development of *H. dulcis* pseudofruits, suggesting that the peak at 90 DAA represents an active physiological response for cellular protection. CGA, the condensation product of caffeic and quinic acids, is present in coffee beverages and, more generally, in fruits and vegetables. This phenolic has attracted attention due to its antioxidant, anti-inflammatory, and antimicrobial activities, besides its role in regulating lipid and glucose metabolism, which can be explored in therapies focused on the management of type 2 diabetes and cardiovascular diseases (Yu et al., 2022).

Contrary to the observations for CGA, but similarly to those of CAT and RES, the levels of CaA and VAN decreased when comparing immature green (30 DAA) and ripe (210 DAA) pseudofruits (Fig. 4b and f). CaA levels reduced from 0.24 to 0.19 mg 100 g⁻¹, while VAN levels dropped from 0.097 to 0.07 mg 100 g⁻¹. CaA values are lower than those reported for *A. crassiflora* (0.99 mg 100 g⁻¹) (Da Costa, Nascimento, et al., 2025) and *C. pubescens* (0.55 mg 100 g⁻¹) (Machado et al., 2025). CaA, commonly found in coffee beverages, exhibits various biological activities described by Yazar et al. (2025) in their extensive literature review: antioxidant, anti-hyperallergic, antimicrobial, anticancer, cytotoxic, anti-inflammatory, anticoagulant, and antidiabetic. In turn, VAN, a volatile phenolic aldehyde responsible for the characteristic aroma of vanilla, is extracted mainly from the seed pods of *Vanilla planifolia* and is widely used by the food and cosmetic industries.

A behavior contrary to that of CaA and VAN was observed for *o*-CuA, whose concentrations rose from 0.13 to 0.21 mg 100 g⁻¹ in immature green (30 DAA) and ripe (210 DAA) pseudofruits, respectively (Fig. 4i). These values are higher than those observed in passion fruit pulp (0.06 mg 100 g⁻¹) (Da Costa et al., 2023).

The presence of the phenolic compounds GA, *p*-CuA, *m*-CuA, RA, and *t*-CiA exclusively in the early stages of development (Fig. 4d, h, j, k, l) may be associated with the intense initial activity of the general phenylpropanoid pathway enzymes (such as PAL and C4H) for the

protection of young tissues against biotic and abiotic stresses, as suggested by Li et al. (2023). On the other hand, the disappearance of these compounds may be linked to their direct utilization as building blocks in the synthesis of structural polymers, such as lignin and complex flavonoids, during the ripening process. Furthermore, the rapid expansion of the tissue promotes a physical dilution effect, reducing concentrations to levels below the analytical detection limit (Zhang et al., 2022).

SA was identified only starting at 90 DAA, fluctuating around 0.075 mg 100 g⁻¹ up to 210 DAA. SA is a phenolic compound found in various fruits and vegetables, such as olives, dates, grapes, acai, and pumpkin; it exhibits antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic activities, as well as protective effects on the heart, liver, and brain, while also contributing to the structural integrity of lignin (Srinivasulu et al., 2018). Since SA is a dimethoxylated derivative of GA, the appearance of the former starting at 90 DAA may be metabolically associated with the consumption and disappearance of the latter starting at 120 DAA, indicating an active late methylation pathway.

The results reveal that *H. dulcis* pseudofruits possess a phenolic profile that varies qualitatively and quantitatively throughout

development. The phenolic compounds confirm this pseudofruit as a metabolically active vehicle with relevant antioxidant and functional potential. These findings consolidate *H. dulcis* pseudofruits as a plant matrix with significant nutritional and bioactive value, although poorly explored, while providing essential bases regarding the biochemical and enzymatic mechanisms that regulate its development.

3.3. Volatile organic compounds (VOCs)

Volatile compounds play a fundamental role in the aroma and sensory acceptance of food products. In *H. dulcis* pseudofruits, 25 volatile compounds were identified (Table 1), showing significant variations throughout development ($p < 0.05$). Chemical characterization was performed by comparing mass spectra with Wiley 8 and NIST libraries, adopting a similarity index $\geq 80\%$ for identity confirmation. The identified compounds were classified into eight chemical categories, with a predominance of alcohols (32%) and terpenes (16%), followed by aldehydes, esters, terpene oxides, acids, ketones, and hydrocarbons (Table 1). Both the concentration and the profile of these constituents

Table 1
Volatile organic compounds identified in *H. dulcis* pseudofruits during development.

Classe Química	Compound	RI Calc	RI Lit	Odor	Area (%) S1	Area (%) S2	Area (%) S3	Area (%) S4	Area (%) S5	Area (%) S6	Area (%) S7
Alcohols	2,3-Butanediol	806	806	fruit. Onion	—	—	—	—	—	—	1 ± 0.01
	(Z)-3-hexenol	854	858	grass	1 ± 0.13 ^a	0.75 ± 0.03 ^d	0.40 ± 0.1 ^f	0.96 ± 0.07 ^b	0.8 ± 0.07 ^c	0.53 ± 0.01 ^e	—
	(E)-2-hexen-1-ol	864	853	green. Leaf. walnut	0.41 ± 0.07 ^a	—	0.16 ± 0.00 ^b	—	—	—	—
	1-hexanol	867	863	resin. Flower. green	3.29 ± 0.22 ^g	4.37 ± 0.41 ^f	11.96 ± 0.7 ^d	22.68 ± 1.3 ^c	35.97 ± 1.6 ^b	43.82 ± 2.7 ^a	6.9 ± 0.08 ^e
	2-heptanol	902	894	mushroom	52.79 ± 2.1 ^a	41.3 ± 2.91 ^b	31.63 ± 2.5 ^c	15.85 ± 0.8 ^d	9.14 ± 0.84 ^e	6.83 ± 0.18 ^f	4.63 ± 1.1 ^g
	1-octen-3-ol	980	982	mushroom	—	—	—	1.14 ± 0.11 ^b	0.8 ± 0.00 ^c	0.49 ± 0.01 ^d	1.31 ± 0.13 ^a
	Lavandulol	1165	1165	herb	0.81 ± 0.14 ^a	0.11 ± 0.03 ^b	—	—	—	—	—
	Borneol	1175	1165	camphor	0.86 ± 0.09 ^b	0.11 ± 0.02 ^c	1.67 ± 0.05 ^a	—	—	—	—
	Camphene	950	953	camphor	0.11 ± 0.04	—	—	—	—	—	—
	Myrcene	989	988	balsamic. Must. pice	—	0.01 ± 0.01	—	—	—	—	—
Terpenes	Limone	1030	1030	citrus. Mint	1.98 ± 0.45 ^b	1.74 ± 0.42 ^c	3.19 ± 0.17 ^a	0.81 ± 0.04 ^e	0.29 ± 0.03 ^f	1.04 ± 0.05 ^d	—
	(Z)-ocimene	1047	1043	citrus. Herb. flower	2 ± 0.16 ^a	0.08 ± 0.04 ^b	—	—	—	—	—
	Hexanal	801	801	grass. Tallow. fat	2.41 ± 0.04 ^e	7.94 ± 0.75 ^a	4.81 ± 0.21 ^c	4.22 ± 0.62 ^d	2.04 ± 0.23 ^f	7.34 ± 0.18 ^b	0.21 ± 0.01 ^g
	(E)-2-hexenal	850	844	green. Leaf	1.6 ± 0.1 ^b	2.36 ± 0.37 ^a	—	0.87 ± 0.06 ^d	0.25 ± 0.02 ^e	1.17 ± 0.05 ^c	—
Aldehydes	Benzaldehyde	964	960	almond. Burnt sugar	—	—	—	—	—	—	0.57 ± 0.09
	Ethyl isovalerate	850	849	apple. Pear	—	—	—	—	—	—	0.21 ± 0.00
	Ethyl hexanoate	998	997	apple peel. Fruit	—	—	—	—	—	—	0.15 ± 0.05
	Methyl salicylate	1195	1190	peppermint	7.26 ± 0.55 ^d	19.3 ± 2.61 ^c	—	20.35 ± 2.1 ^b	20.39 ± 1.9 ^a	—	—
Terpene oxides	1,8-cineole	1033	1030	mint. Sweet	0.63 ± 0.12 ^a	—	—	0.24 ± 0.07 ^b	—	—	—
	(E)-linalool oxide	1088	1084	flower	0.63 ± 0.03	—	—	—	—	—	—
Acids	Isovaleric acid	847	827	sweat. Acid. rancid	—	—	—	—	—	—	1.05 ± 0.11
	2-Methylbutanoic acid	853	873	cheese. Sweat	—	—	—	—	—	—	0.27 ± 0.03
	2-heptanone	888	889	soap	3.50 ± 0.31 ^c	2.38 ± 0.26 ^e	12.58 ± 0.2 ^a	3.95 ± 0.3 ^b	3.11 ± 0.09 ^d	1.98 ± 0.01 ^f	—
Ketones	3-Octanone	985	979	herb. Butter. resin	—	—	—	—	—	—	0.47 ± 0.13
	Styrene	892	892	balsamic. Gasoline	—	0.55 ± 0.14 ^b	1.41 ± 0.05 ^a	0.34 ± 0.02 ^d	—	0.38 ± 0.05 ^c	—

showed significant variations during development, reflecting an intense reprogramming of the secondary metabolism.

Values represent the mean and standard deviation. Results were expressed as a percentage of the total area. Different letters indicate significant differences ($p < 0.05$) by analysis of variance (ANOVA), followed by Tukey's test. ND: not detected; RI: calculated retention index; RI Lit: retention index found in the literature.

Alcohols constituted the predominant class of volatiles across all analyzed stages. Among them, 2-heptanol and 1-hexanol were the most abundant; the former showed higher concentrations in the early stages (30, 60, and 90 DAA), while the latter predominated in the final stages (120 to 210 DAA) (Table 1). Both compounds exhibited opposite kinetic profiles: 1-hexanol showed a progressive increase, whereas 2-heptanol demonstrated a linear decline during ripening. The increase in 1-hexanol levels is likely associated with the activation of the lipoxygenase (LOX) pathway, contributing to the expression of herbaceous sensory notes (Han & Barringer, 2016). Biologically, this phenomenon is attributed to the loss of cellular compartmentalization during ripening, which facilitates contact between LOX enzymes and their lipid substrates (polyunsaturated fatty acids), resulting in the biosynthesis of these volatiles (Bate & Rothstein, 1998). This process is corroborated by firmness loss data and SEM micrographs (Figs. 2k and 3). Furthermore, the decreasing behavior of 2-heptanol reinforces the metabolic relevance of this class in tropical fruits, analogous to observations in passion fruit maturation (Da Costa et al., 2023).

A clear metabolic reorganization of alcohols was observed, in which six-carbon (C6) defense volatiles such as (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol, derived from the cleavage of fatty acid hydroperoxides by the enzyme hydroperoxide lyase (HPL) were restricted to the early stages (Zhou et al., 2021). As maturation progressed, these compounds gave way to volatiles of greater aromatic complexity, such as 1-octen-3-ol (characteristic of fungal/earthy notes) and 2,3-butanediol. This transition suggests a shift in metabolic investment: the plant reduces the synthesis of herbivory signals (Green Leaf Volatiles - GLVs) as the pseudofruit accumulates energy reserves. This hypothesis is supported by data for soluble solids, sugars, and starch (Figs. 2 j, p, q), where an increase in the former and a decline in the latter are observed, respectively. This profile indicates a transition toward a chemical phenotype that favors attractivity for dispersers, a pattern also validated by the phenolic metabolism (Fig. 4) discussed previously. Moreover, the presence of these alcohols in immature tissues is fundamental, as they serve as substrates for the subsequent synthesis of ripening-related volatile esters (Ortiz et al., 2011).

Changes in the volatile profile during development are further corroborated by the dynamics of terpenes and aldehydes. While limonene persisted throughout ontogeny, indicating continuous biosynthesis via the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways for defense and attraction (Aharoni et al., 2006; Rosenkranz et al., 2021), other terpenes and C6 aldehydes, such as hexanal and (E)-2-hexenal, predominated only in the early stages. The reduction of these "green" aldehydes (Feussner & Wasternack, 2002) in favor of the late accumulation of benzaldehyde, which is typically floral (Lv et al., 2024), marks a sensory migration from an herbaceous profile to a floral bouquet at full maturity. The presence of benzaldehyde, a phenolic aldehyde derived from the phenylpropanoid pathway (shikimate pathway), is consistent with the detection of vanillin (4-hydroxybenzaldehyde) in the phenolic profile (Fig. 4d), evidencing metabolic coordination between soluble and volatile compounds.

Simultaneously, the consolidation of the mature pseudofruit's characteristic volatile profile is driven by the biosynthesis of esters, such as ethyl isovalerate and ethyl hexanoate. The exclusive detection of these compounds in the fully ripe stage (S7) (Table 1) suggests temporal enzymatic coordination, where the initial activity of alcohol dehydrogenases (ADH) provides the necessary substrates for alcohol acyltransferases (AAT) in the final phase (Qian et al., 2019), a pattern also observed in tropical fruits such as pequi (Da Costa et al., 2024).

These data converge with results reported for 'Golden Reinders' apples, where the activity of these enzymes is directly related to the increase in esters during ripening. This reinforces that volatile ester production results, in part, from the increased availability of precursors, such as alcohols and acyl-CoA, for AAT action (Ortiz et al., 2011).

Finally, the minor volatile profile elucidates the physiological transition of the pseudofruits. The presence of terpene oxides restricted to the early stages, followed by the accumulation of isovaleric and 2-methylbutanoic acids at maturity, signals a metabolic shift toward the degradation of branched-chain amino acids (BCAAs), such as leucine and isoleucine. This transition reflects the intensification of catabolic processes and late oxidative activity (Bizzio et al., 2022). Similarly, the replacement of 2-heptanone by 3-octanone in the ripe stage reflects late metabolic events involving lipid degradation and membrane oxidation (El Hadi et al., 2013; Schwab et al., 2008). Taken together, these variations demonstrate that maturation in *H. dulcis* pseudofruits acts as the primary metabolic modulator, replacing primary defense mechanisms with an aromatic complexity aimed at palatability and seed dispersal.

3.4. Proteomics

In *H. dulcis* pseudofruits, approximately 496 proteins were identified across the seven developmental stages analyzed. Among these, 222 exhibited significant variations in abundance ($q < 0.1$ and $FC > 2$), with differences reaching up to 8781-fold between developmental stages (Supplementary Table 1). This subset of proteins showed the following abundance distribution per stage: 15 in stage S1, 3 in stage S2, 2 in stage S3, 31 in stage S4, 84 in stage S5, 33 in stage S6, and 54 in stage S7.

The identified proteins were classified into 30 functional categories, mostly associated with biological and metabolic processes (Fig. 5a). Due to their participation in multiple metabolic pathways and different cellular compartments, some proteins were linked to more than one biological process. Furthermore, Principal Component Analysis (PCA) (Fig. 5b), performed using the three replicates of the 496 proteins for each stage, demonstrated that the proteomic profiles of the replicates for each stage clustered consistently, evidencing high data reproducibility.

Functional enrichment analysis revealed that the proteins accumulated in *H. dulcis* pseudofruits are involved in 10 biological processes, with emphasis on those related to responses to temperature and abiotic stimuli (Fig. 4a). Complementarily, the KEGG pathway analysis (Fig. 5c) highlighted that central carbon metabolism was strongly impacted, notably carbon fixation in photosynthetic organisms and glyoxylate and dicarboxylate metabolism, which showed the highest levels of fold enrichment and statistical significance.

The processes with the highest number of proteins were biological processes (135) and metabolic processes (112). Among these, 56 proteins were selected based on their metabolic functions in the fruits, and a heatmap was generated to visualize their distribution and abundance (Fig. 6a).

The topological analysis of the protein-protein interaction network (Fig. 6b) revealed a scale-free architecture, characterized by the presence of critical structural proteins known as hubs. The TIM protein was identified as the primary central node of the system, exhibiting the maximum values for Degree (12.00) and Betweenness Centrality (0.14), as indicated by the node size scale and deep purple coloring, respectively. This centrality suggests that TIM plays an essential regulatory role in coordinating signaling flow between peripheral clusters. Proteins such as GAPC1, GAPC2, and MDH showed intermediate centrality values (approximately 0.07), acting as communication bridges between the central core and more specialized sub-networks, such as the cluster formed by CSD1, CSD2, and CAT2, which demonstrated lower topological dependence within the global context of the network.

3.4.1. Photosynthesis

Four isoforms of Rubisco (EC 4.1.1.39) were identified, three of which showed peak expression at stage 5 (S5), with an increase of up to

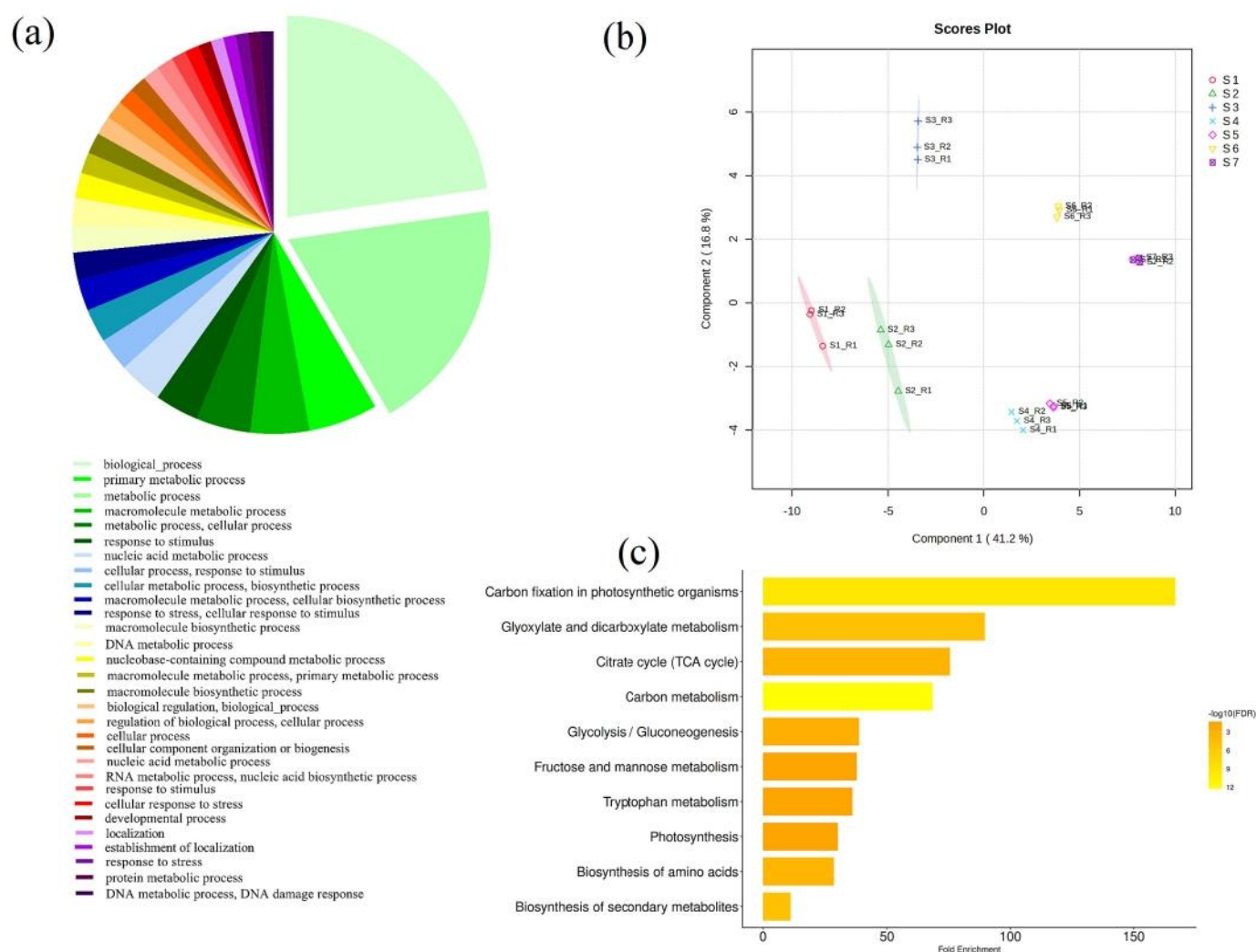


Fig. 5. Functional Annotation of biological processes (a), PCA (b), and Functional Enrichment Analysis of identified proteins based on the KEGG database (c) for differentially abundant proteins across the seven developmental stages (30 to 210 DAA) of *H. dulcis* pseudofruits.

