UNIVERSIDADE FEDERAL DE GOIÁS (UFG)
ESCOLA DE AGRONOMIA (EA)
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA
DE ALIMENTOS

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Aproveitamento integral dos resíduos da jabuticaba

GOIÂNIA
2023

UNIVERSIDADE FEDERAL DE GOIÁS
ESCOLA DE AGRONOMIA

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Referência: Processo nº 23070.006175/2023-05

BRUNA MELO MIRANDA

Aproveitamento integral dos resíduos da jabuticaba

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Escola de Agronomia da Universidade Federal de Goiás, como requisito para a obtenção do título de doutor em Ciência e Tecnologia de Alimentos.
Área de concentração: Ciência e Tecnologia de Alimentos

Orientador: Prof. Dr. Flávio Alves da Silva
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Miranda , Bruna Melo
Aproveitamento integral dos resíduos da jabuticaba [manuscrito] /Bruna Melo Miranda . - 2023.
64 f.: il.

Orientador: Prof. Dr. Flávio Alves da Silva; co-orientadora Dra. Kátia Flávia Fernandes .
Tese (Doutorado) - Universidade Federal de Goiás, Escola de Agronomia (EA), Programa de Pós-Graduação em Ciência e Tecnologiade Alimentos, Goiânia, 2023.
Bibliografia.

1. subproduto agroindustrial. 2. polissacarídeo péctico. 3. antocianina. 4. amido. 5. caracterização química. I. Silva, Flávio Alvesda, orient. II. Título.

CDU 63

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ATA DE DEFESA DE TESE

Ata Nº 151 da sessão de Defesa de Tese de **Bruna Melo Miranda** que confere o títulode **Doutora** em **Ciência e Tecnologia de Alimentos**, na área de concentração em Ciência e Tecnologia de Alimentos.

Aos quinze dias do mês de fevereiro de dois mil e vinte e três, a partir das nove horas via videconferência, realizou-se a sessão pública de Defesa de Tese intitulada "Aproveitamento integral dos resíduos da jabuticaba". Os trabalhos foram instalados pelo Orientador, Doutor Flávio Alves da Silva (EA/UFG) com a participação dos demais membros da Banca Examinadora: Doutora Gardênia Martins de Sousa (EA/UFG), membro titular interno; Doutora Cristiane Maria Ascari Morgado (UEG), membro titular externo; Doutora Nara Rúbia Rodrigues do Nascimento Silva (Unifan/ICS), membro titular externoe Doutor Cláudio Fernandes Cardoso (EA/UFG), membro titular externo. Durante a argüição os membros da banca não fizeram sugestão de alteração do título do trabalho. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido a candidata aprovada pelos seus membros. Proclamados os resultados pelo Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora.

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Referência: Processo nº 23070.006175/2023-05

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AGRADECIMENTOS

Primeiramente gostaria de agradecer a Deus por tudo que proporcionou em minha vida, por me permitir chegar até aqui e continuar tendo fé e esperança.

Agradeço minha família, meu pai Jerônimo Paulo de Miranda, minha mãe Valéria Amaral de Melo Miranda e minha irmã Gabriela Amaral Rocha, que sempre estiveram ao meu lado, me apoiando e ensinando valores importantes para a vida. Sem vocês, esse sonho não seria possível.

Agradeço também meus amigos, que trilharam ao meu lado esse longo caminho, me incentivando nos momentos difíceis e fazendo tudo o que era possível para me apoiar e ajudar. Em especial, os meus queridos amigos de laboratório, a amizade de vocês foi um dos melhores presentes que recebi durante o doutorado.

Aos meus orientadores, Dr. Flávio Alves da Silva e Dra. Kátia Flávia Fernandes, minha enorme e eterna gratidão. Obrigada por acreditarem em mim, e pela generosidade em transmitir tantos conhecimentos e ensinamentos que foram essenciais para minha formação, enquanto profissional e pessoa. Minha admiração por vocês é gigante.

E finalmente, gostaria de agradecer aos excelentes exemplos de pesquisadores e professores que tive o prazer de encontrar nessa jornada, a experiência de vocês me ajudaram a crescer e contribuíram para minha formação acadêmica, científica e didática.

A todos, minha sincera gratidão.