632-fold (Fig. 6a, $q < 0.1$). Although fruits are primarily metabolic sinks, the presence of chlorophyll in the dermal tissue of *H. dulcis* pseudofruits supports a local photosynthetic capacity. The exacerbated expression of Rubisco at the onset of ripening (S5) suggests that photosynthesis within the pseudofruit itself provides essential ATP, NADPH, and carbon skeletons for metabolite biosynthesis and the intense sugar accumulation observed (Fig. 2o; Garrido et al., 2023). This endogenous activity reinforces the enzyme's role in assisting the synthesis of starch and storage compounds during the metabolic transition to ripening (Jiang et al., 2020).

Photosystems I and II (PSI and PSII) are the primary components of the electron transfer process, playing a fundamental role in converting absorbed energy into chemical energy during photosynthesis (Rane et al., 2021). In the analyzed pseudofruits, specific enzymatic isoforms for PSI and PSII were identified, showing higher expression at stages S4 and S6, respectively. Consequently, these results suggest an increase in photosynthetic activity within the pseudofruits during these developmental stages. These increases may be associated with the rise of seven Rubisco isoforms in S5, the enzyme responsible for carbon fixation in the photosynthetic process.

One isoform of triose phosphate isomerase (TPI, EC 5.3.1.1) and another of phosphoglycerate kinase (PGK, EC 2.7.2.3) were identified in the studied pseudofruits, showing higher expression at stage S5 (Fig. 6a), coinciding with the peak expression of Rubisco and reinforcing the synchrony in carbon metabolism. TPI and PGK are fundamental for

the interconversion of triose phosphates produced in the Calvin cycle, ensuring energy homeostasis and the carbon supply for the biosynthesis of metabolites essential for development (Li et al., 2014).

This enzymatic coordination suggests that, at S5, the pseudofruit prioritizes the partitioning of trioses toward the formation of organic matter and reserves. Conversely, the decline in the expression of TPI, PGK, and Rubisco in the final stages (S6 and S7) signals a reduction in autotrophic activity and primary metabolism at the peak of maturity. Understanding these local photosynthetic dynamics in fruits is strategic for genetic improvement and the management of microenvironmental conditions aiming at productivity and quality (Garrido et al., 2023).

3.4.2. Glycolysis: Glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase

Six isoforms of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) showed significant variations in expression throughout the cycle (Fig. 6a), suggesting a regulatory role in the development of *H. dulcis* pseudofruits (Choi et al., 2022). The oscillation of these isoforms reflects the metabolic adaptation of tissues to the energetic and biosynthetic demands of the pseudofruit. Depending on the stage, GAPDH can act both in the glycolytic flux for energy generation and in the gluconeogenic pathway for starch and sugar synthesis, highlighting its importance in carbon partitioning during ripening (Luo et al., 2020).

Two isoforms of pyruvate kinase (PK, EC 2.7.1.40) were identified in

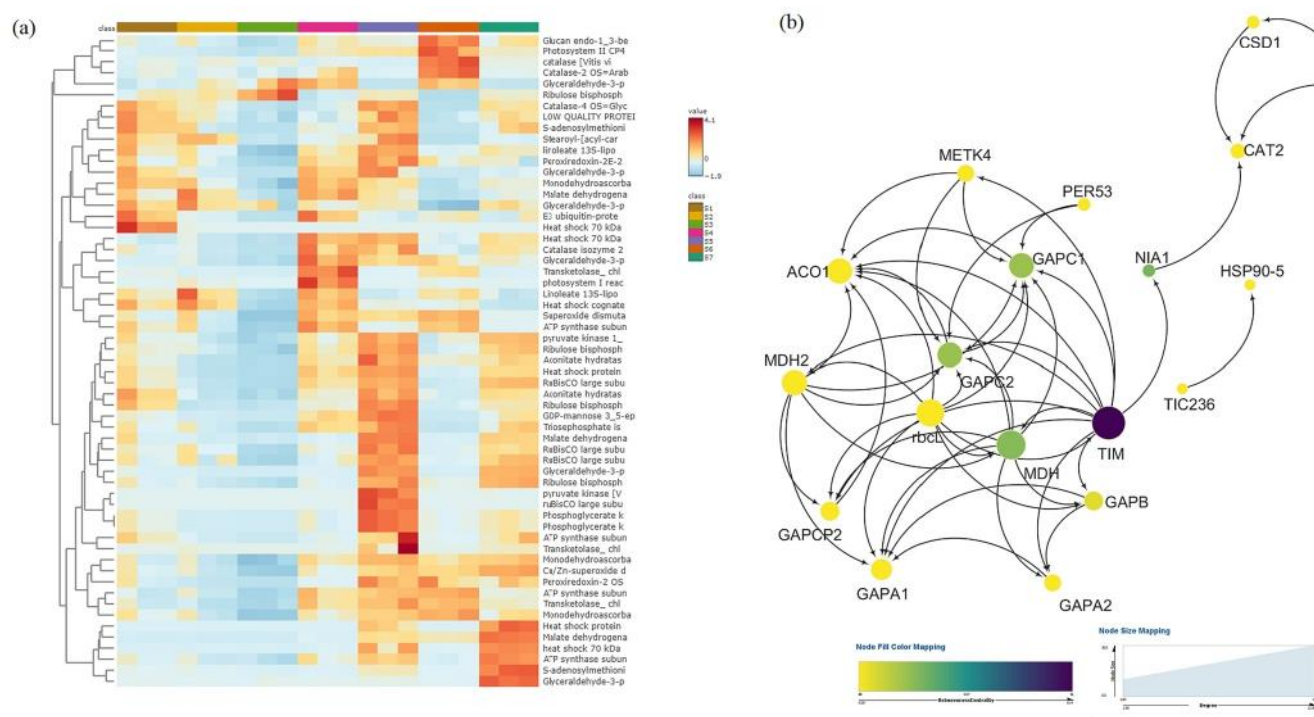


Fig. 6. (a) Heatmap of differentially abundant proteins and (b) Protein-Protein Interaction (PPI) network with mapping of Degree and Betweenness Centrality parameters.

the pseudofruits of the Japanese raisin tree, with higher expression at S5 and lower expression during the early developmental stages (S1 and S3) (Fig. 6a). PK catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, generating ATP, making it an essential enzyme in the final step of glycolysis (Ambasht & Kaystha, 2002). Its lower expression in early stages compared to S5 suggests an intensification of glycolysis at the onset of ripening, a period of high energy demand. Conversely, its lower expression in the initial stages may indicate that, during this period, energy metabolism may rely on other pathways, such as the pentose phosphate pathway, as well as mitochondrial oxidative phosphorylation based on lipids and/or amino acids.

3.4.3. Pentose phosphate pathway

Three isoforms of transketolases (TK, EC 2.2.1.1) were identified in the pseudofruits, with higher expression at S5 and lower expression during the early developmental stages (S1 and S3) (Fig. 6a). TK is the primary regulatory enzyme of the non-oxidative branch of the pentose phosphate pathway, playing a fundamental role in the interconnection between this pathway and glycolysis, in addition to being essential for the synthesis of coenzymes, vitamins, and nucleotide precursors (Kochetov & Solovjeva, 2014). Its higher expression at S5 supports the previously presented data, suggesting an increase in energy demand at the onset of ripening.

3.4.4. Krebs cycle and oxidative phosphorylation

Two isoforms of aconitate hydratase (also known as aconitase, EC 4.2.1.3) were identified in the pseudofruits, showing higher expression at S5 and lower at S3 (Fig. 6a). These enzymes are responsible for the reversible isomerization of citrate to isocitrate via the cis-aconitate intermediate (Yun et al., 2010). Furthermore, aconitase can regulate the carbon flux between organic acid and sucrose metabolism (Carrari et al., 2003). As the fruit ripens, the conversion of organic acids may be reduced, leading to sugar accumulation. The activity of this enzyme may be a regulatory factor in this process, which is supported by the change in acidity of the analyzed pseudofruits (Fig. 2m).

Three isoforms of malate dehydrogenase (MDH, EC 1.1.1.37)

exhibited peak expressions at S4, S5, and S7 (Fig. 6a). Although MDH acts in the interconversion of oxaloacetate into malate, a central component of sensory quality (Yao et al., 2011), its high expression did not follow the same proportion as the titratable acidity (Fig. 2m). This divergence indicates that malate dynamics in *H. dulcis* are governed by complex multigenic control, where MDH may act in both the synthesis and oxidation of the acid to supply other metabolic pathways, as observed in apples (Zhang et al., 2022).

Five isoforms of ATP synthase (EC 3.6.3.14) were identified, with maximum expression fluctuating between stages S4 and S7 (Fig. 6a). Responsible for the final step of oxidative phosphorylation (Liu et al., 2021), the variation of this enzyme reflects the fluctuations in cellular energy supply required to regulate ripening and senescence (Wang et al., 2013). Systemically, the abundance profile of enzymes from the Calvin cycle, glycolytic pathways, and the Krebs cycle confirms that the onset of ripening (S5) is the stage of highest energetic and biosynthetic demand in *H. dulcis* pseudofruits.

3.4.5. Stress, defense, and antioxidant system

Fruits are dynamically exposed to stress factors that trigger defense mechanisms, typically associated with antioxidant systems. Superoxide dismutase (SOD, EC 1.15.1.1) constitutes the first line of defense against reactive oxygen species (ROS), rapidly converting them into H_2O_2 and O_2 (Gill et al., 2015). Two SOD isoforms were identified, showing higher expression in the final developmental stages (S6 and S7) and lower expression in S3 (Fig. 6a). The increased expression of this enzyme in the final stages of the pseudofruit of the Japanese raisin tree is consistent with the greater accumulation of free radicals that occurs during senescence. Therefore, SOD action is vital for free radical scavenging and combating aging. Indeed, SOD expression is an indicator that the plant is likely employing antioxidant mechanisms to protect its cells (Masia, 1998).

Due to its toxic nature, the H_2O_2 produced by SOD or other mechanisms must be eliminated. Thus, Catalase (CAT, EC 1.11.1.6) is a highly expressed enzyme, especially in specific plant cell types, and plays a fundamental role in the plant antioxidant system (Mhamdi et al., 2010).

Its primary function is to eliminate H_2O_2 generated during mitochondrial electron transport, fatty acid oxidation, and, most importantly, photorespiration, under both normal and stress conditions (Sharma & Ahmad, 2014). In the analyzed pseudofruits, four catalase isoforms were identified; two showed higher expression at stages S4 and S5, while the other two were more expressed in S6 (Fig. 6a). These patterns suggest a dynamic response to oxidative stress throughout development. Their expression at these stages may be linked to intense metabolic changes, adaptation to environmental stress, ripening and senescence processes, or defense against biotic stresses (Masia, 1998; Rodrigues-Ruiz et al., 2019).

Peroxiredoxin 2 (Prx2, EC 1.11.1.15) is an antioxidant enzyme that utilizes cysteine residues to decompose peroxides. In the analyzed pseudofruits, two isoforms of this enzyme were identified, with peak expression observed at stage S5 and the lowest at S3 (Fig. 6a). The difference in expression between these stages exceeds 150-fold, highlighting significant variation in Prx2 regulation during development. These results suggest a possible increase in ROS production or a link to physiological ripening processes, such as changes in cellular respiration, pigment synthesis, and alterations in secondary metabolism.

Heat shock proteins (HSPs) are highly conserved across living organisms throughout evolution. Although structurally related, they perform distinct functions within the cell. Several HSPs can coexist in a single cell, distributed across compartments such as the endoplasmic reticulum, mitochondria, and cytosol (Tutar & Tutar, 2011). In the analyzed pseudofruits, six isoforms were identified: four belonging to the HSP70 family, one to the HSP80 family, and two to the HSP90 family (Fig. 6a). These isoforms showed significant variations in expression between stages S1, S4, S5, and S7. These results suggest that, during development, the pseudofruits were exposed to thermal stress, likely associated with temperature variations. Additionally, stress factors such as drought, high irradiation, salinity, or other abiotic stresses can interfere with HSP expression (Al-Whaibi, 2011), justifying the observed variations in the Japanese raisin tree pseudofruits.

Another protein related to biotic and abiotic stress signaling found in *H. dulcis* pseudofruits was E3 ubiquitin ligase (EC 2.3.2.27). One isoform of this protein was identified, with higher expression at stage S1 (Fig. 6a). This may indicate an early response to stress or preparation for future physiological demands related to development and environmental stress (Mao et al., 2022).

3.4.6. Vitamin C

One isoform of GDP-mannose 3,5-epimerase (GME, EC 5.1.3.18) was identified in the Japanese raisin tree pseudofruits, showing higher expression at S5 and lower at S3. This enzyme is crucial in the reversible conversion of GDP-D-mannose to GDP-L-galactose (Celi et al., 2023; Smirnoff et al., 2001) within the synthesis pathway of 2,3-enediol-L-gulonic acid, commonly known as vitamin C or ascorbic acid (AA). This pathway, known as the D-mannose/L-galactose or Smirnoff-Wheeler pathway, is recognized as the most likely route for AA production in plants (Smirnoff et al., 2001). The lower expression at S3 follows the initial decline of vitamin C occurring at S2 and extends until S4 (Fig. 2q). The peak expression at S5 coincides with the rise in vitamin C between S4 and S5 (Fig. 2q). These results reinforce the role of GME in vitamin C biosynthesis.

In addition to its important role in AA synthesis, GME also acts in the synthesis of cell wall compounds. Indeed, the GDP-L-galactose produced from GDP-D-mannose by GME is used not only for vitamin C production but also in the biosynthesis of glycoproteins and cell wall polysaccharides, playing an essential role in plant development (Smirnoff et al., 2001; Wang et al., 2015).

The AA produced by plants is recognized for its specific function in redox reactions in response to oxidative stress (Celi et al., 2023). Through the action of ascorbate oxidase or ascorbate peroxidase, it is oxidized to monodehydroascorbic acid (MDHA) and dehydroascorbic acid (DHA), and is regenerated back to AA by the enzymes MDHA

reductase and DHA reductase, using glutathione and $\text{NADPH} + \text{H}^+$ as reducing agents (Noctor & Foyer, 1998).

Three isoforms of MDHA reductase (MDHAR, EC 1.6.5.4) were found in the pseudofruits of the Japanese raisin tree, with the highest expression occurring at developmental stages S4, S5, and S6, and the lowest at stage S3 (Fig. 6a). Over the same interval, a variation in vitamin C content was observed (Fig. 2q), suggesting the involvement of this enzyme in ascorbic acid regeneration in response to oxidative stress in the Japanese raisin tree pseudofruits.

3.4.7. Ethylene

In the analyzed pseudofruits, two isoforms of S-adenosylmethionine synthase (SAMS, EC 2.5.1.6) were identified (Fig. 6a), showing peak expression at developmental stages S5 and S7, with a 340-fold increase compared to stage S3, which exhibited the lowest enzyme expression. SAMS plays a fundamental role in ethylene biosynthesis, catalyzing the conversion of L-methionine and ATP into S-adenosylmethionine (SAM), an essential intermediate in general cellular metabolism. SAM serves as a precursor for polyamines during the juvenile phase of fruits, as well as for 1-aminocyclopropane-1-carboxylic acid (ACC), which subsequently gives rise to ethylene, the fruit ripening hormone (Machado et al., 2025). It also acts as a donor of methyl groups essential for the metabolism of various plant organs. Thus, the increase in SAMS expression in the final stages of development suggests its role in ethylene synthesis and the ripening of the Japanese raisin tree pseudofruits, as well as in the methylation of metabolites, such as pectic substances.

3.4.8. β -glucans

In the pseudofruits under study, an isoform of the enzyme Glucan β (1–3) glucosidase (GBG) was identified, with peak expression at stage S6. GBGs, classified as *endo*- β (1–3) glucanases (EC 3.2.1.39) and *exo* β (1–3) glucanases (EC 3.2.1.58), catalyze the hydrolysis of β (1–3) glycosidic bonds in β (1–3) -D-glucans (Ramos & Malcata, 2010). This enzyme acts in cell wall degradation, and its levels increase significantly during the defense process against fungal pathogens (Kebede & Kebede, 2021). *H. dulcis* is recognized as a vehicle for polysaccharides, whose presence has been associated with the hypoglycemic effects of the pseudofruit (Zhu et al., 2025). Therefore, the presence of an isoform of this enzyme supports its potential depolymerizing activity in mature fruits. The peak activity of GBG at S6 may be associated with the decline in pseudofruit firmness between S6 and S7, given that β (1–3) glucans are considered cell wall polysaccharides.

3.5. Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

The results of the physicochemical, metabolic, and proteomic analyses of *H. dulcis* pseudofruits across seven developmental stages were subjected to multivariate analysis, performed considering the 53 most significant variables (top features), where PC1 and PC2 cumulatively explain 64.8% of the total data variation (40.2% and 24.6%, respectively; Fig. 7a). Considering a loading cutoff of 0.02 (Supplementary Table 2), the variables responsible for the separation in PC1 were: PSII, TPI, PGK, GAPDH, PK, TK, aconitate hydratase, MDHs, CAT, HSPs (70, 82, 90–5), E3 ubiquitin, Prx2, SOD, linoleate lipoxygenase, MDHAR, GME, SAMS, GBG, isovaleric acid, 2-methylbutanoic acid, 1-hexanol, benzaldehyde, 1-octen-3-ol, 3-octanone, ethyl hexanoate, *o*-CuA, mass, density, soluble pectin, total pectin, pH, sugar, anthocyanins, starch, and soluble solids. In fact, the visual analysis of Fig. 7a confirms that these compounds are associated with developmental stages S4 to S7, situated opposite to RuBisCO, stearoyl-desaturase, hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal, (*E*)-2-hexen-1-ol, 2-heptanone, styrene, 2-heptanol, camphene, limonene, 1,8-cineole, (*Z*)-ocimene, (*E*)-linalool oxide, lavandulol, borneol, methyl salicylate, GA, CAT, CGA, CaA, VAN, *p*-CA, RES, L*, C*, h°, TA, Vit C, total carotenoids, total chlorophyll, and firmness, which characterize the fruits in stages S1 to S3.

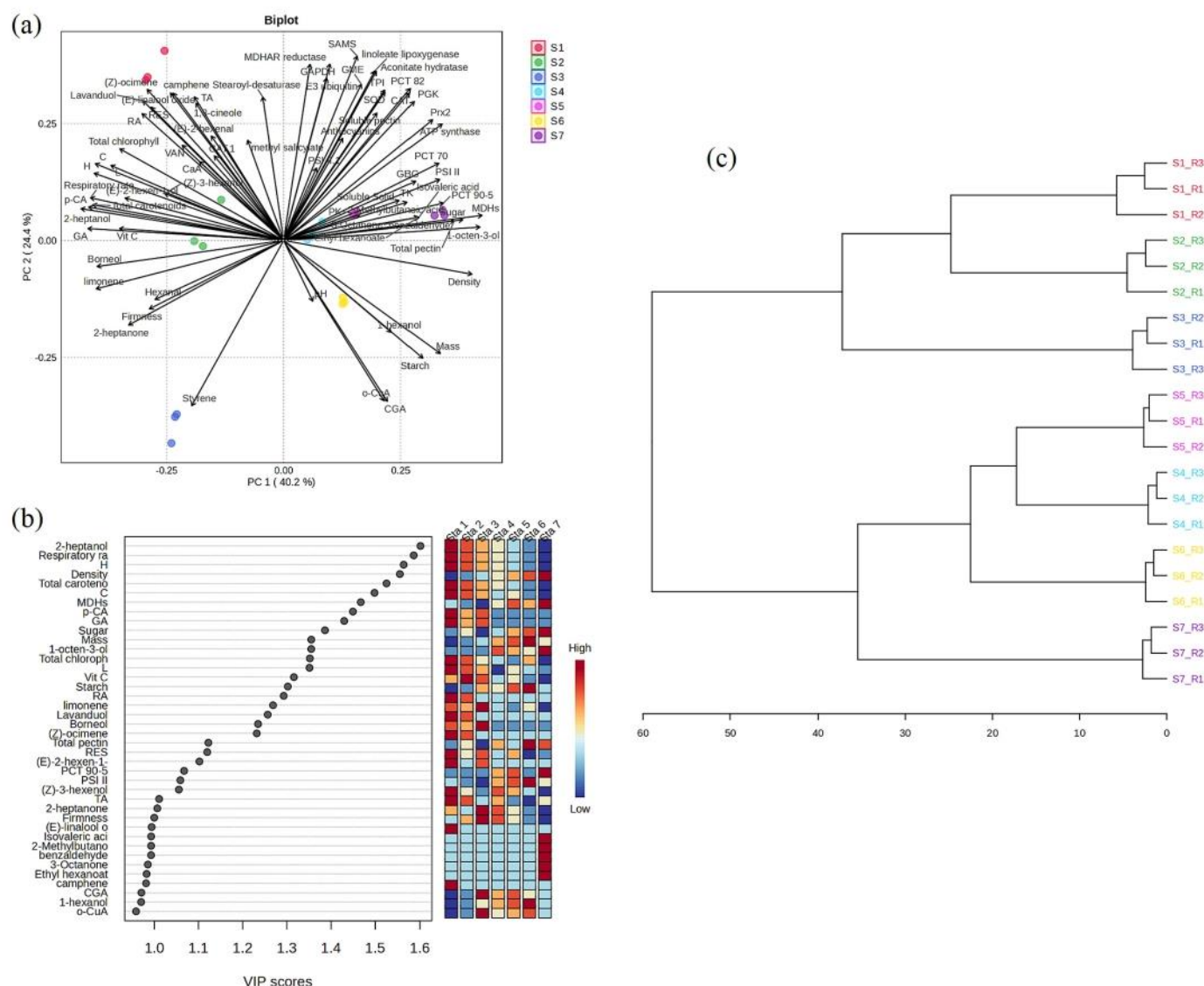


Fig. 7. (a) Biplot (PCA), (b) Variable Importance in Projection (VIP Score), and (c) Hierarchical Cluster Analysis (HCA) dendrogram.

The VIP (Variable Importance in Projection) score analysis (Fig. 7b) identified that the metabolic transition during the ripening of *H. dulcis* is primarily governed by changes in the volatile profile (2-heptanol) and respiratory physiology. The high VIP values for density and Hue angle (h°) corroborate the observed physical transformations; statistically, these variables showed high loadings on Comp 1, establishing themselves as the most discriminating phenological events between stages. Furthermore, the activity of the malate dehydrogenase (MDH) enzyme contributed significantly to this separation, reinforcing the reorganization of central metabolism during pseudofruit development.

Hierarchical Cluster Analysis (HCA, Fig. 7c) corroborated the PCA and sPLS-DA results (Figs. 7a and b), segmenting the samples into two main clusters: the first, grouping the early stages (S1 to S3) and the second encompassing all stages of maturation, including ripening (S4 to S7). This separation is supported by the sign inversion in the loadings of variables such as starch and biomass (mass) relative to phenolic compounds (*p*-CA, GA), marking a profound metabolic transition starting from stage S4. The high similarity between biological replicates reinforces the robustness of the obtained proteomic and metabolic profiles.

3.6. Multi-omics integration: Correlations between proteins and metabolites

Pearson correlation analysis (Fig. 8, Supplementary Table 3) reinforced the covariance structure between the PCA, allowing for the identification of specific associations at the molecular level. A broad spectrum of coefficients was observed, ranging from negligible associations to extremely strong correlations ($r > 0.99$; $p < 0.05$), such as those verified between the metabolites 2-methylbutanoic acid and isovaleric acid, and between *p*-CA and GA. These correlations suggest metabolic co-regulation or enzymatic regulation, validating the integration of the proteomic and metabolic datasets.

The results reveal a highly coordinated system between energy metabolism, antioxidant defenses, and cell wall restructuring during pseudofruit development. Central metabolism acted as the primary integration axis for the datasets. The GAPDH enzyme stood out due to an extremely strong coupling ($r > 0.9$) with MDHAR, SAMS, and linoleate lipoygenase, suggesting that glycolytic flux is intrinsically linked to ethylene signaling and redox responses. Complementarily, TPI and PGK showed strong ($r > 0.7$) to very strong ($r > 0.9$) correlations with ATP synthase, aconitate hydratase, and Prx2, indicating a rigorous synchrony between energy production (ATP) and the neutralization of reactive oxygen species generated by respiratory metabolism. MDHs exhibited a

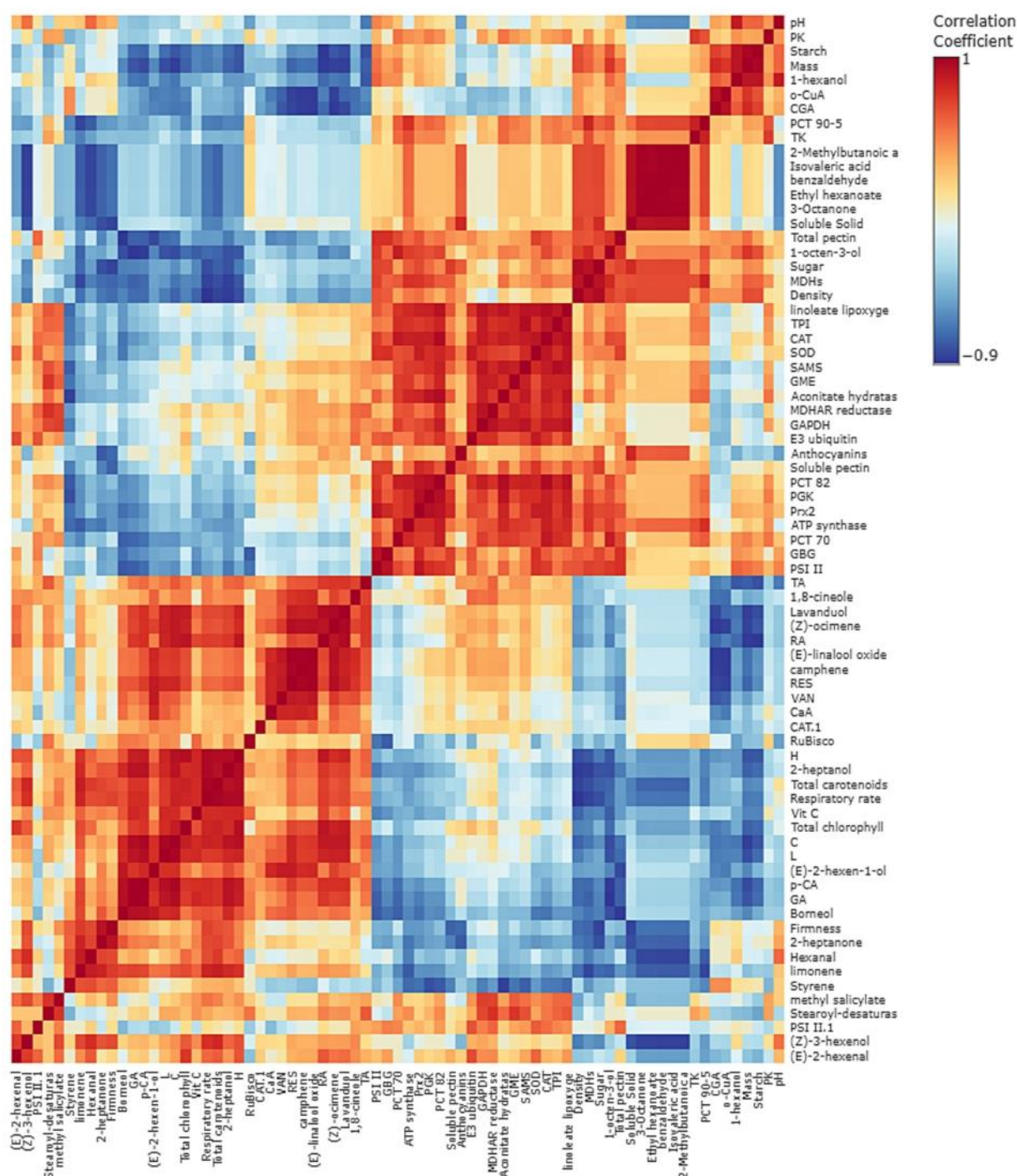


Fig. 8. Pearson correlation matrix between physicochemical variables, metabolites, and proteins during the ripening of *H. dulcis* pseudofruits.

very strong correlation ($r > 0.9$) with density and sugar content, as well as moderate associations with various volatiles (isovaleric and 2-methylbutanoic acids), consolidating their role in flavor transition and biomass accumulation.

Proteins of the photosynthetic apparatus showed correlations marking the decline of autotrophy and the progression of programmed senescence. Rubisco correlated moderately ($r > 0.5$) with vitamin C, GA, and borneol, while photosystems PSI and II presented associations with stress markers (SOD, CAT) and cell wall degradation (total pectin). Notably, the PSII protein demonstrated a very strong link ($r > 0.9$) with beta-glucans (GBG) and Prx2, reinforcing the interdependence between thylakoid membrane stability and the antioxidant defense system.

The antioxidant defense system exhibited the densest network of strong interactions ($r > 0.7$), where SOD, CAT, and Prx2 varied in close synchrony with E3 ubiquitin ligase, indicating fine-tuned regulation of proteolysis and redox homeostasis. Vitamin C biosynthesis, represented by the GME enzyme, showed a strong correlation ($r > 0.7$) with ATP synthase and GAPDH, suggesting that the fruit's antioxidant potential is dependent on the energy supply derived from primary metabolism. CAT, in particular, presented a very strong correlation with Heat Shock Proteins, linking chemical defense to the physical integrity of the fruit.

Cell wall dynamics were monitored via total and soluble pectin, which showed moderate to strong correlations with most metabolic enzymes and stress proteins. The SAMS enzyme, a key player in ethylene

synthesis, presented very strong associations ($r > 0.9$) with GAPDH and TPI, in addition to strong correlations with aconitate hydratase and GME. This pattern evidences that the hormonal signaling that triggers ripening is metabolically coupled to both carbon supply and ascorbate biosynthesis, as well as pectin restructuring, ensuring that softening and aroma development occur in a synchronized manner.

4. Conclusion

The multi-omics study of *H. dulcis* pseudofruit development reveals a highly coordinated biological system, where maturation is defined by a strategic redirection of carbon and energy flux. This process spans 180 days, with the critical metabolic transition occurring from stage S4 (120–150 DAA) and culminating in effective ripening at S7.

Multivariate analysis (PCA and sPLS-DA) consolidated the existence of three distinct phases: chemical defense (S1–S2), metabolic transition (S3–S6), and sensory maturation (S7). This stratification is governed by a dense network of molecular interactions, with central metabolism (glycolysis and the Krebs cycle) acting as the integrating axis. The strong synchrony observed between glycolytic enzymes (GAPDH, TPI, PGK) and the antioxidant defense system (SOD, CAT, Prx2) demonstrates that the pseudofruit maintains fine-tuned regulation of redox homeostasis and energy supply to sustain secondary metabolite synthesis and cell wall restructuring.

Determining phenological events, such as the reduction in Hue angle (H) and the increase in density, were statistically validated by high VIP scores and Comp 1 loadings, highlighting the central role of the malate dehydrogenase (MDH) enzyme in flavor transition and biomass accumulation. Simultaneously, the evolution of the volatile profile from green notes in early stages to complex esters at maturity evidences a specialization of sensory attributes aimed at attractivity for dispersers.

The robustness of the proteometabolic integration is confirmed by strong Pearson correlations, which revealed the coupling between hormonal signaling (via the SAMS enzyme), polysaccharide degradation, and ascorbate synthesis. Together, these results not only decipher the molecular determinants of programmed senescence in *H. dulcis* but also provide a solid scientific basis for selecting the ideal harvest stage, enabling the strategic technological and nutritional utilization of this pseudofruit to meet the demands of the functional food industry.

CRedit authorship contribution statement

Gilson Gustavo Lucinda Machado: Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Elda Eller:** Writing – review & editing, Visualization. **Carlos Henrique Milagres Ribeiro:** Methodology, Investigation. **Carlos Alexandre Rocha da Costa:** Methodology, Investigation. **Sidney Vasconcelos do Nascimento:** Methodology, Investigation. **Sayure Mariana Raad Nahon:** Methodology, Investigation. **Alice de Paula de Sousa Cavalcante:** Methodology, Investigation. **Isa Rebecca Chagas da Costa:** Methodology, Investigation. **Rafael Borges da Silva Valadares:** Methodology, Investigation. **Elisângela Elena Nunes Carvalho:** Methodology, Investigation. **Eduardo Valério de Barros Vilas Boas:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochms.2026.100407>.

Data availability

Data will be made available on request.

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**ARTICLE 2 - POLYSACCHARIDES FROM *Hovenia dulcis*: AN
INTEGRATED APPROACH TO EXTRACTION,
CHARACTERIZATION, AND *IN VIVO* APPLICATION**

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Article

Polysaccharides from *Hovenia dulcis*: An Integrated Approach to Extraction, Characterization, and In Vivo Application

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Highlights

What are the main findings?

- Polysaccharides from Brazilian *Hovenia dulcis* (HDPs-BR) are suitable for edible coatings.
- Those coatings applied alone or in combination with sodium alginate (SA) and eugenol (Eug) improved strawberry storage.

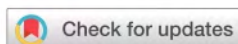
What are the implications of the main findings?

- Treatments with edible coatings based on HDPs-BR promoted better quality retention through 15 days of storage.
- Those coatings can be tested on other fruits to increase their shelf life.

Abstract

Bioactive polysaccharide-based edible coatings are promising strategies for maintaining postharvest fruit quality. This study aimed to extract and characterize crude polysaccharides from Brazilian *Hovenia dulcis* (HDPs-BR) and evaluate their potential as edible coatings, alone or combined with sodium alginate (SA) and eugenol (Eug), for strawberry preservation. HDPs-BR showed an extraction yield of $5.31 \pm 0.19\%$, with $62.68 \pm 1.90\%$ total sugars, $24.95 \pm 0.39\%$ uronic acids, and $5.33 \pm 0.23\%$ proteins. They also contained phenolic compounds and exhibited antioxidant activity. Coating performance depended on formulation and storage period. After 15 days, HDPs-BR 0.5% provided the highest firmness, whereas HDPs-BR 1% favored phenolic and anthocyanin retention and red color. SA-containing formulations showed attribute-dependent benefits: HDPs-BR 0.5% + SA 0.5% promoted high phenolic content, while its combination with Eug 0.1% improved luminosity and antioxidant activity at specific storage periods. HDPs-BR 1% + Eug 0.1% maintained a red color and showed high anthocyanin content and antioxidant activity. Overall, HDPs-BR demonstrated potential as a bioactive edible coating matrix, with formulations containing SA and Eug providing additional benefits depending on the quality attribute evaluated.

Keywords: edible coating; strawberry; postharvest; carbohydrate; bioactive



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1. Introduction

Hovenia dulcis Thunb., commonly known as the Japanese raisin tree, is a perennial fruit-bearing species of the Rhamnaceae family, native to East Asia [1–4], and widely used in traditional medicine in countries such as Japan, China, and Korea [5]. All parts of this tree, including pseudofruits, seeds, leaves, roots, and bark, have been reported to possess medicinal properties [6,7]. Among them, the pseudofruit is the most commonly consumed part due to its sweet taste [8,9] and pear-like aroma, which makes it appealing for human consumption [10].

The pseudofruit, formed through modification of the peduncle, exhibits a twisted shape, coiled appearance, and reddish-brown color when ripe. It can be consumed fresh or in processed forms, such as wines, juice, fermented alcoholic beverages, preserves, jams, and raisins, and as an antioxidant in meat products [2,11–13]. However, its use remains relatively unknown in Western countries, such as Brazil [14], representing a promising source for further studies, particularly due to its high sucrose content and functional compounds.

In recent years, plant polysaccharides have gained attention due to their antioxidant, bacteriostatic, antitumor, antiviral, immunomodulatory, anti-inflammatory, anti-radiation, probiotic, and antidiabetic properties, as well as their technological applications as gelling and emulsifying agents, foaming agents, and thickeners [3]. Polysaccharides from *Hovenia dulcis* have attracted significant scientific interest because of their potential hepatoprotective effects and ability to alleviate hangover symptoms, in addition to being promising candidates for the development of functional foods, pharmaceuticals, and edible packaging [7].

Among the most promising applications of plant polysaccharides is their use in edible packaging systems. Edible films and coatings produced from natural biopolymers such as polysaccharides, proteins, and lipids have gained considerable attention as environmentally friendly alternatives for food preservation. These materials act as semipermeable barriers to moisture and gas exchange, reducing physiological deterioration while helping maintain the sensory, nutritional, and microbiological quality of fresh products, thereby extending their shelf life [15,16]. Nevertheless, the effectiveness of edible coatings depends on several factors, including the physicochemical properties of the biopolymer, the characteristics of the coated commodity, and the coating application method [17].

Among fresh horticultural products, strawberry (*Fragaria × ananassa*) represents an excellent model for evaluating innovative edible coating technologies because of its high commercial value, worldwide consumption, and extreme perishability. Although strawberries are classified as non-climacteric fruits, they exhibit a very short postharvest life due to their high respiration rate, elevated moisture content, mechanical fragility, and susceptibility to fungal decay, resulting in substantial quantitative and qualitative losses during storage and commercialization [18–20]. Refrigerated storage is commonly employed to delay deterioration; however, refrigeration alone is often insufficient to preserve fruit quality throughout the distribution chain [21]. Consequently, edible coatings have become an effective complementary strategy for reducing water loss, delaying microbial and physiological deterioration, and preserving the physicochemical and sensory attributes of strawberries [22,23]. Furthermore, the incorporation of natural bioactive compounds into polysaccharide-based coatings has opened new opportunities for developing multifunctional preservation systems with enhanced antioxidant and antimicrobial properties.

In light of these considerations, the present study aimed to extract polysaccharides from the pseudofruits of *Hovenia dulcis*, develop edible coatings based on these biopolymers, and evaluate their effectiveness in preserving the postharvest quality of strawberries (*Fragaria × ananassa*). To the best of our knowledge, this is the first study to investigate the use of polysaccharides extracted from *Hovenia dulcis* pseudofruits as a matrix for edible

coatings applied to strawberries, providing insights into their potential as sustainable biomaterials for postharvest preservation and expanding the technological applications of this underutilized species.

2. Materials and Methods

2.1. Extraction and Characterization of Polysaccharides

2.1.1. Plant Material

Approximately 10 kg of edible pseudofruits of *Hovenia dulcis* were harvested in the city of Santana do Garambéu, located in the Campos das Vertentes region, Minas Gerais, Brazil (Latitude: 21°34'30" S, Longitude: 44°4'49" W, 1044.52 m above sea level), at the mature stage (pseudofruits exhibiting a reddish-brown coloration and a pleasant, sweet aroma, often compared to that of a pear). The samples were placed in low-density polyethylene bags and transported to the Postharvest Fruit and Vegetable Laboratory at the Federal University of Lavras. Upon arrival, the pseudofruits were washed under running water; pseudofruits showing signs of pathogen infection, pest damage, defects, or seeds were removed; and the remaining material was sanitized in 100 ppm sodium hypochlorite for 30 min and subsequently dried in air-circulation ovens. Pseudofruits showing signs of pathogen infection, pest damage, defects, or the presence of seeds were discarded. The remaining material was sanitized in a 100 ppm sodium hypochlorite solution for 30 min and subsequently dried in a forced-air circulation oven at 45 °C until constant weight was achieved, which required approximately 7 days. During this period, the material was turned daily and periodically ground using an industrial blender as moisture loss permitted in order to reduce particle size and improve the uniformity and efficiency of the drying process.

2.1.2. Extraction, Deproteinization, and Purification of Polysaccharides

The polysaccharides were extracted using hot water, following the method previously described by Liu et al. (2020) [24]. Subsequent deproteinization was carried out using the Savage method, according to Huang, Yang, and Wang (2010) [25], while purification followed the protocol of Huang et al. (2020) [26]. After purification, the dried polysaccharides were transferred to high-density polyethylene containers, to which 100 mL of distilled water was added. The solution was homogenized on a shaker table for 1 h and then refrigerated for 48 h to ensure the complete dissolution of the polysaccharide residues. Subsequently, the samples were transferred to an ultrafreezer, where they remained until the lyophilization step, performed as described by Meira et al. (2023) [27]. Additional details regarding the extraction, deproteinization, and purification procedures are provided in Supplementary Materials S1. The obtained polysaccharides were designated as *Hovenia dulcis* polysaccharides from Brazil (HDPs-BR) and stored in vacuum-sealed low-density polyethylene bags until analysis. The extraction yield (%) was calculated according to Equation (1).

$$\text{Extraction yield (\%, } \frac{W}{W}) = \frac{\text{Dry HDPs} - \text{BR mass(g)}}{\text{Mass of pretreated } H.\text{Dulcis powder (g)}} \times 100 \quad (1)$$

2.2. Characterization of Polysaccharides

2.2.1. Color

Color was measured using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) in the CIE L*, a*, b*, C*, and h° color spaces using CIE standard illuminant D65, the 2° standard observer, and a d/0 (diffuse illumination/0° viewing) geometry.

2.2.2. Chemical Composition

The total carbohydrate content was determined using the sulfuric acid–phenol colorimetric method, with glucose as the standard [28]. The total uronic acid content was determined following the method of McCready and McComb (1952) [29] and quantified as described by Bitter and Muir (1962) [30], using galacturonic acid as the standard. Protein content was estimated using the Bradford assay with BSA as the standard [31]. Results were expressed as grams per 100 g of dry weight ($\text{g } 100 \text{ g}^{-1} \text{ DW}$).