RESUMO

A indústria de alimentos tem buscado alternativas para produzir alimentos mais saudáveis e com propriedades funcionais, sem perder as qualidades físico-químicas e sensoriais, exigidas pelos consumidores. A extração de polissacarídeos e compostos bioativos de matrizes vegetais, principalmente de resíduos/subprodutos gerados pela indústria é uma excelente alternativa econômica e sustentável para tal finalidade. Para a recuperação e utilização de tais compostos, é fundamental compreender os mecanismos relacionados à sua extração, composição, estrutura e interações que podem acontecer em meios alimentícios reais. Neste sentido, o objetivo deste trabalho foi extrair compostos dos resíduos de jaboticaba, como antocianinas e polissacarídeo péctico da casca e amido da semente, além disso, realizar a caracterização química e tecnológica destes compostos. Durante o processo de extração da antocianina foram avaliados os melhores parâmetros para o processo (pH, tempo, temperatura e aplicação de enzimas) e proposto sua aplicação em um filme halacromico. Durante a extração do polissacarídeo péctico, também extraído da casca de jaboticaba, os parâmetros de tempo, temperatura e pH foram avaliados e o material caracterizado quanto a sua composição de monossacarídeos por ressonância magnética nuclear e cromatografia gasosa, também foi determinado o grau de esterificação, cor, atividade antioxidante, capacidade emulsificante e propriedades reológicas. O amido extraído da semente foi estudado com relação a sua composição (teor de amilose, amilopectina, compostos fenólicos, proteínas) e estrutura (raio X, calorimetria de varredura diferencial, cromatografia de exclusão por tamanho e cromatografia de troca aniônica de alto desempenho) Os resultados obtidos demonstraram que a antocianina foi incluída com sucesso em uma matriz polimérica para formulação de um filme sensível ao pH, que responde com variação de cor de acordo com o pH do meio. Foi observado que o polissacarídeo péctico possui alto teor de galactose na sua composição e excelente atividade antioxidante e emulsificação. Já o resíduo de sementes, foi possível obter um amido puro, classificado como do tipo Cc , com alto teor de amilose e cadeias intermediárias de amilopectina. Deste modo, os resíduos de jaboticaba se apresentaram como excelentes materiais para obtenção de compostos de alto interesse industrial, com diversas possibilidades para aplicações.

Palavras-Chave: subproduto agroindustrial, polissacarídeo péctico, antocianina, amido, caracterização química.

ABSTRACT

The food industry has been looking for alternatives to produce healthier foods with functional properties, without losing the physical-chemical and sensory qualities demanded by consumers. The extraction of polysaccharides and bioactive compounds from plant matrices, mainly from waste/by-products generated by the industry, is an excellent economic and sustainable alternative for this purpose. For the recovery and use of such compounds, it is essential to understand the mechanisms related to their extraction, composition, structure and interactions that can occur in real food environments. In this sense, the objective of this work was to extract compounds from jaboticaba residues, such as anthocyanins and pectic polysaccharide from the peels and starch from the seed, in addition to carrying out the chemical and technological characterization of these compounds. During the anthocyanin extraction process, the best parameters for the process (pH, time, temperature and application of enzymes) were evaluated and their application in a halachromic film was proposed. During the extraction of the pectic polysaccharide, also extracted from jaboticaba peel, the parameters of time, temperature and pH were evaluated and the material characterized as to its composition of monosaccharides by nuclear magnetic resonance and gas chromatography, the degree of esterification was also determined, color, antioxidant activity, emulsifying capacity and rheological properties. The starch extracted from the seed was studied in relation to its composition (amylose, amylopectin, phenolic compounds, proteins) and structure (X-ray, differential scanning calorimetry, size exclusion chromatography and high-performance anion exchange chromatography). The results obtained demonstrated that anthocyanin was successfully included in a polymeric matrix for the formulation of a pH-sensitive film, which responds with color variation according to the pH of the medium. It was observed that the pectic polysaccharide has a high galactose content in its composition and excellent antioxidant activity and emulsification. As for the seed residue, it was possible to obtain a pure starch, classified as the Cc type, with a high amylose content and intermediate chains of amylopectin. Thus, the jaboticaba residues are presented as excellent materials for obtaining compounds of high industrial interest, with several possibilities for applications.

Key words: agroindustrial by-product, pectic polysaccharide, anthocyanin, starch, chemical characterization.

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO

A jaboticaba (*Plinia cauliflora*), fruta originária da região Centro-sul do Brasil, apresenta-se na forma de pequenas bagas, com diâmetro de 3 a 4 cm, casca negra e polpa branca aderida à semente. A fruta apresenta sabor adocicado e adstringente, devido ao seu alto teor de açúcares e ácidos. Relatos na literatura tem associado o consumo da jaboticaba com vários benefícios a saúde, devido à sua composição química, com grande abundância de polifenóis (ABULQUERQUE et al., 2020). Durante o processamento da jaboticaba, a casca e a semente são geralmente descartadas, podendo representar até 50% do fruto *in natura* (MARTINS et al., 2011). Uma boa alternativa para o emprego deste resíduo é a recuperação de suas substâncias para aplicações com finalidades tecnológicas.

Uma grande quantidade de compostos bioativos está presente na casca da jaboticaba, sendo um dos principais as antocianinas, que estão entre os pigmentos naturais de maior distribuição no reino vegetal, pertencentes à classe dos flavonoides. Além disso, as antocianinas estão associadas a diversas bioatividades, como antioxidante, antiinflamatória, antimutagênica e antimicrobiana (ALEZANDRO et al., 2013) e apresentam um espectro de cores que varia do vermelho ao azul dependendo do pH, sendo uma excelente alternativa de pigmento natural, visto que existe o interesse na busca por fontes naturais desses compostos devido a constante busca de melhoria da qualidade e segurança alimentar, sendo concomitante a preocupação com o uso excessivo de corantes sintéticos.

Além das antocianinas presentes na casca, as paredes celulares vegetais consistem em uma matriz polissacarídica, que protege os compartimentos internos das células e desempenham inúmeras funções no desenvolvimento da planta (KLAASSE; TRINDADE, 2020). Eles são compostos principalmente por celulose, hemicelulose e pectinas, polissacarídeos que atraem muita a atenção industrial, pois oferecem melhorias tecnológicas aos produtos, apresentando funcionalidades em relação ao incremento de viscosidade, melhoria na textura e atividade emulsificante (KIENTEKA et al., 2018).