2.2.3. Sugar Profile

Soluble sugars were quantified by high-performance liquid chromatography (HPLC). The supernatant was diluted with ultrapure water and filtered through a $0.22 \mu\text{m}$ Merck Millipore Express™ nylon membrane before injection into the chromatographic system. Analyses were performed using a Shimadzu Prominence HPLC system equipped with a Shim-pack SCR-102HG analytical column protected by an SCR-102HG guard column. The column was maintained at 50°C , and 5 mM perchloric acid was used as the mobile phase at a flow rate of $0.600 \text{ mL min}^{-1}$. Sugars were detected using a refractive index detector (RID-20A). Sucrose, glucose, and fructose were identified by comparing their retention times with those of authentic analytical standards and quantified using external calibration curves. Results were expressed as $\text{g } 100 \text{ g}^{-1}$ dry weight (DW).

2.2.4. Phenolic Compounds and Antioxidant Activity

Extract Preparation

This procedure was performed according to the method described by Waterhouse (2002) [32], using 50% methanol and 70% acetone as extraction solvents for phenolic compounds. The resulting extracts were subsequently used for the determination of total phenolic content and antioxidant activity.

Determination of Total Phenolic Compounds and Antioxidant Capacity

The total phenolic content was determined using the Fast Blue method as described by Medina (2011) [33], and results were expressed as milligrams of gallic acid equivalents (GAE) g^{-1} of dry sample. Total flavonoid content was determined following Arvouet-Grand et al. (1994) [34], using 2% aluminum chloride (AlCl_3) in methanol, and results were expressed in mg of quercetin per g of dry sample.

Antioxidant activity was evaluated using different methods: the Phosphomolybdenum assay, based on the reduction of Mo^{6+} to Mo^{5+} [35], with results expressed as mg ascorbic acid g^{-1} of dry sample; the ABTS assay, based on scavenging of the ABTS⁺ radical by an antioxidant, with results expressed as μMol TROLOX equivalents (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of dry sample [36]; and the FRAP assay, which evaluates antioxidant capacity based on the reduction of the ferric complex (Fe^{3+} -TPTZ) to the ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants, as described by Pulido, Bravo, and Saura-Calixto (2000) [37], with results expressed as μM ferrous sulfate per g of dry mass ($\mu\text{M FeSO}_4 \text{ g}^{-1} \text{ DW}$).

2.2.5. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Vibrational spectroscopy analyses in the infrared region were performed using a Shimadzu FTIR spectrometer model 8201A with Fourier transform infrared (FTIR) over a spectral range of $400\text{--}4000 \text{ cm}^{-1}$, with a resolution of 4 cm^{-1} and 64 scans, using KBr pellets [8].

2.2.6. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was conducted by weighing approximately 10 mg of the material into an alumina crucible and heating from 25 to 800 °C at 10 °C/min under a nitrogen atmosphere [8]. The equipment consists of a balance, furnace, sample holder, temperature sensor, and furnace temperature programmer (DTG-60A/60AH), a recording system (TA-60WSO), and a furnace flow and atmosphere controller (FC-60A).

2.2.7. Scanning Electron Microscopy (SEM)

Observations were performed using an ultra-high-resolution field emission gun scanning electron microscope (FEG-SEM) (Tescan-Clara, Brno, Czech Republic) operating at an acceleration voltage of 20 kV. HDPs-BR samples were mounted on holders using double-sided adhesive tape and subsequently coated with gold via evaporation (Baltec, SCD 050, Balzers, Liechtenstein) [38].

2.3. In Vivo Application of Polysaccharides

2.3.1. Plant Material

Strawberries of the cultivar Karina (*Fragaria* × *ananassa*) were harvested in the Algarve region (Faro) in February 2025 and immediately transported to the postharvest laboratory at the University of Algarve. Upon arrival, the fruits (at the 100% red ripening stage) were selected based on the absence of pathogens and disease symptoms, washed under running water, and randomly assigned to the different treatments.

2.3.2. Chemicals

Food-grade sodium alginate (SA) and glycerol (GLY) (Fisher Scientific, Schwerte, Germany) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Ultrapure water was obtained using a Milli-Q filtration system. Calcium chloride and pure eugenol (4-allyl-2-methoxyphenol), the major bioactive compound of clove essential oil, were also purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

2.3.3. Preparation of Emulsions

The formulation and concentrations used in the present study were selected based on preliminary assays performed with *Hovenia dulcis* polysaccharides (HDPs-BR). These preliminary evaluations assessed the performance of different polysaccharide and eugenol concentrations, and the formulations showing the most promising physicochemical characteristics and coating performance were selected for the subsequent in vivo experiments.

Hovenia dulcis polysaccharides exhibit non-Newtonian behavior and low viscosity, which may limit their application in coating systems [7]. Therefore, they were combined with sodium alginate, a polysaccharide widely used in the food industry, recognized for its high viscosity and ability to form stable gels [39]. This combination aimed to evaluate possible interactions between the biopolymers while improving the rheological properties of the coating by increasing the viscosity of the resulting system.

Sodium alginate (SA) at 1% (*w/w*) was dissolved in Milli-Q water at 70 °C under continuous stirring until completely dissolved. The solution was then cooled to 25 °C and used as the base for preparing alginate-containing coating solutions. The eugenol (Eug) concentration of 0.1% (*w/w*) was selected based on previous studies reporting its minimum inhibitory concentration [40], as well as on the preliminary assays conducted in the present study. All coating solutions were supplemented with 1% glycerol (GLY) as a plasticizer.

Emulsions containing 1% (*w/w*) SA were prepared as described by Rojas-Grau et al. (2005) [41] by dissolving SA and 1% GLY in Milli-Q water under stirring until the so-

lution became homogeneous and translucent. The coating-forming solutions were then homogenized using an Ultra Turrax T25 (IKA, Staufen, Germany).

Emulsions containing 1% (*w/w*) HDPs-BR were prepared by dissolving HDPs-BR and 1% GLY in Milli-Q water at 70 °C under continuous stirring until completely dissolved. The solution was subsequently cooled to 25 °C, after which Eug was incorporated into the respective treatments using a T-18 Ultra Turrax (IKA, Staufen, Germany). Solutions containing 0.5% SA + 0.5% HDPs-BR were obtained by mixing equal volumes of the corresponding 1% emulsions, as presented in Table 1.

Table 1. List of resulting combinations.

List of Combinations		
N.	Description	Code
1	Untreated fruit	Control
2	<i>Hovenia dulcis</i> polysaccharides from Brazil 0.5%	HDPs-BR 0.5%
3	<i>Hovenia dulcis</i> polysaccharides from Brazil 0.5% + Eugenol 0.1%	HDPs-BR 0.5% + Eug 0.1%
4	<i>Hovenia dulcis</i> polysaccharides from Brazil 1%	HDPs-BR 1%
5	<i>Hovenia dulcis</i> polysaccharides from Brazil 1% + Eugenol 0.1%	HDPs-BR 1% + Eug 0.1%
6	<i>Hovenia dulcis</i> polysaccharides from Brazil 0.5% + Sodium alginate 0.5%	HDPs-BR 0.5 + SA 0.5%
7	<i>Hovenia dulcis</i> polysaccharides from Brazil 0.5% + Sodium alginate 0.5% + Eugenol 0.1%	HDPs-BR 0.5% + SA 0.5% + Eug 0.1%

2.3.4. Dipping Application

Treatments containing HDPs-BR without SA, with or without Eug, were applied in a single step. The fruits were immersed in the respective coating solution for 2 min, after which the excess solution adhering to the fruit surface was removed by manual centrifugation for 1 min using a salad spinner.

All treatments containing SA, with or without Eug, were applied using a two-step procedure. First, the fruits were immersed in the respective coating solution for 1 min, followed by manual centrifugation for 1 min to remove excess solution. Subsequently, the fruits were immersed in a 1% (*w/v*) calcium chloride (CaCl₂) solution for 1 min to promote ionic crosslinking of sodium alginate. The excess CaCl₂ solution was then removed by manual centrifugation for 1 min. Thus, the HDPs-BR 0.5% + SA 0.5% + Eug 0.1% treatment followed the same two-step application procedure used for the HDPs-BR 0.5% + SA 0.5% treatment, including the CaCl₂ crosslinking step.

After coating application, the fruits were weighed, identified, and placed in perforated polyethylene trays (14 × 9 × 5 cm). The trays were stored in a cold chamber at 1 °C and 90–95% relative humidity for 15 days. Evaluations were performed after 5, 10, and 15 days of storage, following an additional 24 h period at room temperature. Four replicates were used for each treatment and storage period.

2.3.5. Fruit Quality Analyses

Color, Texture, Weight Loss, Decay Rate, and Soluble Solids

Fruit color was determined using the CIE (Commission Internationale de l'Éclairage) system. Measurements were performed using a PCE-CSM1 colorimeter in the CIELab color space (L*, a*, and b*). For each fruit, two measurements were taken at distinct and opposite points in the equatorial region, and the mean value was used for statistical analysis. Color was evaluated on day 0, prior to the application of the treatments to the strawberries, and subsequently at 5-day intervals throughout the 15-day storage period. Strawberry firmness was measured at the equatorial region using a 4 mm diameter probe, with a compression

speed of 1 mm s^{-1} and a penetration depth of 7 mm, using a Chatillon TCD200 texture analyzer coupled to a DFIS 50 digital force gauge (John Chatillon & Sons, Ametek Inc., Largo, FL, USA). Results were expressed in Newtons (N).

Fruit weight was measured at the beginning of storage (day 0) and after 5, 10, and 15 days of refrigerated storage, following an additional 24 h period at room temperature. Mass loss was calculated as the percentage of weight reduction relative to the initial fruit weight using the following Equation (2):

$$\text{Weight loss (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100 \quad (2)$$

The decay rate was determined as the percentage of fruits showing visible signs of decay, with four independent replicates per treatment, each consisting of eight fruits. Inspections were conducted every 5 days over a total of 15 days of storage. Fruits were classified as infected if they exhibited visible lesions, characterized by brown spots, softening in damaged areas, visible mold, or wilting. The percentage of fruit decay was calculated as the number of decayed fruits divided by the initial number of fruits, multiplied by 100. Results were expressed as the percentage of infected fruits.

Next, 10 g of fruits per replicate were weighed and homogenized using a T-18 Ultra Turrax (IKA, Staufen, Germany), followed by centrifugation (Universal 320-Hettich Zentrifugen) at 3000 g for 5 min. The supernatant (juice) was used to measure total soluble solids ($^{\circ}\text{Brix}$, expressed as %) using a digital refractometer (Atago Co. Ltd., Tokyo, Japan).

Total Phenolic Compounds, Anthocyanins, and Antioxidant Activity

Total phenolic compounds were determined using the Folin–Ciocalteu reagent, with gallic acid as the standard, as described by Singleton and Rossi (1965) [42], with some modifications. Briefly, 20 μL of the samples, 100 μL of 10% (*v/v*) Folin–Ciocalteu reagent, and 80 μL of sodium carbonate solution (75 g/L) were added to 96-well microplates. The reaction was maintained for 30 min at room temperature, and absorbance readings were performed at 765 nm using a spectrophotometer. Results were expressed as milligrams of gallic acid equivalents per milliliter (mg GAE/mL).

Total anthocyanin content was quantified using the differential pH method, as described by Sun et al. (2009) [43] and Saldaña et al. (2021) [44]. For the acidic buffer solution (pH 1.0), 0.186 g of KCl was dissolved in 100 mL of distilled water, and the pH was adjusted to 1.0 ± 0.05 using hydrochloric acid (HCl). The pH 4.5 buffer solution was prepared by dissolving 5.443 g of sodium acetate trihydrate in 100 mL of distilled water, with the pH similarly adjusted using HCl.

Strawberry liquid extracts were diluted at a 1:50 ratio and then mixed with the buffer solutions at a 1:1 (*v/v*) ratio. Absorbance readings of the diluted samples were recorded using a spectrophotometer at wavelengths of 520 and 700 nm for both solutions (pH 1.0 and pH 4.5). Anthocyanin content was calculated based on Equation (3) as proposed by the referenced authors.

$$\text{Anthocyanin content } \left(\frac{\text{mg}}{\text{L}} \right) = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\epsilon \times 1} \quad (3)$$

where $A = (A_{520} - A_{700} \text{ nm})$ at pH 1.0— $(A_{520} - A_{700} \text{ nm})$ at pH 4.5; molecular weight (MW) = 449.2 g/mol of cyanidin-3-glucoside equivalent (Cy3GE); dilution factor (DF) = 50; $\epsilon = 26,900$ (molar extinction coefficient in L/mol.cm for Cy3GE); 1 represents the path length (in cm); and 10^3 is the conversion factor from grams to milligrams. Anthocyanin content was expressed as milligrams of Cy3GE per liter of extract.

Antioxidant activity was assessed using the ferric reducing antioxidant power (FRAP) assay, as described by Benzie and Strain (1996) [45], with adaptations to reagent and sample volumes for 96-well microplates. The FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), a 10 mM solution of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and a 20 mM solution of ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in a 10:1:1 (*v/v/v*) ratio. The reagent was protected from light and heated to 37 °C prior to use. For the assay, 180 μL of the FRAP reagent was added to 20 μL of the sample or standard solution in 96-well microplates. The mixture was incubated at 37 °C for 30 min, and absorbance was measured at 593 nm (Synergy HTX, BioTek, USA). Results were calculated based on a ferrous sulfate (FeSO_4) standard curve and expressed as micromoles of Fe^{2+} equivalents per milliliter ($\mu\text{mol Fe}^{2+} / \text{mL}$).

Antioxidant activity assessed through the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging assay was performed as described by Brand-Williams et al. (1995) [46], with some modifications. A 0.1 mM DPPH stock solution was prepared in methanol and kept in the dark at room temperature. The working solution was obtained by diluting the stock solution with methanol until an absorbance of approximately 0.9 ± 0.02 at 517 nm was reached. For the assay, 195 μL of the DPPH working solution was added to 25 μL of the strawberry juice extract or standard solution in 96-well microplates. The mixture was incubated in the dark at room temperature for 30 min to allow the reaction to proceed. Absorbance was measured at 517 nm (Synergy HTX, BioTek, Santa Clara, CA, USA) to determine the residual DPPH concentration. Results were expressed as antioxidant activity equivalent to Trolox per milliliter. Blanks were prepared using methanol in place of the sample, and a control containing only the DPPH solution was used to measure the maximum absorbance.

Microbial Count

Procedures for the enumeration of mesophilic aerobic bacteria, psychrophilic bacteria, molds, and yeasts were performed according to Guerreiro et al. (2015) [40]. Ten grams of each sample were added to 90 mL of peptone water (Oxoid) and homogenized using a Classic/Panoramic homogenizer (IUL Instruments, Barcelona, Spain), followed by serial decimal dilutions. For the enumeration of molds and yeasts, Dichloran Rose Bengal Chloramphenicol (DRBC) agar (Biokar, Paris, France) was used, whereas Plate Count Agar (Biokar, Paris, France) was used for the enumeration of psychrotrophic and mesophilic aerobic bacteria. Culture plates were incubated at 25 ± 1 °C for 72 h for molds and yeasts, at 30 ± 1 °C for 24 h for mesophilic aerobic bacteria, and at 6.5 ± 1 °C for 7–10 days for psychrotrophic bacteria. Results were expressed as Log_{10} colony-forming units (CFU) per gram of fresh weight [47].

2.4. Experimental Design and Statistical Analysis

Polysaccharide analyses were performed in triplicate, and the results are expressed as mean $\pm$ standard deviation.

The *in vivo* experiment was conducted using a completely randomized design (CRD) in a 7×4 factorial arrangement, consisting of seven coating treatments and four storage periods (0, 5, 10, and 15 days). Each treatment $\times$ storage period combination included four replicates, with each replicate consisting of four fruits (16 fruits per treatment at each storage period).

Data obtained from the *in vivo* experiment were analyzed using IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA). A two-way analysis of variance (ANOVA) was performed considering coating treatment and storage period as fixed factors. Whenever a significant treatment $\times$ storage period interaction was detected ($p < 0.05$), simple effects

were evaluated using Duncan's multiple range test ($p < 0.05$) by comparing treatments within each storage period and storage periods within each treatment.

Results from the strawberry quality analyses were autoscaled prior to principal component analysis (PCA), which was performed using OriginPro 2018 (version 9.5, OriginLab Corporation, Northampton, MA, USA). Graphical representations were generated using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA).

3. Extraction and Characterization of Polysaccharides

3.1. Color, Chemical Composition, and Sugar Profile

Following hot water extraction followed by freeze-drying, the crude polysaccharides from Brazilian *Hovenia dulcis* (HDPs-BR) showed an average extraction yield of 5.31 ± 0.19 along with a reddish-yellow coloration (Figure 1, Table 2).

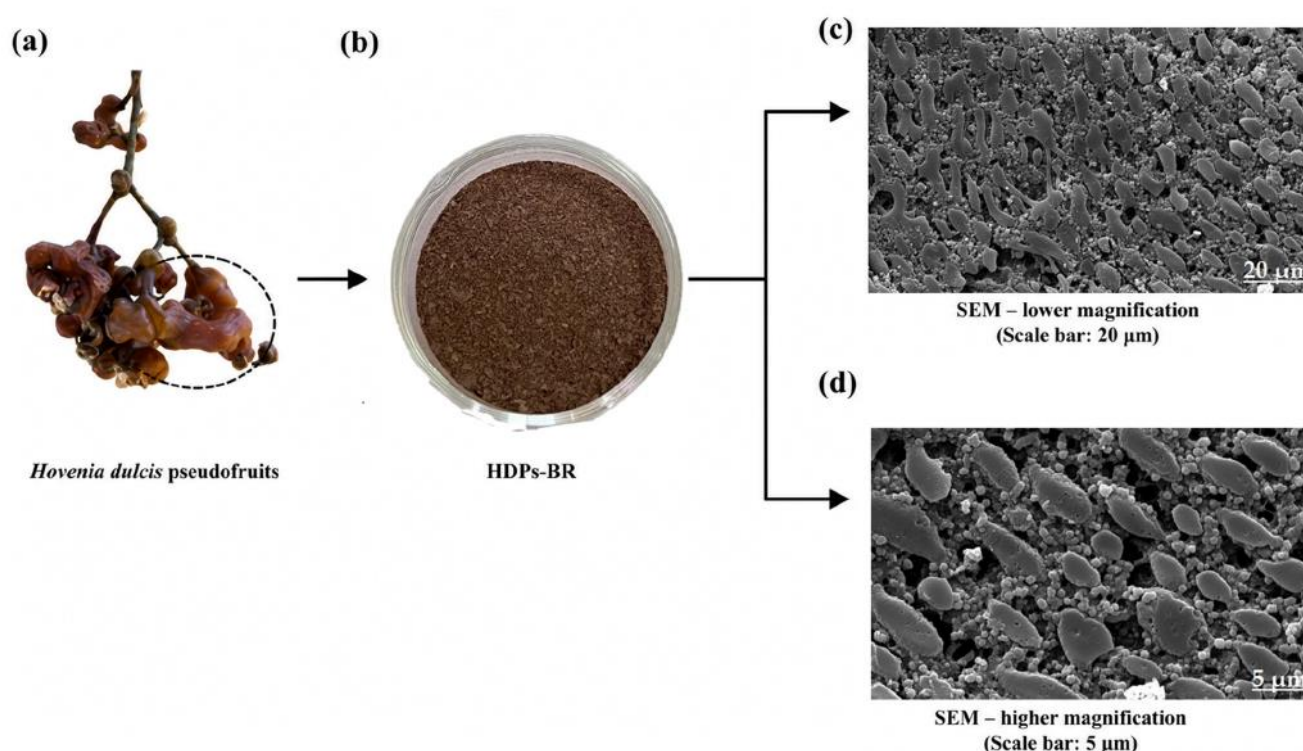


Figure 1. Raw material and microstructure of polysaccharides extracted from Brazilian *Hovenia dulcis* (HDPs-BR). (a) *Hovenia dulcis* pseudofruits; (b) HDPs-BR; (c) scanning electron microscopy (SEM) image at lower magnification; and (d) SEM image at higher magnification. HDPs-BR: polysaccharides extracted from Brazilian *Hovenia dulcis*.

Table 2. Physical and chemical characterization of the extracted polysaccharides.