Outro resíduo que é descartado após o processamento, é a semente da fruta, que por sua vez, pode ser utilizada como fonte de outro tipo de polissacarídeos, o amido. A busca por novas fontes de amido tem sido interesse de pesquisa dos últimos anos, devido as diferentes aplicações

que podem ser aplicadas aos diferentes tipos de amido nos mais diversos setores, como indústrias alimentícias, químicas, farmacêuticas entre outros (MENZEL et al., 2015).

De fato, nos últimos anos, tem aumentado o interesse por diferentes fontes de polissacarídeos, principalmente aqueles provenientes de fontes ricas em compostos bioativos, pois podem apresentar propriedades químicas adicionais, aumentando seu potencial de aplicação (HAMMAMI et al., 2018). Entretanto, o uso industrial de polissacarídeos exige o entendimento da relação estrutura-funcionalidade, o que é possível por meio de técnicas que fornecem informações de ambos os parâmetros simultaneamente.

Deste modo, o objetivo deste trabalho é extrair compostos dos resíduos de jaboticaba, como antocianinas e polissacarídeo péctico da casca, e amido das sementes. Além disso, realizar a caracterização química e tecnológica destes compostos como uma alternativa sustentável e rentável.

2. REVISÃO BIBLIOGRÁFICA

2.1 Jabuticaba

2.1.1 Informações gerais

Jaboticaba, ou jabuticaba, é uma baga brasileira pertencente à Família *Myrtaceae* e gênero *Myrciaria*, que também é denominado como *Plinia*. O nome jaboticaba é derivado das palavras iapoti'kaba e jabotin da língua indígena Tupi que significam, respectivamente, “botão de fruta” e “gordura de tartaruga”. A fruta é nativa da região centro/sul do Brasil, mas também pode ser encontrada no Paraguai e Argentina. A jabuticabeira apresenta porte médio, de 6 a 9 metros, sua copa possui formas variadas e folhas avermelhadas/verdes quando novas (CITADIN et al., 2010).

Com formato globuloso e diâmetro de 3 a 4 cm, a jaboticaba possui casca lisa, firme e brilhante, que muda de verde para roxo escuro/preto durante o amadurecimento, com polpa esbranquiçada, agridoce e ligeiramente ácida, aderida a uma até quatro sementes no seu interior (ABULQUERQUE et al., 2020). É caracterizada por uma floração e posterior frutificação nos ramos e troncos, e devido sua maturação ocorrer enquanto está ligada à planta-mãe, é considerada uma fruta não-climatérica, sendo a coloração preta-avermelhada da casca um indicativo do seu grau ideal de maturação (ANDRADE et al., 2021), como pode ser observado na (Figura 1).

Figura 1. Fotografias representativas da jabuticabeira: A) floração, B) frutificação e C) frutas maduras. (Autor, 2020).

A jabuticabeira costuma florescer duas vezes por ano, sendo a safra no Brasil normalmente de agosto a novembro, durante a primavera, podendo variar de acordo com a espécie, região e condições climáticas (SALOMÃO et al., 2018). Geralmente os frutos apresentam curta vida útil, de apenas três dias após a colheita, devido a intensa perda de água, deterioração microbiana e fermentação da polpa (GARCIA, 2017). Deste modo, embora a jaboticaba seja uma fruta muito apreciada em todo o Brasil, sua alta perecibilidade prejudica sua comercialização *in natura*, sendo uma alternativa sua industrialização em produção de produtos alimentícios, tais como sucos, geleias, vinagres, licores e vinho (INADA et al., 2021).

2.1.2 Resíduos da jabuticaba

A indústria de alimentos é responsável pela geração de alta quantidades de subprodutos durante o processamento, sendo que na maioria das vezes esses resíduos são descartados de forma inapropriada no meio ambiente. No entanto, os subprodutos agro-alimentares podem ser considerados matérias-primas promissoras para a produção de energia, aditivos alimentos e polissacarídeos funcionais, sendo a jaboticaba um excelente exemplo de material que pode ser explorado nesta vertente (DA ROSA et al., 2023).

O Brasil tem uma produção anual média de 5.000 toneladas de jaboticaba, sendo essa estimativa apenas dentro do comércio formal, não incluindo o valor referente ao consumo doméstico ou informal (MASSA et al., 2022). Normalmente, a forma mais comum de consumo é da fruta *in natura*, entretanto, devido ao alto valor nutricional, sabor agradável e curta vida útil, o processamento industrial de produtos derivados da jaboticaba se mostra como uma excelente alternativa.

De forma resumida, o processamento industrial de jaboticaba consiste em seleção, lavagem, sanitização e despolpamento em máquinas, que simultaneamente separam a polpa do subproduto. A polpa é embalada e comercializada ou destinada à formulação de sucos, geleias, licores, sorvetes e balas. Deste modo, no processamento industrial da jaboticaba, a polpa é responsável pela maior participação de mercado, enquanto cascas e sementes são considerados os subprodutos (FIDELIS et al., 2021).

Aproximadamente 50% do peso do fruto da jaboticaba consiste em cascas e sementes (Figura 2), que normalmente são descartadas sem qualquer recuperação ou utilização. Quando esses subprodutos são despejados no meio ambiente sem tratamento ou controle de descarte, liberam muitos componentes, que têm sido associados à água eutrofização, emissões de gases de efeito estufa (GEE), entre outros efeitos colaterais ambientais (ROSA et al., 2022).

Figura 2. Fotografias representativas: A) fruto de jaboticaba B) resíduos do processamento. (Autor, 2020).

Além dos danos sócio-ambientais gerados pelos resíduos agroindustriais, é no subproduto do processamento da jaboticaba onde se encontra a maior concentração de compostos fenólicos, fibras, proteínas e vitaminas, se tratando de um material rico com diversas rotas tecnológicas viáveis para a geração de novos produtos de valor agregado.

Portanto, novas alternativas envolvendo o reaproveitamento desses materiais estão sendo identificados e avaliados, com o objetivo de reduzir o impacto ambiental e, ao mesmo tempo, agregar valor aos subprodutos, como a recuperação de biomoléculas (flavonoides, antocianinas, carboidratos e etc) para incorporação em formulações industriais, nas mais amplas áreas industriais da medicina, farmácia e alimentos. (FERNANDES et al., 2020; ROSA et al., 2022).

2.2 Fitoquímicos da jabuticaba

As frutas são excelentes fontes de fitoquímicos, e dentre a infinidade de compostos encontrados nessas fontes, algumas classes se destacam por seus efeitos positivos à saúde e também por seu papel na conservação dos alimentos. Os polifenóis, um dos principais fitoquimicos encontrados nas frutas, são metabólitos secundários do metabolismo vegetal que são produzidoss por uma interação com fatores intrinsecos e extrinsicos do proprio vegetal. Em geral, esta categoria de compostos pode ser classificada em ácidos fenólicos (esqueleto C6-C1 e C6-C3 para os ácidos hidroxibenzóico e hidroxicinâmico, respectivamente), flavonoides

(esqueleto C6-C3-C6), estilbenos (esqueleto C6-C2 -C6 esqueleto), lignanas (C6-C3)2) e outros polifenóis (MUNEKATA et al., 2023).

Desta forma, atualmente há um interesse crescente na atividade biológica desses fitoquímicos presentes na frutas, uma vez que desempenham um papel muito importante no organismo humano. A Jaboticaba tem sido relatada na literatura como uma fruta com rico perfil fitoquímico, apresentando alta atividade bilógica in vitro e in vivo, devido seus altos teores de compostos voláteis, antocianinas, flavonóides, taninos e outros compostos fenólicos (PALUDO et al., 2022). Os fitoquímicos mais estudados da jaboticaba são os polifenóis e de acordo com a literatura, o perfil fenólico da jaboticaba é composto principalmente por ácido fenólico, taninos e flavonóides, sendo o principal deste último grupo, as antocianinas.

2.2.1 Antocianinas

As antocianinas são compostos solúveis em água, pertencentes ao grupo flavonoides, presentes naturalmente em uma grande variedade de flores, folhas, hortaliças, frutas e grãos, apresentando coloração do avermelhado ao arroxeado (DE MEJIA et al., 2020).

Sua estrutura básica é composta por quinze carbonos, constituídas por três anéis: sendo um anel aromático ligado a um anel heterocíclico que contém um oxigênio e um terceiro anel aromático. Os diferentes grupos ligados em R, assim como os ácidos a eles ligados, caracterizam as diferentes moléculas de antocianinas (Figura 3) (LOPES, 2007).

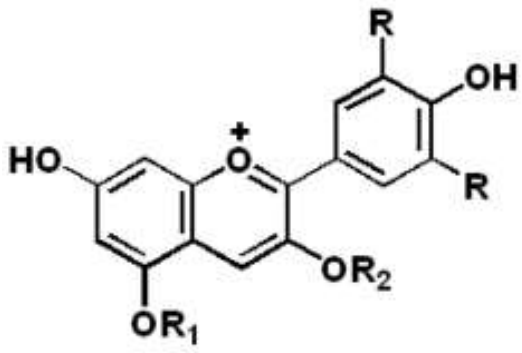

Figura 3: Estrutura básica das antocianinas.
Fonte: (LOPES, 2007)

De acordo com o pH do meio, as antocianias podem assumir diferentes configurações químicas e consequentemente, diferentes colorações, como o cátion flavilium (vermelho), anidrobase quinonoidal (azul), pseudobase carbinol (incolor) e chalcona (incolor ou levemente amarelada) (HOFFMANN et al., 2022).

Devido à essa habilidade das antocianinas de mudarem de cor de acordo com o pH, elas possuem alto potencial para aplicação, podendo ser usadas em alimentos, cosméticos, tecidos e outros. Segundo Hoffmann et al. (2022), uma das aplicações mais discutidas recentemente é seu uso em formulações de embalagens, pois a antocianina pode ser usada como um método visual simples para detectar a deterioração dos alimentos, sendo um excelente indicador para embalagens inteligentes, pois, durante o armazenamento, os alimentos geram ácidos orgânicos voláteis e/ou aminas voláteis em resposta ao crescimento de microrganismos, que alteram o pH, modificando a cor da embalagem (LUCHESE et al., 2017).

Outra aplicação das antocianinas é como corante natural, pois, somente pigmentos aprovados pela Administração de Alimentos e Medicamentos dos EUA (FDA) podem ser utilizados em formulações alimentícias. Na Europa, a antocianina já vem sendo aplicada e possui o código E, Antocianina (E-163), devido ao reconhecimento de seus diversos benefícios (NABI et al., 2023).