Variables	Values
Color	
L*	48.56 ± 0.42
h°	65.45 ± 0.055
C*	27.24 ± 0.11
Proteins (g 100 g ⁻¹)	5.33 ± 0.23
Galacturonic acid (g 100 g ⁻¹)	24.95 ± 0.39
Total sugars (g 100 g ⁻¹)	62.68 ± 1.90
Sucrose (g 100 g ⁻¹)	8.01 ± 0.29
Glucose (g 100 g ⁻¹)	1.69 ± 0.05
Fructose (g 100 g ⁻¹)	4.12 ± 0.075

Results were expressed as means $\pm$ standard deviation, with all values presented on a dry weight (DW) basis.

Scanning electron microscopy (SEM) analysis (Figure 1b,c) revealed that HDPs-BR exhibit a predominantly ellipsoidal morphology, covered by uniformly distributed spheres, presenting a rough surface and the presence of pores. The protein, uronic acid, and total sugar contents were 5.33 ± 0.23 , 24.95 ± 0.39 , and $62.68 \pm 1.90 \text{ g } 100^{-1}$, respectively (Table 2).

Sugar profile analysis by HPLC allowed the identification of one disaccharide and two monosaccharides: sucrose, glucose, and fructose, with concentrations of 8.01 ± 0.29 , 1.69 ± 0.05 , and $4.12 \pm 0.075 \text{ g } 100^{-1}$, respectively (Table 2), totaling a sum of individual sugar concentrations of $13.82 \text{ g } 100^{-1}$.

3.2. Total Phenolic Compounds and Antioxidant Activity of the Polysaccharides

Total phenolic analysis by the Fast Blue method, as well as that of flavonoids, evidenced the presence of phenolic compounds in the polysaccharides, with concentrations of 241.73 ± 0.76 and $11.53 \pm 0.36 \text{ mg g}^{-1}$, respectively (Table 3).

Table 3. Total phenolics and antioxidant activity of HDPs-BR.

Analyses	
Fast Blue (mg g^{-1} of gallic acid)	241.73 ± 0.76
Flavonoids (mg g^{-1})	11.53 ± 0.36
Phosphomolybdenum (mg of ascorbic acid g^{-1})	93.69 ± 0.67
ABTS (μM of trolox g^{-1})	723.40 ± 4.05
FRAP ($\mu\text{M FeSO}_4 \text{ g}^{-1}$)	$18,459.19 \pm 436.89$

Results were expressed as means $\pm$ standard deviation, with all values presented on a dry weight (DW) basis.

In addition to the presence of total phenolic compounds, the polysaccharides exhibited significant antioxidant activity (Table 3), with values of $93.69 \pm 0.67 \text{ mg g}^{-1}$ by the phosphomolybdenum method, $723.39 \pm 4.05 \mu\text{M Trolox g}^{-1}$ by the ABTS method, and $18,459.19 \pm 436.89 \mu\text{M FeSO}_4 \text{ g}^{-1}$ by the FRAP method.

3.3. FTIR and TGA of HDPs-BR

In the FTIR analysis, six main spectral bands were identified. Initially, a broad band was observed in the region around 3390 cm^{-1} , followed by a band near 2930 cm^{-1} . Following the spectrum, a strong absorption was detected at 1645 cm^{-1} and a subsequent peak at 1251 cm^{-1} . Additionally, the profile revealed a peak at 1075 cm^{-1} and a complementary band at 1034 cm^{-1} (located within the range of $1000\text{--}1200 \text{ cm}^{-1}$) (Figure 2a).

Concurrently, the TGA analysis (Figure 2b) revealed that the first mass loss of the *Hovenia dulcis* polysaccharide occurred in the range between 27°C and 150°C . Above this temperature, a new stage of mass loss began, with thermal decomposition concentrated mostly between 150°C and 350°C . Upon reaching the 350°C plateau, it was found that the residual mass of the polysaccharide stabilized at approximately 43%, decreasing gradually from this point up to the limit of 800°C .

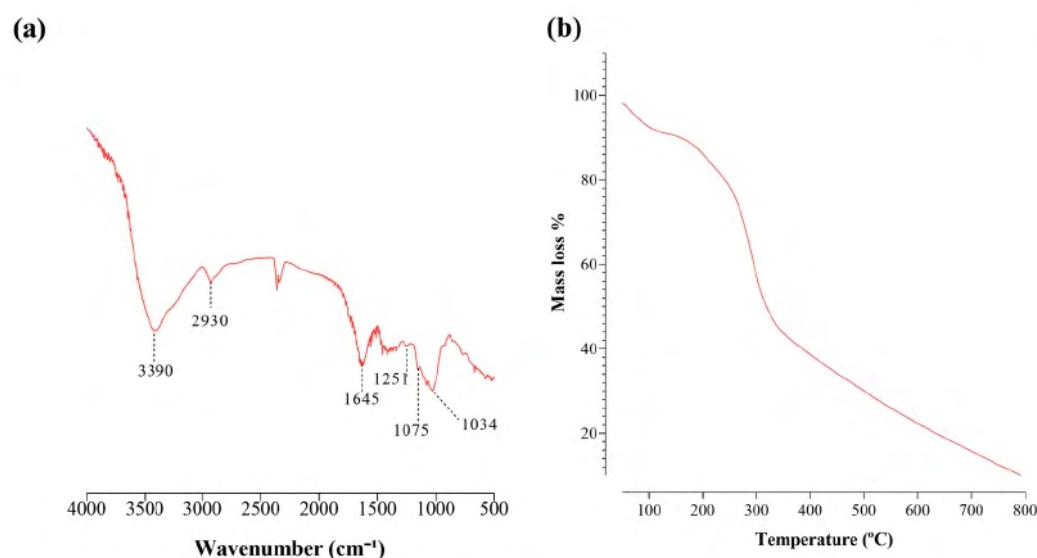


Figure 2. Structural and thermal characterization of polysaccharides extracted from Brazilian *Hovenia dulcis* (HDPs-BR): (a) Fourier-transform infrared (FTIR) spectrum and (b) thermogravimetric analysis (TGA) curve.

4. Fruit Quality Analyses

4.1. Color, Texture, Soluble Solids, Weight Loss, and Decay Rate

Fruit color was evaluated on day 0, before the application of the treatments to the strawberries, and subsequently every 5 days during 15 days of storage. The results of the color analysis (Figure 3a,b) showed a significant interaction between treatment and storage period ($p < 0.05$) on strawberry color over time (Figure 4).

On the 15th day of storage, fruits treated with HDPs-BR 0.5% + SA 0.5% + Eug 0.1% showed the highest L^* values, differing significantly from the control and the treatment containing only HDPs-BR 0.5%, while the remaining treatments showed intermediate values and did not differ significantly from either the highest- or lowest-value groups (Figure 3a).

Regarding red color intensity (a^*), the highest values were observed in fruits treated with HDPs-BR 1% and HDPs-BR 1% + Eug 0.1% on the 15th day of storage (Figure 3b). The HDPs-BR 1% + Eug 0.1% treatment showed no significant variation in a^* values throughout the storage period.

For firmness, significant differences among treatments were observed on the 15th day of storage ($p < 0.05$). Fruits coated with HDPs-BR 0.5% showed the highest firmness values, differing significantly from the control and the other treatments (Figure 3c). Furthermore, HDPs-BR 0.5% resulted in higher firmness than HDPs-BR 1%.

Soluble solids content was only slightly affected by the different edible coatings during storage (Figure 3d), although small differences among treatments were observed at some storage periods. In contrast, storage period had a significant effect ($p < 0.05$), with soluble solids increasing in several treatments by the end of the experimental period.

The coatings did not significantly reduce weight loss compared with the control. However, weight loss increased significantly throughout storage in all treatments, including the control (Figure 3e).

Regarding the decay index (Figure 3f), fruits coated with HDPs-BR 0.5% and HDPs-BR 1% showed the lowest values after 15 days of storage, with no decayed fruits observed in these treatments. Although these values were numerically lower than those of the control, they did not differ significantly from the control ($p > 0.05$). In contrast, the highest decay

indices were observed for HDPs-BR 0.5% + SA 0.5% and HDPs-BR 1% + Eug 0.1%, which differed significantly from the treatments showing the lowest decay values.

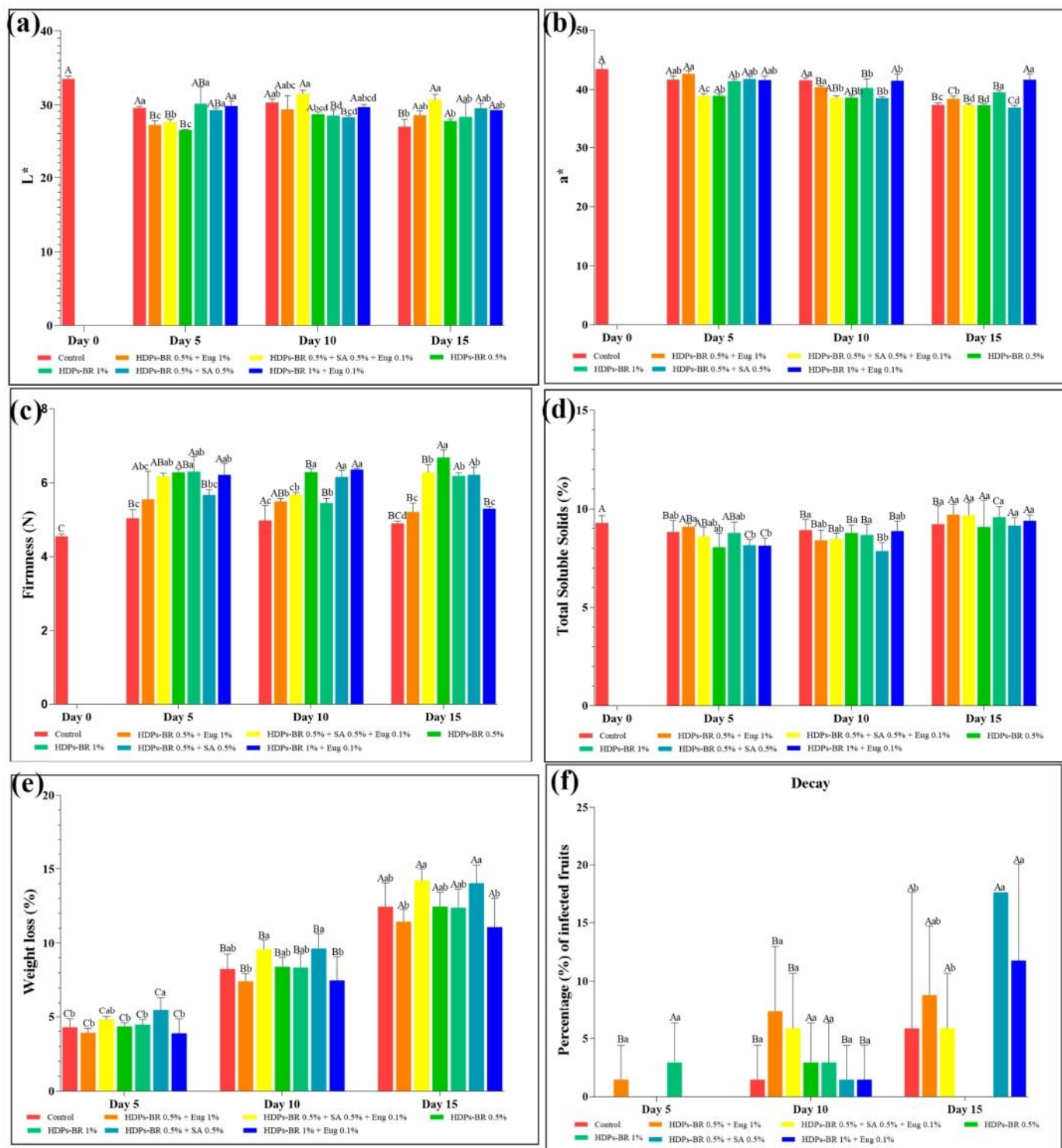


Figure 3. Changes in the physicochemical quality and decay of strawberries subjected to different coating treatments during storage: (a) L* value; (b) a* value; (c) firmness; (d) soluble solids; (e) weight loss; and (f) decay. Values are expressed as mean $\pm$ standard deviation ($n = 4$). Different lowercase letters indicate significant differences among treatments within the same storage period, whereas different uppercase letters indicate significant differences among storage periods within the same treatment, according to Duncan's multiple range test ($p < 0.05$). HDPs-BR: polysaccharides extracted from Brazilian *Hovenia dulcis*; Eug: eugenol; SA: sodium alginate.

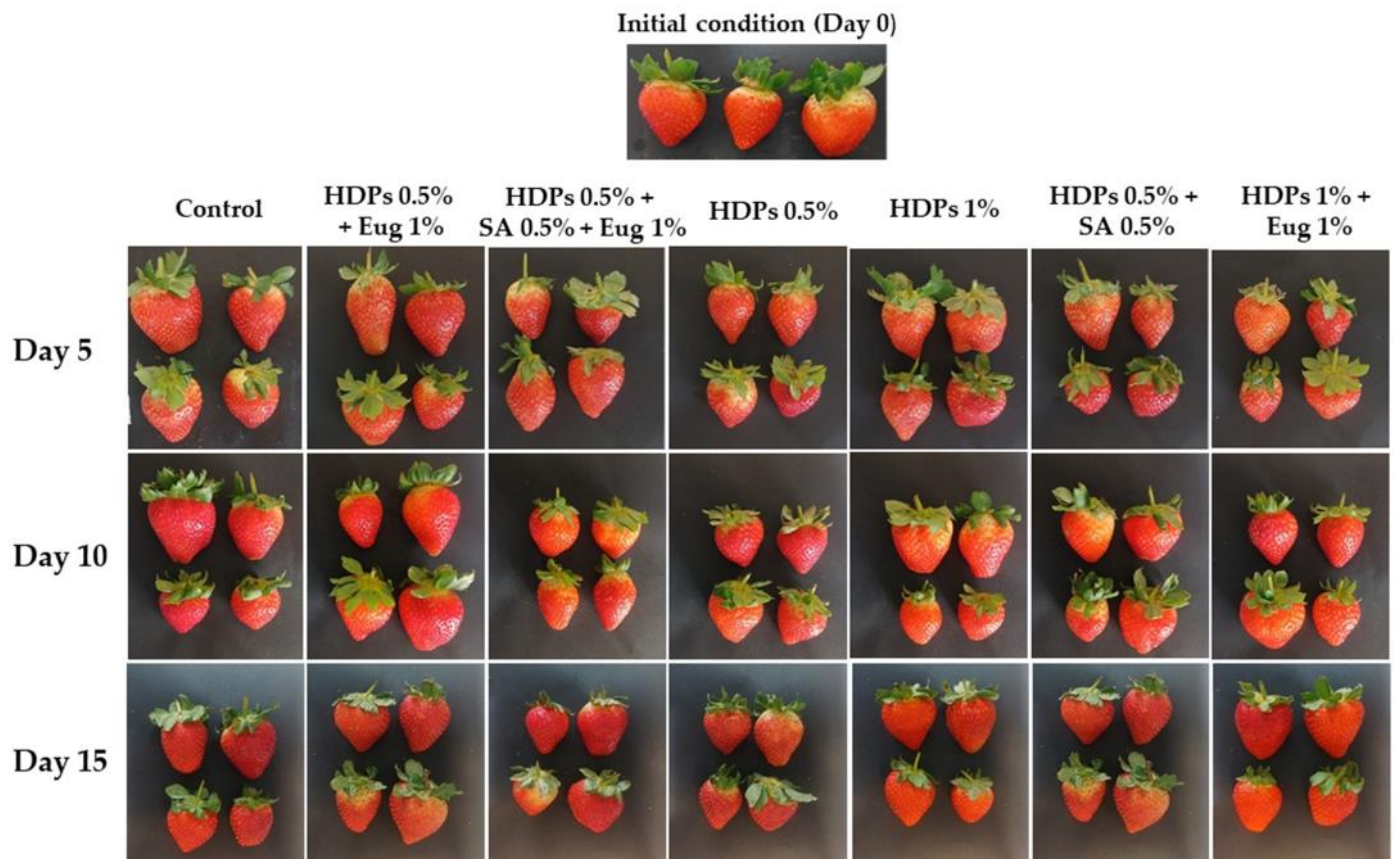


Figure 4. Visual appearance of strawberries subjected to different coating treatments during 15 days of refrigerated storage. Photographs show representative fruits at 0, 5, 10, and 15 days of storage. HDPs—BR: Polysaccharides extracted from Brazilian *Hovenia dulcis*; Eug: eugenol; SA: sodium alginate.

4.2. Total Phenolics, Anthocyanins, and Antioxidant Activity

Total phenolic content showed different patterns among treatments throughout storage (Figure 5a). On day 5, significant differences were observed among treatments, with fruits treated with HDPs-BR 1% showing the highest total phenolic content. At the end of storage, on day 15, the control showed the lowest total phenolic content, whereas all coated fruits exhibited higher values, particularly those treated with HDPs-BR 1% and HDPs-BR 0.5% + SA 0.5% ($p < 0.05$). In addition, variations in total phenolic content were observed throughout storage, with patterns differing according to treatment.

Anthocyanin content also showed different patterns among treatments throughout storage (Figure 5b). On day 5, significant differences were observed among some treatments, with the control showing a higher anthocyanin content than certain coated fruits. However, on day 10, no significant differences were observed among treatments ($p > 0.05$). At the end of storage, on day 15, the HDPs-BR 0.5% + Eug 0.1%, HDPs-BR 1%, HDPs-BR 0.5% + SA 0.5%, and HDPs-BR 1% + Eug 0.1% treatments showed the highest anthocyanin contents and did not differ significantly from one another. Changes in anthocyanin content throughout storage varied according to treatment. In particular, the HDPs-BR 1% + Eug 0.1% treatment showed a higher anthocyanin content on day 15 than on day 10.

The antioxidant activity of strawberries was evaluated using the FRAP and DPPH assays, with the results presented in Figure 5c,d. Antioxidant activity determined by the FRAP assay showed a treatment- and storage period-dependent pattern (Figure 5c). On day 5, differences were observed among the coatings, although some treatments did not differ significantly from the control. On day 10, the HDPs-BR 0.5% + SA 0.5% + Eug 0.1% treatment showed the highest antioxidant activity, which was significantly higher than

that of the control ($p < 0.05$). At the end of storage, HDPs-BR 1% + Eug 0.1% showed the highest numerical FRAP value, although it did not differ significantly from some of the other coating treatments.

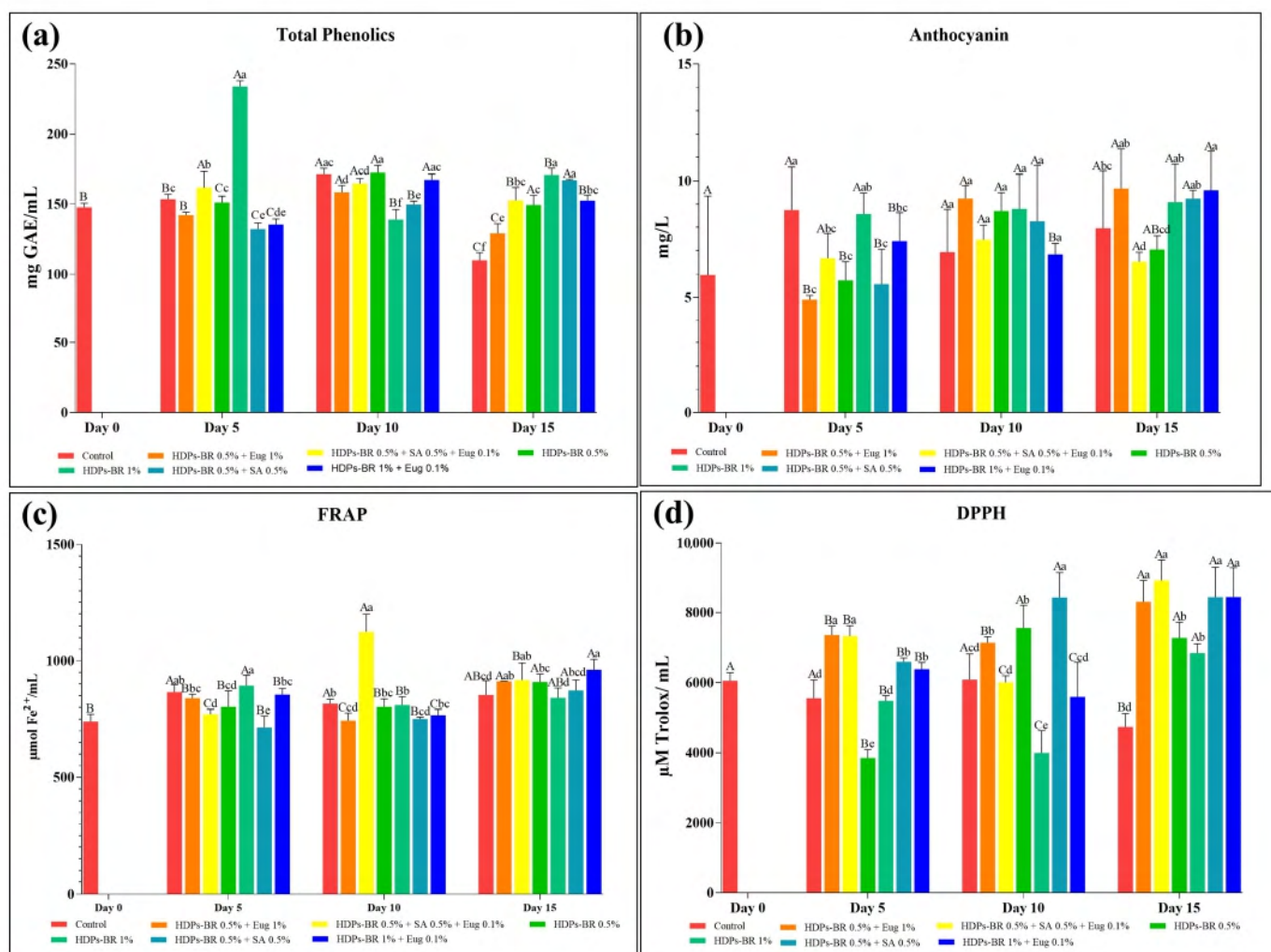


Figure 5. Bioactive compounds and antioxidant activity of strawberries subjected to different coating treatments during storage: (a) total phenolic content; (b) total anthocyanin content; (c) ferric reducing antioxidant power (FRAP); and (d) DPPH radical scavenging activity. Values are expressed as mean $\pm$ standard deviation ($n = 4$). Different lowercase letters indicate significant differences among treatments within the same storage period, whereas different uppercase letters indicate significant differences among storage periods within the same treatment, according to Duncan's multiple range test ($p < 0.05$). HDPs-BR: polysaccharides extracted from Brazilian *Hovenia dulcis*; Eug: eugenol; SA: sodium alginate.