2.3 Polissacarídeos da jabuticaba

Os polissacarídeos são macromoléculas naturais e os principais componentes das paredes celulares vegetais, protegendo os compartimentos internos das células e desempenhando inúmeras funções no desenvolvimento da planta (KLAASSE & TRINDADE, 2020). A matriz polissacarídica da casca é composta majoritariamente por celulose, hemicelulose e pectinas, já as sementes, além das fibras, encontram-se também outro tipo de polissacarídeo, amplamente utilizado pela indústria, o amido.

A utilização de resíduos agroindustriais para a obtenção de polissacarídeos é um dos assuntos mais discutidos na ciência, com conceitos modernos de biorrefinaria, promovendo a diversificação do uso desses polímeros em diversos bioprodutos incorporando conceitos de sustentabilidade e a economia circular. Essas macromoléculas foram selecionadas devido à sua importância industrial e valor aplicações funcionais e biológicas que têm despertado interesses de mercado, como para a produção de medicamentos, cosméticos e embalagens sustentáveis (SOUZA et al., 2022).

2.3.1 Heteropolissacarídeo rico em galactose

Estudos detalhados dedicados aos carboidratos da parede celular vegetal, compreendendo completamente sua estrutura e funcionalidade, ainda são raros e difíceis de encontrar na literatura, embora tais polissacarídeos sejam componentes ativos importantes responsáveis pela atividade biológica e propriedades funcionais dos alimentos (OGNYANOV et al., 2020). Sabe-se que tais polissacarídeos ricos em galactose, também denominados de polissacarídeos pécticos ou substâncias pécticas, são normalmente compostos por galactose, manose, glicose, arabinose e raminose, apresentando um comportamento similar ao da pectina.

Em geral, tais substâncias pécticas são polissacarídeos ácidos, que desempenham papel importante na parede celular primária e na lamela média. Do ponto de vista industrial, são amplamente utilizados como espessantes, estabilizantes, emulsificantes, capazes de aumentar a viscosidade e controlar atividade de água do meio (CHEN et al., 2022). As mais variadas aplicações podem ser exemplificadas, como, agente gelificante em geleias, estabilizante em bebidas lácteas ácidas e emulsificante em pastas, sorvetes e carnes emulsionadas (MARAN et al., 2013).

Entretanto, para entender melhor a estrutura desses polissacarídeo e avaliar sua melhor aplicação, é necessário o desenvolvimento de técnicas de caracterização e elucidação molecular, através de minusciosas análises quimicas, como ressonância magnética nuclear, cromatografia, infra vermelho, reologia entre outras (LI; FANG; ZHANG, 2007).

2.3.2 Amido

O amido é o carboidrato de reserva mais importante para as plantas, importante componente nutricional nos alimentos e um dos principais ingredientes dos alimentos básicos para o consumo humano. Em termos de composição química, o amido é composto por dois polissacarídeos, amilose e amilopectina, sendo a amilose uma molécula linear de unidades D-glucopiranosil ligadas a α-1,4, com alguns ramos de α-1,6 ligações, enquanto a amilopectina é uma molécula altamente ramificada (Figura 4) (BULEON et al., 1998).

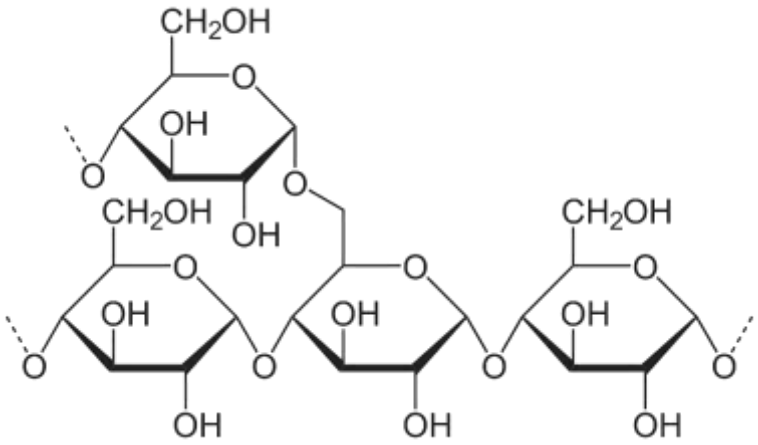

Figure 4. Modelo da estrutura da molécula de amido.
Fonte: Adaptação (MARTIN; SMITH, 1995).

As principais fontes comerciais de amido são cereais e tubérculos, e são amplamente utilizados na indústria de alimentos como espessante, gelificante, estabilizador e até substituto de gordura. Entretanto, devido as demandas crescentes para produção e uso tecnológico de amidos, se faz necessário a busca por novas alternativas de sua obtenção. Além disso, as pesquisas sobre amidos não convencionais estão relacionadas com a produção sustentável, o uso de subprodutos e melhores propriedades tecnológicas sobre amidos comuns, que podem apresentar certas desvantagens, como baixa resistência ao cisalhamento e solubilidade, resistência térmica e instabilidade congelamento-descongelamento, retrogradação e propriedades de fácil digestão. Tais essas características indesejadas do amido nativo limitaram severamente sua aplicação prática na indústria alimentícia (TAGLIAPIETRA et al., 2021).

Desta forma, os amidos não convencionais, são uma alternativa para a indústria. E por esta razão, uma análise detalhada desses amidos deve ser realizada para propor uma aplicação tecnológica adequada.

3 OBJETIVOS

3.1 Objetivo geral

Extrair compostos dos resíduos de jaboticaba, como antocianinas e polissacarídeos pécticos da casca, e amido das sementes. Realizar a caracterização química e tecnológica destes compostos.

3.2 Objetivos específicos

- Realizar extração de antocianinas das cascas de jaboticaba
- Quantificar as antocianinas nos extratos
- Aplicar as antocianinas em filmes com indicação de pH
- Extrair polissacarídeos pécticos das cascas de jaboticaba
- Caracterizar quimicamente o polissacarídeo
- Analisar ação antioxidante e emulsificante do polissacarídeo péctico
- Analisar o comportamento reológico do polissacarídeo péctico
- Extrair amido das sementes de jaboticaba
- Calcular o rendimento do amido extraído
- Caracterizar a composição química do amido

REFERÊNCIAS

ALBUQUERQUE, B. R.; PEREIRA, C.; CALHELHA, R. C.; ALVES, M. J.; ABREU, R. M.; BARROS, L.; FERREIRA, I. C. Jabuticaba residues (Myrciaria jaboticaba (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. **Food chemistry**, v. 309, p. 125735, 2020.

ALEZANDRO, M. R., GRANATO, D., GENOVESE, M. I. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. **Food Research International,** *54*(1), 650-659, 2013

ANDRADE, N. N., STRINGHETA, P. C., GARCÍA-ROMERO, E., GÓMEZ-ALONSO, S. Elaboração e caracterização de fermentado alcoólico de jabuticaba (Plinia jaboticaba) produzido artesanalmente. **Research, Society and Development**, v. 10, n. 4, p. e3010413799-e3010413799, 2021.

BULEON, A., COLONNA, P., PLANCHOT, V., BALL, S. Starch granules: structure and biosynthesis. **International journal of biological macromolecules,** *23*(2), 85-112, 1998.

CHEN, X., YANG, J., SHEN, M., CHEN, Y., YU, Q., XIE, J. Structure, function and advance application of microwave-treated polysaccharide: A review. **Trends in Food Science & Technology,** 2022.

CITADIN, I., DANNER, M. A., SASSO, S. A. Z. Jabuticaba trees (Plinia sp.). **Revista Brasileira de Fruticultura,** 32(2), 343–656, 2010.

DA ROSA, R. G., SGANZERLA, W. G., BARROSO, T. L. C. T., CASTRO, L. E. N., BERNI, M. D., FORSTER-CARNEIRO, T. Sustainable bioprocess combining subcritical water pretreatment followed by anaerobic digestion for the valorization of jabuticaba (Myrciaria cauliflora) agro-industrial by-product in bioenergy and biofertilizer. **Fuel**, v. 334, p. 126698, 2023.

DE MEJIA, E. G., ZHANG, Q., PENTA, K., EROGLU, A., LILA, M. A. The colors of health: Chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. **Annu. Rev. Food Sci. Technol,** 11(1), 145-182, 2020.

FERNANDES, F. A., FONTELES, T. V., RODRIGUES, S., DE BRITO, E. S., TIWARI, B. K. Ultrasound-assisted extraction of anthocyanins and phenolics from jabuticaba (Myrciaria cauliflora) peel: Kinetics and mathematical modeling. **Journal of food science and technology**, 57(6), 2321-2328, 2020.

FIDELIS, M., SANTOS, J. S., ESCHER, G. B., ROCHA, R. S., CRUZ, A. G., CRUZ, T. M., GRANATO, D. Polyphenols of jabuticaba [Myrciaria jaboticaba (Vell.) O. Berg] seeds incorporated in a yogurt model exert antioxidant activity and modulate gut microbiota of 1, 2-dimethylhydrazine-induced colon cancer in rats. **Food Chemistry,** 334, 127565, 2021.

GARCIA, L. G. C. (2017). Desenvolvimento fisiológico e conservação pós-colheita de jabuticaba. 97 f. **Tese de Doutorado**: Escola de Agronomia, Universidade Federal de Goiás.

HAMMAMI, N., GARA, A. B., BARGOUGUI, K., AYEDI, H., ABDALLEH, F. B., BELGHITH, K. Improved in vitro antioxidant and antimicrobial capacities of polysaccharides isolated from Salicornia arabica. **International journal of biological macromolecules**, 120, 2123-2130, 2018.

HOFFMANN, T. G., ANGIOLETTI, B. L., BERTOLI, S. L., DE SOUZA, C. K. Intelligent pH-sensing film based on jaboticaba peels extract incorporated on a biopolymeric matrix. **Journal of Food Science and Technology,** 59(3), 1001-1010, 2022.

INADA, K. O. P., LEITE, I. B., MARTINS, A. B. N., FIALHO, E., TOMÁS-BARBERÁN, F. A., PERRONE, D., MONTEIRO, M. Jaboticaba berry: A comprehensive review on its polyphenol composition, health effects, metabolism, and the development of food products. **Food Research International,** 147, 110518, 2021.

LI, YAN-QUN; FANG, LU; ZHANG, KE-CHANG. Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured Ganoderma lucidum. **Carbohydrate Polymers**, v. 68, n. 2, p. 323-328, 2007

LOPES, T., XAVIER, M., QUADRI, M. G., QUADRI, M. Antocianinas: uma breve revisão das características estruturais e da estabilidade. **Current Agricultural Science and Technology,** 13(3), 2007.