For antioxidant activity determined by the DPPH assay (Figure 5d), on day 5 of storage, the HDPs-BR 0.5% + Eug 0.1% and HDPs-BR 0.5% + SA 0.5% + Eug 0.1% treatments showed higher antioxidant activity than the control. On day 10, HDPs-BR 0.5% + SA 0.5% showed the highest antioxidant activity, while other coatings, including HDPs-BR 0.5% and HDPs-BR 0.5% + Eug 0.1%, also showed higher values than the control. At the end of storage, all coated fruits exhibited significantly higher antioxidant activity than the control ($p < 0.05$), indicating that the differences between coated fruits and the control became more pronounced as storage progressed.

4.3. Microbial Count

The count of aerobic mesophilic microorganisms increased during storage up to day 10 in both the control and the HDPs-BR treatments (Figure 6a). However, on day 15, microbial counts decreased compared with day 10, with no microbial growth detected in some treatments.

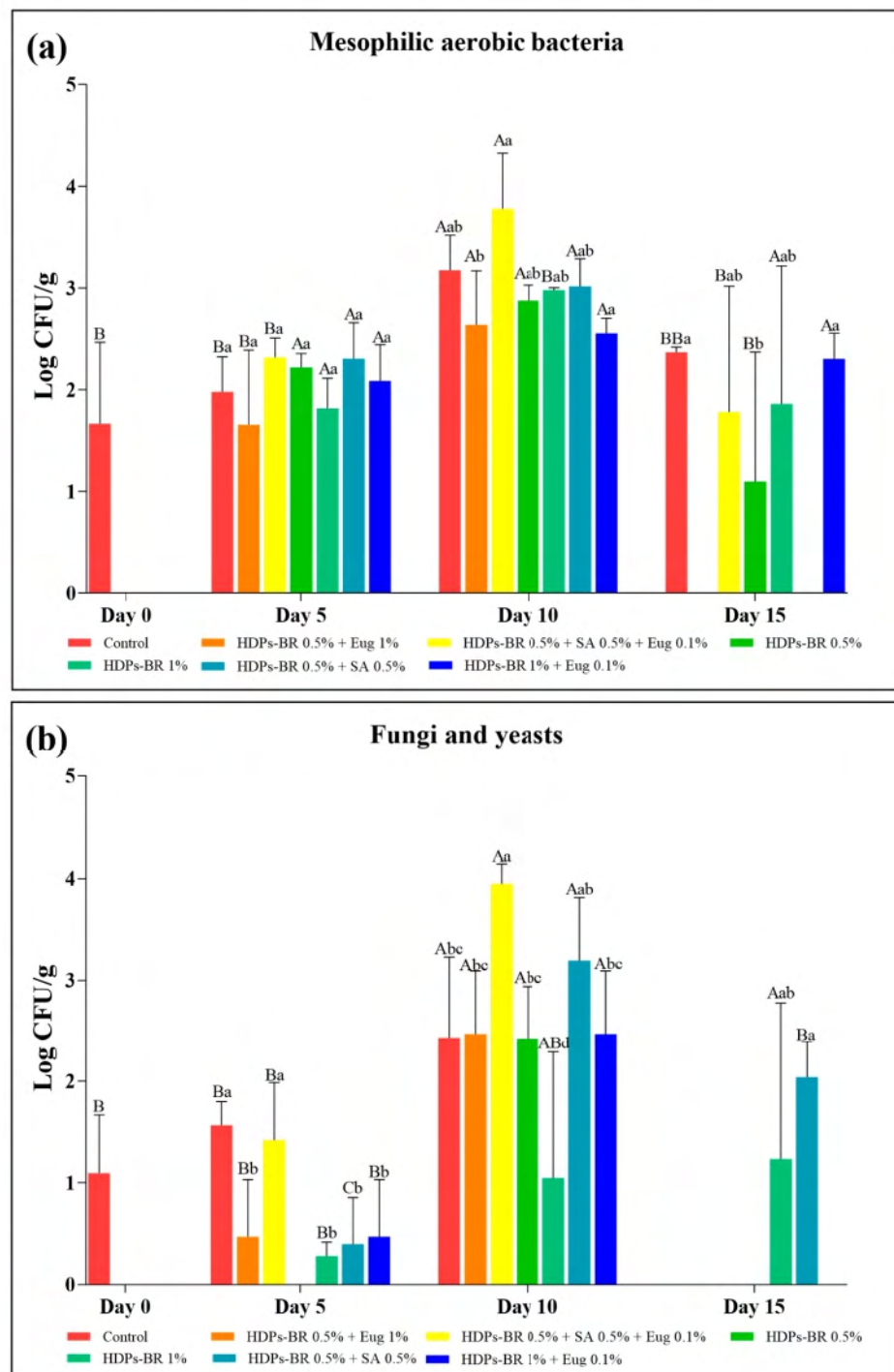


Figure 6. Microbial counts in strawberries subjected to different coating treatments during storage: (a) mesophilic aerobic bacteria and (b) fungi and yeasts. Values are expressed as mean $\pm$ standard deviation ($n = 4$). Different lowercase letters indicate significant differences among treatments within the same storage period, whereas different uppercase letters indicate significant differences among storage periods within the same treatment, according to Duncan's multiple range test ($p < 0.05$). HDPs-BR: polysaccharides extracted from Brazilian *Hovenia dulcis*; Eug: eugenol; SA: sodium alginate.

Fungal and yeast counts generally increased during storage, reaching the highest values on day 10 for most treatments (Figure 6b). However, on day 15, a marked reduction in fungal load was observed, with no mold or yeast growth detected in most treatments, including the control. The exceptions were the HDPs-BR 1% and HDPs-BR 0.5% + SA 0.5% treatments, which still showed fungal growth at this time point. Most coating treatments showed lower fungal counts than the control on day 5 of storage, whereas on day 10, only the HDPs-BR 0.5% + SA 0.5% + Eug 0.1% treatment showed a reduction compared with the control.

No psychrotrophic bacterial growth was observed during the fruit storage period.

4.4. Principal Component Analysis (PCA)

The quality attributes of fruits subjected to the different HDPs-BR-based edible coatings were further evaluated using principal component analysis (PCA). The first two principal components (PC1 and PC2) explained 56.2% of the total variance in the data, accounting for 36.9% and 19.3%, respectively (Figure 7).

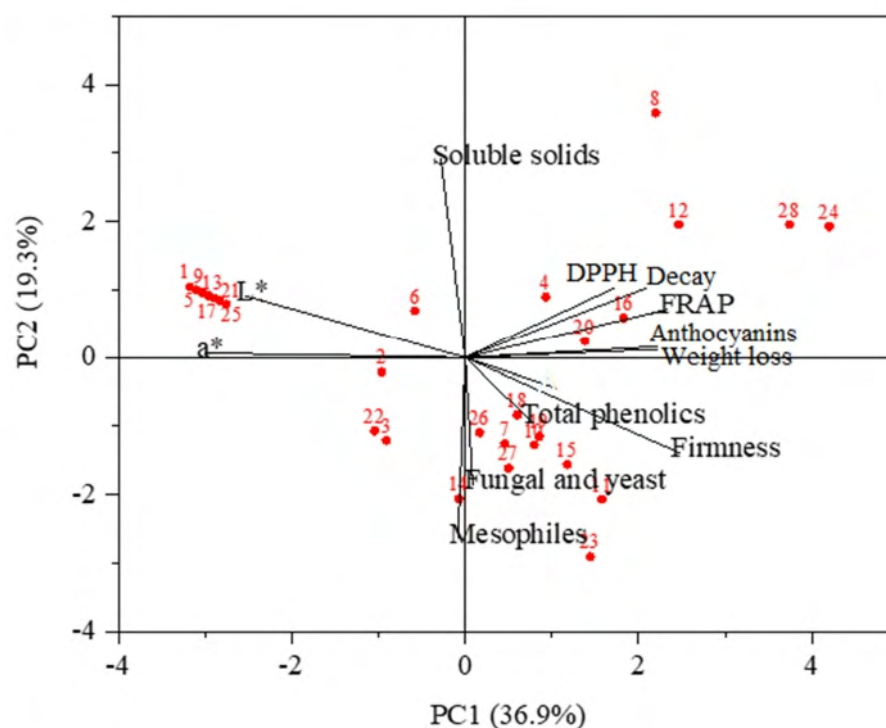


Figure 7. Individual Principal Component Analysis. Caption: 1—Control (Day 0); 2—Control (Day 5); 3—Control (Day 10); 4—Control (Day 15); 5—HDPs-BR 0.5% + Eug 0.1% (Day 0); 6—HDPs-BR 0.5% + Eug 0.1% (Day 5); 7—HDPs-BR 0.5% + Eug 0.1% (Day 10); 8—HDPs-BR 0.5% + Eug 0.1% (Day 15); 9—HDPs-BR 0.5% + SA 0.5% + Eug 0.1% (Day 0); 10—HDPs-BR 0.5% + SA 0.5% + Eug 0.1% (Day 5); 11—HDPs-BR 0.5% + SA 0.5% + Eug 0.1% (Day 10); 12—HDPs-BR 0.5% + SA 0.5% + Eug 0.1% (Day 15); 13—HDPs-BR 0.5% (Day 0); 14—HDPs-BR 0.5% (Day 5); 15—HDPs-BR 0.5% (Day 10); 16—HDPs-BR 0.5% (Day 15); 17—HDPs-BR 1% (Day 0); 18—HDPs-BR 1% (Day 5); 19—HDPs-BR 1% (Day 10); 20—HDPs-BR 1% (Day 15); 21—HDPs-BR 0.5% + SA 0.5% (Day 0); 22—HDPs-BR 0.5% + SA 0.5% (Day 5); 23—HDPs-BR 0.5% + SA 0.5% (Day 10); 24—HDPs-BR 0.5% + SA 0.5% (Day 15); 25—HDPs-BR 1% + Eug 0.1% (Day 0); 26—HDPs-BR 1% + Eug 0.1% (Day 5); 27—HDPs-BR 1% + Eug 0.1% (Day 10); 28—HDPs-BR 1% + Eug 0.1% (Day 15).

Considering absolute loading values ≥ 0.25 (Table 4), PC1 showed positive associations with weight loss (0.439), decay (0.349), firmness (0.332), FRAP (0.322), anthocyanins (0.312), and DPPH (0.257), whereas a^* (−0.41654) and L^* (−0.353) were negatively associated with this component. A positive association with soluble solids (0.567) and negative associations

with aerobic mesophilic microorganisms (−0.525), fungi and yeasts (−0.370), and firmness (−0.271) mainly characterized PC2. Thus, PC1 primarily described variation related to color attributes, physical quality, antioxidant activity-related variables, weight loss, and decay, whereas PC2 was more strongly associated with soluble solids and microbial counts.

Table 4. Loading matrix of principal component analysis.

PCA Loading	PC1 (36.9%)	PC2 (19.3%)
L*	−0.35277	0.19326
a*	−0.41654	0.02807
Firmness	0.33159	−0.27146
Soluble solids	−0.03033	0.56749
Weight loss	0.43918	0.01926
Fungal and yeast	0.01109	−0.3698
Mesophiles	−0.01233	−0.52478
Decay	0.34863	0.23642
Total phenolics	0.09601	−0.17149
Anthocyanins	0.31224	0.0329
FRAP	0.32231	0.13321
DPPH	0.25669	0.22244

The score distribution further indicated that, on day 15, the HDPs-BR 1%, HDPs-BR 0.5%, HDPs-BR 0.5% + SA 0.5%, HDPs-BR 0.5% + SA 0.5% + Eug 0.1%, HDPs-BR 1% + Eug 0.1%, and HDPs-BR 0.5% + Eug 0.1% treatments were predominantly positioned on the positive side of PC1. Accordingly, their distribution was associated with the combined contribution of firmness, anthocyanins, FRAP, DPPH, weight loss, and decay, which showed positive loadings on this component, in contrast to L* and a*, which were negatively associated with PC1.

5. Discussion

5.1. Extraction and Characterization of Polysaccharides

5.1.1. Color, Chemical Composition, and Sugar Profile

The extraction yield obtained for HDPs-BR ($5.31 \pm 0.19\%$) was lower than the value of $6.89 \pm 0.21\%$ reported by Yang et al. (2019, 2022) [8,48]. This difference may be related to variations in the characteristics of the raw material and extraction conditions. Factors associated with the geographical origin of the plant material, environmental conditions, developmental stage, and intrinsic chemical composition may influence the relative abundance, solubility, and extractability of different polysaccharide fractions. In addition, extraction conditions can affect mass transfer and the selective solubilization of polysaccharide fractions, resulting in differences in both extraction yield and composition. Hot-water extraction, although one of the most common and accessible methods for obtaining polysaccharides, requires a substantially longer extraction time, being approximately nine times slower than solvent-based methods [8]. Therefore, differences between the HDPs-BR obtained in the present study and polysaccharides previously characterized from *Hovenia dulcis* may reflect both the characteristics of the raw material and the conditions employed during extraction.

The heterogeneous and porous structure observed by SEM suggests the presence of an irregular matrix with regions potentially accessible to interactions with water and other compounds. Such morphology may be relevant to the technological behavior of HDPs-BR, since the organization and surface characteristics of a polysaccharide matrix can influence hydration and interactions with other constituents of coating formulations. In this context, the presence of hydroxyl groups from carbohydrate chains and carboxyl groups associated

with uronic acids may favor intermolecular interactions, including hydrogen bonding, potentially affecting the organization of the polymeric matrix. These characteristics could be relevant to the application of HDPs-BR in edible coating systems. However, SEM morphology alone is insufficient to demonstrate water-holding capacity or barrier properties, and specific analyses would be required to confirm these functional characteristics.

The protein and uronic acid contents were similar to those previously reported for *Hovenia dulcis* polysaccharides, which ranged from 2.48 to 5.28 g 100 g⁻¹ for proteins and from 14.58 to 21.19 g 100 g⁻¹ for uronic acids. In contrast, the total sugar content determined in the present study was higher than the range of 40.77–50.81 g 100 g⁻¹ reported by Yang et al. (2020, 2022) [3,48]. Rather than representing only a quantitative difference, this variation may indicate differences in the relative composition of the polysaccharide fractions recovered. Extraction conditions can favor the solubilization of particular carbohydrate fractions and consequently modify the relative proportions of sugars, uronic acids, proteins, and other compounds associated with the extracted material. Furthermore, to the best of our knowledge, this is the first study to characterize polysaccharides from *Hovenia dulcis* collected in southeastern Brazil (Minas Gerais). Thus, differences associated with geographical origin and the characteristics of the plant material may also have contributed to the observed chemical composition.

These compositional characteristics may be relevant to the technological and functional behavior of HDPs-BR. The relatively high carbohydrate content, together with the presence of uronic acids, may influence hydration and intermolecular interactions within the polymeric matrix. Moreover, differences in chemical composition may affect the rheological behavior and organization of HDPs-BR when used in coating-forming systems. Therefore, the compositional differences observed between HDPs-BR and previously characterized *Hovenia dulcis* polysaccharides [8,42,43] may also contribute to differences in their physicochemical and functional behavior.

Previous studies have indicated that *Hovenia dulcis* polysaccharides predominantly contain galactose, glucose, and arabinose [3,8,18,24,48]. In the present study, however, galactose and arabinose could not be quantified due to the unavailability of the corresponding reference standards. Because chromatographic identification and quantification rely on comparison with reference standards, the sum of the individually identified sugars (13.82 g 100 g⁻¹) should not be interpreted as the actual total sugar content of HDPs-BR. Instead, the difference between this value and the total sugar content reflects, at least in part, the incomplete characterization of the monosaccharide profile. Considering that galactose and arabinose have previously been reported as major constituents of *Hovenia dulcis* polysaccharides [3,8,18,24,48], their absence from the quantified profile likely contributed to the underestimation of the sum of individually identified monosaccharides. Therefore, the total sugar assay and the HPLC monosaccharide profile should be regarded as complementary measurements rather than directly equivalent determinations.

5.1.2. Total Phenolic Compounds and Antioxidant Activity of the Polysaccharides

Phenolic compounds are characterized by structures containing one or more aromatic rings and hydroxyl groups, which are directly related to their ability to donate electrons or hydrogen atoms and, consequently, to their antioxidant activity. Flavonoids constitute an important class of phenolic compounds characterized by a C6-C3-C6 backbone.

The Fast Blue method used for total phenolic determination is based on the coupling of phenolic compounds with a diazonium salt, resulting in the formation of an azo complex. The Fast Blue salt contains a diazonium group (-N=N⁺-), which reacts preferentially with activated positions of the phenolic structure. Coupling generally occurs at positions activated by hydroxyl groups, and when these positions are occupied, substitution may

occur at the ortho position relative to the activating group [33]. This reaction mechanism allows the method to detect phenolic compounds associated with the polysaccharide matrix.

Although no specific studies using the Fast Blue method to quantify total phenolics in this exact matrix were found, the high phenolic content observed in HDPs-BR may be related to the presence of phenolic fractions associated with the polysaccharide structure. Previous studies have identified approximately 13 phenolic compounds in *Hovenia dulcis* polysaccharides, including protocatechuic acid, gallic acid, p-hydroxybenzoic acid, ampelopsin, quercetin-7,4'-diglucoside, dihydroquercetin, rutin, myricitrin, quercetin, kaempferol, 5-methylmyricitrin, and naringenin [24]. Therefore, the phenolic content detected in the present study is consistent with the occurrence of naturally associated phenolic constituents in *Hovenia dulcis* polysaccharide fractions.

The association of phenolic compounds with polysaccharides may occur through non-covalent interactions, including hydrogen bonding, hydrophobic interactions, and ionic interactions, as well as through associations involving proteins present in the matrix [49]. These interactions can be influenced by heating and processing conditions, potentially favoring the retention of phenolic compounds within the extracted polysaccharide fraction. Thus, the phenolic content of HDPs-BR may not represent only free phenolic molecules but also phenolic compounds associated with macromolecular components of the extract. This association may be functionally relevant because it can influence both the physicochemical behavior and the antioxidant properties of the polysaccharide matrix.

Previous studies have also reported pronounced antioxidant activity for *Hovenia dulcis* polysaccharides, particularly regarding hydroxyl radical scavenging capacity [3,8,50]. Although the analytical methods and units used in those studies differ from those employed in the present work, these reports support the bioactive potential of *Hovenia dulcis* polysaccharide fractions. In general, the antioxidant activity of polysaccharides depends on several structural and compositional characteristics, including chemical composition, molecular weight, and monosaccharide profile [8]. In the present study, the antioxidant activity of HDPs-BR may therefore reflect the combined contribution of the polysaccharide backbone and associated phenolic compounds rather than the action of a single constituent.