KLAASSEN, M. T., TRINDADE, L. M. RG-I galactan side-chains are involved in the regulation of the water-binding capacity of potato cell walls. **Carbohydrate polymers,** 227, 115353, 2020.

KIENTEKA, S. S., CORRÊA, F. M. L., Oliveira, P. C. L. Characterization of cell wall polysaccharides from Sicana odorifera fruit and structural analysis of a galactan-rich fraction pectins as side chains**. Carbohydrate polymers,** 197, 395-402, 2018.

MARAN, J. P.; MEKALA, V.; MANIKANDAN, S. Modeling and optimization of ultrasound-assisted extraction of polysaccharide from Cucurbita moschata. **Carbohydrate polymers**, Worcester, v. 92, n. 2, p. 2018-2026, 2013.

MARTINS, S. MUSSATTO , S.I., MARTÍNEZ-AVILA, G., MONTAÑEZ-SAENZ, J., AGUILAR,C.N., TEIXEIRA, J.A. Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. **Biotechnology Advances**, v. 29, n. 3, p. 365-373, 2011.

MARTIN, C.; SMITH, A. M. Starch biosynthesis. **The plant cell**, v. 7, n. 7, p. 971, 1995.

MASSA, N. M. L., DE OLIVEIRA, S. P. A., RODRIGUES, N. P. A., MENEZES, F. N. D. D., DOS SANTOS LIMA, M., MAGNANI, M., DE SOUZA, E. L. . In vitro colonic fermentation and potential prebiotic properties of pre-digested jabuticaba (Myrciaria jaboticaba (Vell.) Berg) by-products. **Food Chemistry**, 388, 133003, 2022.

MENZEL, C., ANDERSSON, M., ANDERSSON, R., VAZQUEZ-GUTIERREZ, J. L., DANIEL, G., LANGTON, M., Effect of varying amylopectin structure and amylose content of starch from genetically modified potatoes. **Carbohydrate polymers**, v. 130, p. 388-397, 2015.

MUNEKATA, P. E., PATEIRO, M., DOMÍNGUEZ, R., NIETO, G., KUMAR, M., DHAMA, K., LORENZO, J. M. Bioactive Compounds from Fruits as Preservatives. **Foods**, v. 12, n. 2, p. 343, 2023.

NABI, B. G., MUKHTAR, K., AHMED, W., MANZOOR, M. F., RANJHA, M. M. A. N., KIELISZEK, M., AADIL, R. M. Natural pigments: Anthocyanins, carotenoids, chlorophylls, and betalains as food colorants in food products. **Food Bioscience**, p. 102403, 2023.

OGNYANOV, M., REMOROZA, C., SCHOLS, H. A., GEORGIEV, Y. N., PETKOVA, N. T., KRYSTYJAN, M. Structural, rheological and functional properties of galactose-rich pectic polysaccharide fraction from leek. **Carbohydrate Polymers**, 229, 115549, 2020.

PALUDO, M. C., DE OLIVEIRA, S. B. P., DE OLIVEIRA, L. F., COLOMBO, R. C., GÓMEZ-ALONSO, S., HERMOSÍN-GUTIÉRREZ, I., GODOY, H. T. Phenolic composition of peels from different Jaboticaba species determined by HPLC-DAD-ESI/MSn and antiproliferative activity in tumor cell lines. **Current Plant Biology**, 29, 100233, 2022.

ROSA, R. G., SGANZERLA, W. G., BARROSO, T. L., BULLER, L. S., BERNI, M. D., FORSTER-CARNEIRO, T. Sustainable production of bioactive compounds from jabuticaba (Myrciaria cauliflora): A bibliometric analysis of scientific research over the last 21 years. **Sustainable Chemistry and Pharmacy**, 27, 100656, 2022.

SALOMÃO, L. C., DE SIQUEIRA, D. L., AQUINO, C. F.; LINS, L. C. Jabuticaba— Myrciaria spp. **Exotic Fruits.** p. 237-244, 2018.

SOUZA, M. A. D., VILAS-BOAS, I. T., LEITE-DA-SILVA, J. M., ABRAHÃO, P. D. N., TEIXEIRA-COSTA, B. E., & VEIGA-JUNIOR, V. F. Polysaccharides in Agro-Industrial Biomass Residues. **Polysaccharides,** 3(1), 95-120, 2022.

TAGLIAPIETRA, B. L., FELISBERTO, M. H. F., SANCHES, E. A., CAMPELO, P. H., CLERICI, M. T. P. S. Non-conventional starch sources. **Current Opinion in Food Science,** 39, 93-102, 2021.

WU, S.; LONG, C.; KENNELLY, E. J. Phytochemistry and health benefits of jaboticaba, an emerging fruit crop from Brazil. **Food Research International**, v. 54, n. 1, p. 148-159, 2013.