The presence of residual phenolic compounds may be particularly relevant to the antioxidant response of HDPs-BR because hydroxyl groups present in phenolic structures can participate in electron- or hydrogen-donation reactions, contributing to radical scavenging and reducing capacity. At the same time, the polysaccharide structure itself may influence the accessibility and reactivity of these associated compounds. Consequently, differences in extraction conditions, molecular organization, monosaccharide composition, and degree of association with phenolic compounds may help explain variations in antioxidant activity among *Hovenia dulcis* polysaccharides reported in different studies [3,8,48,50].

However, the present study did not isolate the contribution of the polysaccharide fraction from that of the associated phenolic compounds. Therefore, the antioxidant activity observed for HDPs-BR should be interpreted as a property of the crude polysaccharide extract as a whole, resulting from the combined contribution of its carbohydrate matrix and associated bioactive constituents. Further purification and structural characterization would be necessary to determine the relative contribution of each fraction to the antioxidant activity.

5.1.3. FTIR and TGA of HDPs-BR

The extracted polysaccharides exhibited spectral profiles similar to those reported in previous studies with the same polysaccharide obtained from other countries and by different extraction methods. This similarity suggests that, despite differences in geographical origin and extraction conditions, the main chemical functionalities of the HDPs-BR matrix

were preserved, indicating a broadly comparable structural organization to that previously described for *Hovenia dulcis* polysaccharides.

The band around 3390 cm^{-1} is indicative of O–H stretching, whose main contribution comes from the hydroxyl groups of the polysaccharide [51]. The abundance of hydroxyl groups is relevant not only for structural identification but also for the physicochemical behavior of the material, since these groups can participate in hydrogen bonding and influence hydration, intermolecular interactions, and the organization of the polymeric matrix [52]. The band around 2930 cm^{-1} is characteristic of polysaccharides, originating from the C–H stretching of alkyl groups, a typical feature of polysaccharidic substances [8,48,50]. The strong absorption at 1645 cm^{-1} was attributed to the asymmetric and symmetric stretching vibrations of C=O, indicating that the polysaccharide is acidic [8,48,51]. This interpretation is consistent with the uronic acid content determined in the chemical characterization and suggests the presence of carboxyl-containing fractions that may contribute to the hydrophilic and ionic characteristics of the matrix.

The absorption peak at 1251 cm^{-1} indicates the presence of sulfate radical moieties in the *Hovenia dulcis* polysaccharides (S=O stretching vibration) [8]. The presence of sulfate groups can confer an anionic character to the polysaccharide matrix, increasing its negative charge density and favoring electrostatic interactions with positively charged species [53]. In addition, sulfate groups can interact with water molecules, thereby influencing hydration and intermolecular interactions within the polymeric matrix [54]. The peak at 1075 cm^{-1} is related to the stretching vibration of the C–O–C glycosidic bond [46], while the band at 1034 cm^{-1} indicates the presence of C–O–C and C–O–H bonds [51]. Together, these absorptions are consistent with a carbohydrate-rich polymeric backbone in which glycosidic linkages and hydroxyl-bearing groups predominate.

In summary, the different observed bands strongly suggest that the extracted polysaccharide consists of acidic polysaccharides with hydroxyl (O–H), alkyl (C–H), carboxyl (C=O), and sulfate residue (S=O) groups, characterizing a matrix similar to those previously reported for *Hovenia dulcis*. From a functional perspective, this combination of hydroxyl, carboxyl, and sulfate-containing groups may favor intermolecular associations and interactions with water and other constituents of coating formulations. Such chemical features may help explain the ability of HDPs-BR to participate in polymeric networks and may influence properties relevant to coating formation, such as hydration, adhesion, and compatibility with other biopolymers [54]. However, FTIR data alone do not allow these functional properties to be quantified, and specific rheological, barrier, and interaction analyses are required to confirm these mechanisms.

Regarding TGA, the first mass loss ($27\text{ }^{\circ}\text{C}$ to $150\text{ }^{\circ}\text{C}$) occurs mainly due to the vaporization of adsorbed and structural water [3,8]. This initial event indicates that part of the water is physically associated with the polysaccharide matrix, which is consistent with the abundance of hydrophilic functional groups identified by FTIR. Above $150\text{ }^{\circ}\text{C}$, devolatilization of the polysaccharide leads to subsequent degradation. This stage is likely associated with the progressive breakdown of the carbohydrate structure, including cleavage and decomposition of glycosidic linkages and other thermally labile components of the matrix. The analyses of degradation behavior and thermal stability indicate that the polysaccharides are composed of a variety of complex polymers with different structures. The broad and progressive nature of the mass loss is therefore consistent with the heterogeneous chemical composition of the crude polysaccharide fraction, in which carbohydrates, uronic acids, proteins, and associated compounds may exhibit different thermal degradation behaviors.

This thermal robustness, characterized by the initial stability up to $150\text{ }^{\circ}\text{C}$ and the high residual mass (43% at $350\text{ }^{\circ}\text{C}$), suggests that the *Hovenia dulcis* polysaccharide or films based on it are suitable for applications where structural integrity at moderate temperatures is es-

sential. Importantly, the thermal range in which the material remains comparatively stable is well above the temperatures used during refrigerated storage and typical postharvest handling, indicating that thermal degradation is unlikely to limit its performance under these conditions. Thus, the material shows promise for the development of edible coatings and bioactive packaging for food preservation, offering greater thermal resistance during processing and storage under ambient or refrigerated conditions. Nevertheless, the TGA results demonstrate thermal stability of the extracted material itself and should not be interpreted as direct evidence of the thermal or mechanical stability of a finished film, since these properties may change after the addition of plasticizers, sodium alginate, eugenol, or other formulation components.

5.2. Fruit Quality Analyses

5.2.1. Color, Texture, Soluble Solids, Weight Loss, and Decay Rate

The higher lightness (L^*) observed on day 15 in fruits treated with HDPs-BR 0.5% + SA 0.5% + Eug 0.1% suggests better preservation of this attribute during storage. This behavior may be related to the formation of a physical barrier by the coating, which may reduce oxygen diffusion and, consequently, limit oxidative browning reactions associated with the activity of enzymes such as polyphenol oxidase and peroxidase [55]. However, the superior performance of this formulation should not be attributed to any of its components individually. The association between HDPs-BR and SA may have modified the physical properties of the coating matrix, while the presence of eugenol may have contributed to limiting oxidative processes. Thus, the observed response likely results from the properties of the formulation as a whole and the physicochemical interactions established among its constituents.

Similarly, the maintenance of a^* values throughout storage in the HDPs-BR 1% + Eug 0.1% treatment indicates greater stability of the red color of the fruits. This behavior may be associated with the protection of the natural pigments of strawberries, particularly anthocyanins, which are susceptible to oxidative degradation and light exposure. A similar result was reported by Lee et al. (2022) [56], who observed less browning and opacity in strawberries coated with cellulose and chitosan nanomaterials supplemented with essential oil compared with the control. In this context, the higher HDPs-BR concentration may have produced a matrix with physical characteristics different from those obtained at 0.5%, thereby modifying the interface between the fruit and the surrounding environment. Its association with eugenol may also have contributed to reducing oxidative reactions capable of affecting pigment stability. However, because eugenol was not evaluated individually, its specific contribution to color preservation cannot be quantified in the present study.

In addition to the possible protective effect of the coatings against oxidative processes, variations in fruit color may be related to the intrinsic coloration of the polysaccharides used in the formulations (Table 2), which exhibited a reddish-yellow hue (Figure 1). This characteristic may have influenced the visual appearance of the coated fruits. Therefore, differences in color parameters likely reflect both physiological changes in the fruits during storage and the optical and physicochemical properties of the coatings themselves. This aspect is particularly important when interpreting L^* and a^* , since the surface layer formed by the coating may modify the interaction of light with the fruit surface in addition to exerting possible effects on the stability of endogenous pigments. Thus, some of the formulations evaluated, particularly HDPs-BR 1% + Eug 0.1%, showed better preservation of red color compared with the control, a behavior similar to that described for other edible coatings applied to strawberries.

Firmness is one of the main quality parameters of fresh fruits and generally decreases in strawberries during storage as a result of changes in the cell wall and loss of tissue

turgor [57]. In the present study, however, the firmness response varied according to treatment and storage period. The control showed no significant change in firmness throughout storage, whereas some coated treatments showed a significant increase in this parameter at specific storage periods. At the end of storage, fruits treated with HDPs-BR 0.5% showed greater firmness than both the control and HDPs-BR 1%, indicating that increasing the polysaccharide concentration did not necessarily result in greater mechanical resistance of the fruits. This result demonstrates that coating performance does not necessarily follow a linear relationship with HDPs-BR concentration. Changes in polymer concentration can modify properties such as viscosity, coating distribution over the fruit surface, and characteristics of the resulting matrix; therefore, a higher concentration does not necessarily result in better texture preservation [58].

Although higher firmness values are frequently associated with better texture preservation, instrumentally determined firmness represents the overall mechanical resistance of the tissue and does not depend exclusively on cell wall integrity. In strawberries, this mechanical response results from the combined contributions of the cell wall, cell-to-cell adhesion, turgor status, and characteristics of epidermal and subepidermal tissues. Thus, different structural changes may produce distinct responses in instrumental firmness measurements. During storage, processes that promote softening, such as degradation of cell wall components and loss of turgor, may occur simultaneously with other structural changes in the tissues. Therefore, the increase or maintenance of firmness observed in some treatments should not be interpreted exclusively as evidence of reduced cell wall degradation, but rather as the result of a combination of structural and water-related changes affecting the mechanical response of the fruit during storage [59].

Higher firmness in strawberries subjected to edible coatings has also been reported by Lee et al. (2022) [56], using cellulose nanomaterials; Kwak et al. (2021) [60], using chitosan-based coatings combined with essential oil and cellulose nanofibers; and Wang et al. (2020) [61], using chitosan-based coatings. Edible coatings can modify water and gas exchange between the fruit and the environment and, consequently, influence the mechanical properties of tissues during storage [55,61,62]. However, in the present study, because the coatings did not significantly reduce weight loss compared with the control, the greater firmness observed in certain treatments cannot be directly attributed to reduced water loss. Furthermore, because specific analyses of the cell wall or its associated enzymes were not performed, it is not possible to establish whether the higher firmness values resulted from reduced cell wall degradation. Therefore, the effect observed for HDPs-BR 0.5% should be interpreted as greater mechanical resistance of the fruits under the evaluated conditions, rather than as direct evidence of reduced senescence or greater cell wall integrity.

The small changes observed in soluble solids may be related to the ripening stage of the strawberries at the time the coatings were applied. Similar behavior was reported by Guerreiro et al. (2015) [57]. In contrast, Al-Hilifi et al. [63] observed greater variation in soluble solids content in strawberries coated with polysaccharide- and protein-based formulations containing hyaluronic acid, with higher values in the control at the end of storage. Lee et al. (2022) [56] also observed a continuous increase in soluble solids during storage, although the rate of increase was higher in uncoated fruits. Kwak et al. (2021) [60], using cellulose nanofibers, reported lower soluble solids contents in coated fruits.

Because the strawberries used in the present study were already ripe when the coatings were applied, physiological processes related to changes in sugar and other soluble solid contents may have already been at an advanced stage, reducing the influence of the coatings on this parameter. In addition, changes in soluble solids during storage may result from competing processes, such as sugar consumption through respiration and solute

concentration resulting from water loss. Therefore, small variations in this parameter cannot be attributed exclusively to the effects of the coatings. Factors such as cultivar and interactions between coating type and the physiological characteristics of the fruits may also contribute to differences among studies.

The high susceptibility of strawberries to postharvest water loss is associated with their thin waxy cuticle and the vapor pressure gradient between fruit tissues and the surrounding atmosphere, contributing to softening, shriveling, and loss of surface gloss [56,64]. Although edible coatings can act as semipermeable barriers and reduce transpiration, this effect was not sufficient to produce significant differences in weight loss compared with the control under the conditions evaluated. This result differs from those reported by Al-Hilifi et al. (2024) [63] and Lee et al. (2022) [56], who observed lower weight loss in strawberries coated with different polysaccharide- and nanomaterial-based formulations. This divergence may be related to the specific properties of each film-forming matrix, since its efficiency as a barrier to water vapor transfer depends on the composition, concentration, continuity, and structural organization of the coating. In the present study, the absence of a significant difference suggests that the HDPs-BR-containing formulations did not form a barrier to water loss sufficiently different from the natural resistance provided by the fruit surface under the storage conditions used.

This result also helps explain why different HDPs-BR concentrations did not show uniform performance across the evaluated parameters. A higher polymer concentration can simultaneously alter viscosity, surface coverage, matrix structure, and mass transfer, but these changes do not necessarily improve all coating properties. Thus, the optimal concentration for maintaining firmness, for example, may not be the same as that required to reduce mass loss or preserve color, demonstrating that postharvest performance depends on the balance among different physicochemical properties of the formulation.

Finally, due to their non-climacteric nature, strawberries are harvested at the eating ripe stage and are highly susceptible to postharvest deterioration because of characteristics such as high moisture content and soft texture [65]. Although HDPs-BR 0.5% and HDPs-BR 1% showed no decayed fruits at the end of storage, the absence of a significant difference compared to the control indicates that this result should be interpreted as a trend rather than as statistical evidence of reduced decay.

This result differs from that reported by Yuan et al. (2020) [66], who observed a significant reduction in the decay of strawberries treated with polysaccharides from *Pythium arrhenomanes* compared with the control. Differences in the nature of the polysaccharides, extraction methods, purification processes, and coating characteristics may contribute to this divergence. These differences are mechanistically relevant because changes in the chemical composition and organization of the polysaccharide matrix can modify properties such as hydration, interaction with the fruit surface, and barrier behavior. Consequently, polysaccharides obtained from different sources or subjected to different extraction processes do not necessarily exhibit the same technological performance when applied as coatings. Nevertheless, the absence of decay observed in fruits coated only with HDPs-BR 0.5% and HDPs-BR 1% suggests the potential of these polysaccharides for postharvest applications and warrants further investigation.

A possible protective effect of HDPs-BR could be associated with the formation of a physical barrier capable of modifying gas exchange and limiting contact between fruit tissues and spoilage microorganisms [65]. However, because weight loss was not significantly reduced, the data do not support attributing this possible protective effect primarily to reduced water loss. Other mechanisms, such as changes in conditions at the fruit-atmosphere interface and in the surface properties of the fruits, may be involved.

In addition, some formulations containing eugenol and/or sodium alginate showed higher decay indices, although this behavior was not consistent across formulations. This result demonstrates that the mere presence of a component with previously recognized properties does not guarantee better performance of the final formulation. Incorporating different components into the coating matrix can alter its structural organization and physicochemical properties, since interactions among constituents can modify viscosity, network formation and density, mechanical resistance, hydrophobicity, and film barrier properties, consequently influencing its performance when applied to the fruit surface [67]. Thus, the observed performance depends on the complete formulation and the proportions of its constituents rather than solely on the individual properties of each ingredient.

Eugenol, in particular, has previously described antimicrobial and antioxidant properties and may therefore contribute to limiting microbiological and oxidative processes in certain formulations [68,69]. However, the effectiveness of eugenol when incorporated into polymeric coatings depends not only on its concentration but also on its distribution within the matrix and its diffusion and release behavior, factors that determine its availability and, consequently, its activity at the surface of the coated product [70]. Thus, the presence of eugenol may contribute to the response of some formulations without necessarily producing the same effect in all of them.

Considering the absence of treatments containing SA and Eug individually, it is not possible to attribute these results to the individual effects of these components or to establish possible interactions between them and HDPs-BR. Consequently, terms such as synergism or specific causal attributions to individual components should be avoided. Combined treatments should be interpreted as complete formulations whose responses result from the collective properties of their constituents. Further studies including individual controls are required to clarify the contribution of each component to the postharvest preservation of strawberries.

5.2.2. Total Phenolics, Anthocyanins, and Antioxidant Activity

Fruits are among the main natural sources of antioxidants, including carotenoids, phenolic compounds, and vitamins. These compounds act as free radical scavengers and are capable of decomposing peroxides, quenching singlet and triplet oxygen, and inhibiting the activity of oxidative enzymes [71]. During postharvest storage, however, the content and activity of these compounds may change as a result of oxidative processes, senescence, and changes in fruit metabolism. In this context, edible coatings may contribute to their preservation by acting as semipermeable barriers between the fruit and the environment, modifying O₂ and CO₂ exchange and respiration rate [72] and, consequently, influencing metabolic and oxidative processes associated with senescence and the degradation of bioactive compounds.

The higher total phenolic contents observed in coated fruits at the end of storage suggest that the coatings may have contributed to the maintenance and/or accumulation of these compounds during storage. These results are consistent with those of Al-Hilifi et al. (2024) [63], who applied hyaluronic acid-based polysaccharide–protein coatings to strawberries and observed a smaller reduction in total phenolic content in coated fruits at the end of storage. Similarly, Farida et al. (2023) [23] found that strawberries coated with different types of edible coatings exhibited higher total phenolic contents than uncoated fruits.

Considering the higher total phenolic contents observed in coated fruits at the end of storage, two hypotheses can be proposed: (i) the coatings may have influenced fruit secondary metabolism, favoring the synthesis and/or maintenance of phenolic compounds, an effect previously described in studies with strawberries [73]; or (ii) the observed values may be related, at least in part, to the composition of HDPs-BR, which exhibited a

high phenolic content as well as significant antioxidant activity, as demonstrated by the phosphomolybdenum, ABTS, and FRAP assays (Table 3). These two mechanisms are not mutually exclusive.

Differences in the responses among formulations may also be related to how HDPs-BR concentration and the presence of SA and eugenol modify the properties of the coating matrix. Changes in polymer concentration can modify hydration, viscosity, surface distribution, and matrix organization, thereby influencing the interaction between the coating and fruit tissues [74]. Therefore, a higher concentration of HDPs-BR does not necessarily result in greater preservation of phenolic compounds, since coating performance depends on the balance among composition, matrix structure, and storage conditions.

Anthocyanins are the main compounds responsible for the characteristic red color of strawberries. The higher contents observed in certain coated fruits at the end of storage are consistent with the results of Yuan et al. (2020) [66], who applied edible coatings based on polysaccharides from *Pythium arrhenomanes* to strawberries and observed greater anthocyanin retention in coated fruits.

Strawberries exhibit the respiratory behavior characteristic of non-climacteric fruits. Although degradation reactions typical of senescence occur during the postharvest period of non-climacteric fruits, changes in anthocyanin metabolism may continue after harvest, even in already ripe fruits, whereas the loss of cellular integrity as senescence progresses may favor pigment degradation. In the present study, because the strawberries were already ripe when the coatings were applied, the higher anthocyanin contents observed at the end of storage should be interpreted primarily in terms of maintenance, reduced degradation, and/or changes in pigment metabolism, rather than necessarily as an exclusive consequence of postharvest synthesis.

In this context, the high anthocyanin contents observed in certain treatments at the end of storage may be associated with the maintenance and/or accumulation of these pigments. Edible coatings may have acted as protective barriers, contributing to a reduction in oxidative processes associated with anthocyanin degradation [75].

Another hypothesis is that coating application may have modulated secondary metabolic pathways in the fruits, favoring the biosynthesis and/or reducing the degradation of these pigments [76]. The relationship between anthocyanin content and a^* values also supports the plausibility of an effect on the preservation of red color in certain treatments, although color parameters may be simultaneously influenced by the natural pigmentation of the fruit and the optical characteristics of the coating layer. Because this behavior was observed across different formulations, it cannot be attributed exclusively to a specific combination of HDPs-BR, SA, or eugenol.

The fact that some formulations showed greater anthocyanin retention than others may reflect differences in the physical and chemical properties of the resulting matrix. HDPs-BR concentration, the presence of SA, and the incorporation of eugenol may modify coating organization and its interaction with the fruit surface, producing different levels of protection against oxidative processes. Thus, the performance of each treatment likely results from the formulation as a whole rather than from a linear relationship between polysaccharide concentration and pigment retention.

The FRAP assay is based on the reduction of the ferric complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants [37], whereas the DPPH method is based on the transfer of electrons or hydrogen atoms from an antioxidant to the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl), resulting in radical neutralization and a consequent change in solution color [77]. Thus, the two methods provide complementary information on the antioxidant capacity of the samples. Consequently, differences between the patterns

observed with FRAP and DPPH are expected, since each method responds differently to the chemical nature, concentration, and reactivity of the antioxidants present in the matrix.

The higher antioxidant activity observed in certain coated fruits may be related to different mechanisms. HDPs-BR themselves contain residual phenolic compounds and exhibit antioxidant activity, while eugenol is a phenolic compound containing a free hydroxyl group [69], which may contribute to the reducing capacity of the formulations. Indeed, studies using polymer-based edible films enriched with eugenol have reported significant increases in antioxidant activity [40,78,79].

Mechanistically, the phenolic hydroxyl group of eugenol can participate in electron- or hydrogen-donation processes, contributing to the neutralization of reactive species and increasing the reducing capacity of the system [79]. However, the contribution of eugenol within the coating depends not only on its presence but also on its incorporation and availability within the polymeric matrix [70]. Different formulations may alter the retention, distribution, and accessibility of this compound at the fruit surface, which may help explain why treatments containing eugenol did not necessarily exhibit identical antioxidant responses.

However, considering the absence of treatments containing eugenol and SA individually, the individual contributions of these components and their possible interactions with HDPs-BR cannot be determined in the present study. Therefore, the results should be interpreted in terms of the performance of the complete formulations evaluated. For this reason, it cannot be stated that the higher antioxidant activity values resulted from synergism between HDPs-BR and eugenol; the data only allow the conclusion that certain formulations containing these components exhibited greater antioxidant capacity under specific storage conditions.

Although the expression of DPPH results used in the present study differs from that employed by Yuan et al. (2020) [66], the findings are consistent, since these authors reported greater antioxidant properties in strawberries coated with polysaccharides from *Pythium arrhenomanes* compared with control samples. Similarly, Al-Hilifi et al. (2024) [63] found that the application of a hyaluronic acid-based edible coating maintained the antioxidant activity of strawberries, whereas uncoated fruits showed a significant reduction in this activity.

The higher antioxidant activity of coated fruits, particularly at the end of storage, may be related to the ability of the coatings to modify conditions at the fruit surface, thereby contributing to limiting oxidative processes associated with the degradation of bioactive compounds, an effect similar to that observed in chitosan-coated strawberries [75]. This mechanism may simultaneously contribute to the maintenance of phenolic compounds and anthocyanins, increasing the availability of compounds capable of participating in the reactions measured by the FRAP and DPPH assays. Thus, the antioxidant response should not be interpreted as an isolated property but rather as the integrated result of the amount, stability, and reactivity of the different bioactive compounds present in the tissues.

In addition, the components of HDPs-BR themselves may have contributed to the observed antioxidant activity, considering their previously demonstrated activity (Table 3). Because the HDPs-BR fraction contains associated phenolic compounds, its application may add a matrix with intrinsic antioxidant potential to the fruit surface. However, without separately determining the antioxidant activity of the fruit tissue and the coating after application, it is not possible to distinguish how much of the observed response derives from endogenous strawberry antioxidants and how much may be related to coating constituents.

Another possibility is that coating application influenced fruit secondary metabolism, favoring the synthesis and/or maintenance of metabolites with antioxidant properties. The higher antioxidant activity observed in certain treatments may also be associated with the maintenance and/or accumulation of bioactive compounds, including phenolic com-

pounds and anthocyanins (Figure 5a,b). The correspondence between higher levels of these compounds and greater antioxidant activity in some treatments provides a biologically plausible interpretation since phenolics and anthocyanins can participate in electron- and hydrogen-transfer reactions. However, this association does not establish direct causality, because other antioxidants present in strawberries may also contribute to FRAP and DPPH responses.

The differences observed among formulations reinforce that coating effectiveness does not simply result from the presence of HDPs-BR, SA, or eugenol but rather from how these components jointly modify matrix properties and the physiological response of the fruits. One formulation may favor greater pigment retention, whereas another may exhibit greater antioxidant capacity at a particular storage period, indicating that there is not necessarily a single optimal formulation for all evaluated attributes. This behavior also helps explain why increasing HDPs-BR concentration or adding eugenol did not produce uniform effects across all variables.

However, these mechanisms should be considered hypotheses, since the present study does not allow differentiation of whether the higher antioxidant activity resulted from preservation of endogenous fruit antioxidants, changes in secondary metabolism, contributions from compounds present in the coatings, or a combination of these factors. Likewise, because specific markers of oxidative stress, antioxidant enzyme activity, or expression of genes related to the biosynthesis and degradation of phenolics and anthocyanins were not evaluated, the proposed mechanisms cannot be directly confirmed. Future studies integrating these analyses may clarify whether HDPs-BR act predominantly as a physical barrier, an additional source of antioxidant compounds, a modulator of secondary metabolism, or through a combination of these mechanisms.

5.2.3. Microbial Count

The reduction in aerobic mesophilic microorganism counts observed in certain treatments at the end of storage is supported by previous studies involving edible coatings applied to strawberries. Temiz and Özdemir (2021) [80] observed a significant reduction in mesophilic counts during strawberry storage using *Lactobacillus rhamnosus* and gelatin films enriched with inulin. Similarly, Pinzon et al. (2020) [19] demonstrated that edible coatings based on banana starch–chitosan and Aloe vera gel reduced mesophilic microorganism counts compared with the control.

The effects observed in the present study may be related to the characteristics of the formulations used. Eugenol has well-recognized bactericidal activity [81] and may have contributed to limiting microbial growth in formulations containing this compound. In addition, coatings containing HDPs-BR and SA may have modified conditions at the fruit surface through the formation of a physical barrier, potentially creating an environment less favorable to the proliferation of aerobic microorganisms. However, because this behavior was not consistent across formulations containing eugenol, HDPs-BR, or SA, and considering the absence of treatments containing eugenol and SA individually, the observed effects cannot be attributed to specific components, nor can potential synergistic interactions be established. Therefore, the results should be interpreted as responses to the complete formulations evaluated.

Regarding fungi and yeasts, significant reductions in fungal load in coated strawberries have also been reported by Popescu et al. (2022) [82] and Li et al. (2024) [83], who evaluated edible formulations based on chitosan combined with essential oils and alginate combined with lactic acid bacteria, respectively. These findings reinforce the potential of edible coatings to modulate the development of spoilage microbiota during storage.

However, the reduction in fungal load observed on day 15 in the present study should be interpreted with caution, since it also occurred in the control. This behavior may be associated with the storage conditions themselves and microbial population dynamics, including nutrient availability, microbiota composition, and the possible accumulation of inhibitory metabolites over time. Therefore, the absence of detectable fungal growth at the end of storage cannot be attributed exclusively to the antifungal activity of eugenol.

Nevertheless, eugenol may have contributed to the inhibition of fungal growth in some formulations, considering its ability to disrupt microbial cell membrane integrity and inhibit microbial proliferation [69]. Antifungal effects associated with the use of eugenol in edible coatings applied to strawberries have also been reported by Guerreiro et al. (2015) [57].

5.3. Principal Component Analysis (PCA)

PCA provided an integrated overview of changes in strawberry quality and showed that the effects of the coatings depended on both formulation and storage period. The distribution of most coated samples evaluated on day 15 on the positive side of PC1 indicates that their overall profiles at the end of storage were characterized by the combined contribution of firmness, anthocyanins, FRAP, DPPH, weight loss, and decay, which showed positive loadings on this component, in contrast to L^* and a^* , which were negatively associated with PC1. However, this distribution should not be interpreted simply as an improvement in overall fruit quality, since PC1 simultaneously integrates desirable attributes, such as firmness and antioxidant activity-related variables, and attributes associated with storage progression, such as weight loss and decay. Instead, the clustering of samples on day 15 suggests that storage period was an important source of multivariate differentiation and that the coatings modulated the magnitude of these changes according to their composition.

From a mechanistic perspective, differences among HDPs-BR-containing formulations may result from changes in the physicochemical characteristics of the coating layer. Polymer concentration can influence solution viscosity, spreading, adhesion, thickness, and gas and water vapor transfer, thereby modifying the microenvironment established at the fruit surface [74]. Consequently, increasing the HDPs-BR concentration from 0.5 to 1% would not necessarily result in a proportional improvement in preservation. Higher polymer concentrations may markedly increase solution viscosity and favor the formation of thicker layers, a characteristic that is not always desirable in edible coatings. Thus, intermediate concentrations may provide a more appropriate balance between viscosity and matrix formation, allowing the development of a functional layer without excessive thickness [58]. This interpretation is consistent with previous studies demonstrating that coating polymer concentration strongly influences surface and barrier properties and, consequently, postharvest performance [75].

The positive contributions of anthocyanins, FRAP and DPPH to PC1 further suggest that changes related to antioxidant activity were important in differentiating the samples during storage. Polysaccharide-based coatings may contribute to the preservation of these compounds through more than one mechanism. By forming a semipermeable layer, the coating may modify O_2 and CO_2 exchange and reduce respiration and oxidative stress, potentially limiting the oxidation of endogenous phenolic compounds [72,75]. In strawberries, polysaccharide-based coatings have also been associated with the maintenance of phenolic compounds and antioxidant capacity, as well as with the modulation of antioxidant enzymes involved in protection against oxidative damage [75]. Therefore, the association of HDPs-BR-coated fruits with anthocyanins and antioxidant activity may reflect both the protective effect of the polymeric barrier and the contribution of phenolic compounds associated with the crude HDPs-BR fraction. However, because the present study did not

determine the migration of phenolic compounds from the coating or the activity of enzymes involved in phenolic metabolism, these mechanisms should be regarded as plausible explanations rather than direct evidence.

The presence of eugenol may have provided an additional active component to the coating system. In alginate- and pectin-based coatings applied to strawberries, formulations containing 0.1% eugenol were among those associated with better preservation of firmness, antioxidant activity, and microbiological quality during refrigerated storage [3]. However, the effect of eugenol cannot be considered independently of the polymeric matrix, since its retention, distribution, release, and accessibility are influenced by interactions with coating components [70]. Thus, differences between HDPs-BR + eugenol and HDPs-BR + SA + eugenol formulations may reflect not only the biological activity of eugenol itself but also differences in the structure and barrier properties of the matrices into which it was incorporated.

The addition of sodium alginate may also modify the organization and functionality of the HDPs-BR matrix rather than simply providing an additive preservative effect. Combining different polysaccharides can modify the thickness and internal organization of the matrix, while the mechanical and water vapor barrier properties of composite films depend on both their composition and the compatibility between their components [84]. These changes may explain why the combined formulations did not necessarily exhibit responses identical to or consistently superior to those obtained with HDPs-BR alone. The preservation efficiency of a coating depends on establishing an appropriate balance between barrier properties and the gas exchange required for fruit physiology; therefore, a formulation that produces a denser or more viscous matrix is not necessarily the most effective for all quality attributes [84].

PC2 represented a distinct source of variation and was characterized mainly by soluble solids on the positive side and by aerobic mesophilic microorganisms, fungi and yeasts, and firmness on the negative side. Thus, this component appears to represent a contrast between soluble solids content and characteristics related to microbial development and tissue firmness, rather than a direct inverse relationship between microbial growth and antioxidant activity. The negative loadings of mesophilic microorganisms and fungi and yeasts indicate that samples positioned on the negative side of PC2 were more strongly characterized by microbial counts, whereas those positioned on the positive side were comparatively more influenced by soluble solids. Because FRAP, DPPH, anthocyanins, total phenolics, weight loss, and decay showed relatively low absolute loadings on PC2, their relationships with this component should be interpreted with caution.

Overall, PCA indicates that the postharvest response of coated strawberries resulted from the combined effects of coating composition, storage period, and multiple physiological, biochemical, and microbiological processes. Rather than indicating a single mechanism responsible for preservation, the multivariate pattern suggests that HDPs-BR concentration and its combination with sodium alginate and/or eugenol modulated different quality attributes through possible changes in barrier properties, oxidative metabolism, structural preservation of tissues, and antimicrobial activity. The fact that combined treatments did not show uniform responses further reinforces that coating functionality depends on interactions among matrix composition, active compounds, and fruit physiology, rather than solely on the concentration of an individual component.

6. Conclusions

Crude polysaccharides extracted from Brazilian *Hovenia dulcis* (HDPs-BR) exhibited relevant chemical and structural characteristics associated to the presence of phenolic compounds and antioxidant activity, supporting their potential as a bioactive matrix for edible

coatings. Their application to strawberries influenced physical, biochemical, and microbiological attributes during refrigerated storage, although the responses varied according to formulation and storage period. Overall, HDPs-BR 0.5% and HDPs-BR 1% showed promising performance, particularly in maintaining firmness, phenolic compounds, and anthocyanins and in limiting fruit decay, whereas HDPs-BR 1% + Eug 0.1% stood out for preserving red color and antioxidant activity-related attributes. Therefore, these formulations represent promising candidates for further optimization and validation as edible coatings for the postharvest preservation of strawberries.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12080000/s1>. Polysaccharide Extraction (Supplementary Materials S1).

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CONSIDERAÇÕES FINAIS

O presente estudo avançou na compreensão das bases fisiológicas, bioquímicas e moleculares que regem o desenvolvimento de pseudofrutos de *Hovenia dulcis* no Brasil e converteu esse conhecimento em uma perspectiva de aplicação tecnológica, demonstrando o potencial de seus polissacarídeos (HDPs-BR) como matriz bioativa para a conservação pós-colheita de frutas. A abordagem integrada revelou que o desenvolvimento dos pseudofrutos se estende por 180 dias, com amadurecimento efetivo aos 150 dias após a antese, marcado por intenso amolecimento, solubilização péctica e conversão de amido em açúcares. Em nível molecular, a identificação de 496 proteínas, das quais 222 foram diferencialmente expressas, evidenciou uma rede proteometabólica dinâmica, na qual o metabolismo energético e as vias fotossintéticas se integram à sinalização do etileno e aos mecanismos de defesa antioxidante para sustentar a transição entre as fases de defesa química, reorganização metabólica e maturação sensorial.

Na vertente tecnológica, os HDPs-BR apresentaram características químicas e bioativas que sustentam sua utilização como matriz para o desenvolvimento de revestimentos comestíveis. Sua aplicação em morangos demonstrou efeitos dependentes da formulação e do período de armazenamento, contribuindo para a manutenção de atributos de qualidade, incluindo firmeza, coloração, compostos fenólicos, antocianinas e atividade antioxidante. A associação dos HDPs-BR ao alginato de sódio e/ou eugenol proporcionou benefícios adicionais para atributos específicos, com destaque para formulações contendo eugenol, que também contribuíram para o controle microbiológico durante o armazenamento.

Em conjunto, os resultados estabelecem uma conexão entre a compreensão dos mecanismos que determinam o desenvolvimento e a qualidade de *Hovenia dulcis* e a valorização tecnológica de seus constituintes. Ao demonstrar simultaneamente a complexidade molecular da maturação e o potencial funcional de seus polissacarídeos, esta tese amplia o conhecimento científico sobre uma espécie ainda subutilizada e fornece bases para sua inserção em estratégias de valorização de recursos biológicos e desenvolvimento de tecnologias sustentáveis para conservação pós-colheita. Os HDPs-BR apresentam-se, portanto, como uma matriz bioativa promissora para o desenvolvimento de revestimentos comestíveis, embora estudos futuros de otimização, escalonamento, avaliação sensorial e validação em diferentes matrizes alimentares sejam necessários para consolidar sua aplicação industrial.

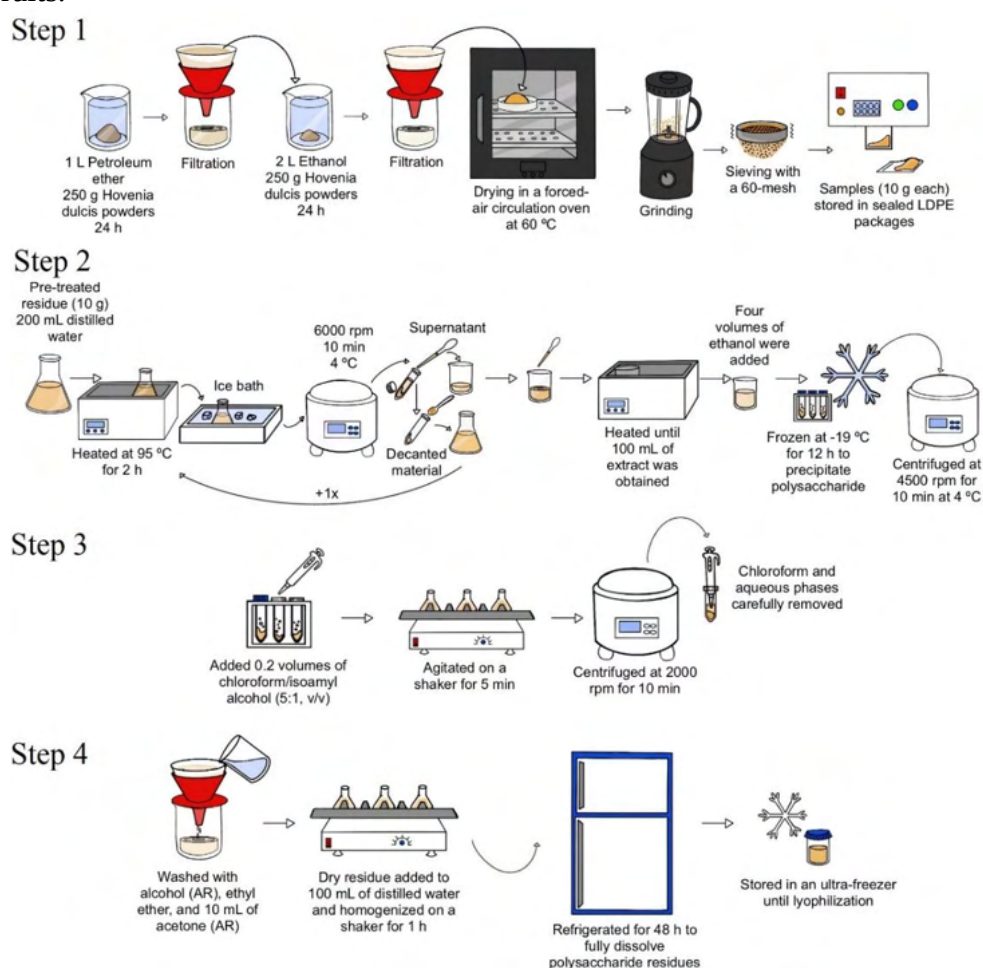
ANNEX

ANNEX 1- Polysaccharide Extraction

The extraction of polysaccharides was carried out using hot water, following a previously described method (Liu et al., 2020) with minor modifications. Briefly, *Hovenia dulcis* pseudofruits were dried in a circulating air oven, ground into powder, sieved through a 60-mesh screen, and stored in hermetically sealed plastic bags until use. The material was then immersed in petroleum ether (1 L/250 g) at room temperature for 24 hours. After filtration, the residue was pretreated with 80% ethanol (2 L/250 g of dry sample) for 24 hours, a step necessary to remove lipids, monosaccharides, pigments, and other small molecular impurities.

The pretreated residues were filtered, dried in a 60 °C oven, ground, and sieved again through a 60-mesh screen. Samples were stored in low-density polyethylene (LDPE) bags, weighing approximately 10 g per bag, which were then sealed and kept at room temperature until further processing (Figure 1 – Step 1).

Figure 1 - Overview of Polysaccharide Extraction and Purification from *Hovenia dulcis* Pseudofruits.



Hot Water Extraction

The pretreated residues (10 g) were transferred to a 500 mL Erlenmeyer flask, 200 mL of distilled water was added, and the mixture was heated in a water bath at 95 °C for 2 hours. After cooling in an ice bath, the material was centrifuged at 6000 rpm for 10 minutes at 4 °C. The supernatant was transferred to a 1 L beaker. The decanted residue was subjected to a second extraction with 200 mL of distilled water in a water bath for another 2 hours, followed by cooling, centrifugation, and addition to the beaker containing the supernatant from the first extraction.

The combined solution was concentrated in a water bath at 100 °C to a final volume of 100 mL. After cooling, four volumes of 80% ethanol (v/v) were added, and the mixture was stored in a freezer (-19 °C) for 12 hours to precipitate the polysaccharides. The precipitate was then centrifuged at 4500 rpm for 10 minutes at 4 °C (Figure 1 – Step 2).

Polysaccharide Purification

Deproteinization was performed using the Savage method, as described by Huang, Yang, and Wang (2010). The crude precipitate was treated with 0.2 volumes of chloroform/isoamyl alcohol (5:1 v/v), stirred for 5 minutes, and then centrifuged at 2000 rpm for 10 minutes. The aqueous phase was carefully separated, and the procedure was repeated 3 to 5 times until no precipitate was observed at the interface (Figure 1 – Step 3).

Polysaccharide Washing

After deproteinization, the residue was transferred to a funnel with filter paper and sequentially washed with PA alcohol (10 mL), ethyl ether (10 mL), and PA acetone (10 mL). The acetone residue was evaporated at room temperature. The dried material was transferred to a plastic container, 100 mL of distilled water was added, and the mixture was homogenized on a shaker for 1 hour and kept in the refrigerator for 48 hours for complete dissolution. Afterwards, the samples were stored in an ultra-freezer until lyophilization of the solutions (Figure 1 – Step 4).

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