CAPÍTULO II

ARTIGO 1: Artigo publicado na Revista *Waste and Biomass Valorization*, disponível em: https://doi.org/10.1007/s12649-022-01688-y

A Halochromic Film Containing *Plinia cauliflora* Peel Anthocyanins Loaded into a Cashew Gum Polysaccharide-Polyvinyl Alcohol Matrix

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Abstract

This study reports the extraction of anthocyanins from *P. cauliflora* peels, its partial characterization and the inclusion into a halochromic film based on cashew gum polysaccharide and polyvinyl alcohol (CGP/PVA). Three kinds of extractions were performed: The classic acidic-alcoholic solvent method; enzymatic method with the cell wall degrading enzymes (cellulase and pectinase) and the combination of these two extraction methods. Time and temperature for extraction varied from 3, 6, 9, 12, and 24 h and 4, 20, 30, and 40 °C, respectively. After extraction, the anthocyanins were fractionated by chromatography and partially characterized by Fourrier transform infrared spectroscopy (FTIR) and regarding their antioxidant activity. Maximum values found in the extraction procedures were 368 ± 1.94 mg 100 g^{-1} (30 °C/9 h); 216.76 ± 2.98 mg 100 g^{-1} (40 °C/3 h) and 707.09 ± 3.97 mg 100 g^{-1}, respectively for acidic-alcoholic solvent, for cellulase and for a combination of solvent and enzyme extraction. The antioxidant activity of the anthocyanins was preserved, showing higher values than the commercial antioxidant BHT. The chromatographic fractionation resulted in 2 peaks, which presented characteristic bands of cyanidin 3-glucoside and delphinidin in FTIR. The inclusion of anthocyanins in CGP/PVA films resulted in a pH-sensitive material changing the color from clear red in acid pH environment to brownish-green color in basic medium. The results of this study were promising and indicate that CGP/PVA-anthocyanin films are good candidates for smart packaging development.

Keywords: Natural pigment, Antioxidant activity, FTIR, Stimuli-responsive.

CAPÍTULO III

ARTIGO 2 : Artigo publicado na Revista *Carbohydrate Polymers*, disponível em: https://doi.org/10.1016/j.carbpol.2020.116821

A galactose-rich heteropolysaccharide extracted from "jaboticaba" (*Plinia cauliflora*) peels

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Abstract

The objective of this work was to extract, identify and characterize a galactose-rich heteropolysaccharide (GH) from "jaboticaba" peel. The best conditions to extract the GH according to a 2^3 full-factorial experimental design were 90 °C/30 min/pH 1.0, resulting in a 32.32 % yield using lyophilized sample. The chemical structure analyzed by GC/MS and NMR spectra (HSQC/HSQC-TOCSY) showed that the main chain of GH consists of a (1→ 4) galactoside branched at carbon 3, containing galactose (67.21 %), glucose (15.78 %), arabinose (9.78 %), rhamnose (2.26 %) and traces of esterified and non-esterified uronic acids. Rheological studies revealed that GH suspensions behave as a Newtonian fluid, with calculated molecular mass of 1.48×10^5 Da. The absolute viscosity of 1 % (w/v) aqueous suspension of GH decreased from 25 mPa s to 10 mPa s in NaCl and 7 mPa s in $CaCl_2$, indicating the polyelectrolyte character of GH.

Keywords: GC/MS chromatography, Relative viscosity, Rheology, Arabinogalactan.

CAPÍTULO IV

ARTIGO 3: Artigo publicado na Revista Food Science and Technology International, disponível em: https://doi.org/10.1177/10820132221100684

Antioxidant and emulsifying properties of a galactose-rich heteropolysaccharide from *Plinia cauliflora* peels

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Abstract

In this study functional properties of a galactose-rich heteropolysaccharide (GH) were accessed. The bands of a galactose-rich polysaccharide were found in FTIR spectra, including those from the fingerprint region. GH was characterized as a dark-red material (L* 25.86 ± 0.75, a* 9.46 ± 1.01, b* 0.65 ± 0.14, Chroma 9.48 ± 1.02) with antioxidant activity of 21.5 ± 0.08, 12.1 ± 0.06 and 0.46 ± 0.04 mmol Trolox Eq mg^{-1} GH in FRAP, DPPH and ABTS, respectively. GH presented 44.9% of esterification degree and 10.73 ± 0. 01 mg of GAE g^{-1}. The production parameters of GH emulsions (GH concentration, time and ultrasound power) were optimized using a 2^3 Central Composite Rotatable Design (CCRD). Emulsion droplets presented particle size (d μm) varying from 0.823 ± 0.065 to 1.926 ± 0.151, polydispersity index (PDI) from 0.10 ± 0.05 to 0.40 ± 0.01 and zeta potential from -29.25 ± 3.98 to -33.75 ± 1.77. Finally, the high emulsifying activity (EA) (96.67%) and emulsion stability (ES) (97.44%) allow suggesting that GH is a promising polysaccharide for food applications.

Keywords: Color measurement; FTIR; PDI; bioactivity; emulsion stability; zeta potential

CAPÍTULO V

ARTIGO 4: Characterization of the pH and sucrose induced gelation of the galactose-rich heteropolysaccharide from the Jaboticaba (*Plinia Cauliflora*) peels

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Abstract

The aim of this study was to elucidate and understand the acid-induced gelling behavior of the Galactose-rich heteropolysaccharide (GH) from jaboticaba peels. For the better understanding, the first test developed was the zeta potential, to elucidate the polysaccharide charge, followed by the viscosity test at different rang of pH and solutions with and without sucrose, and rheology analyzes performed with 60% sucrose and different concentrations of GDL to evaluate the gelling behavior of GH. The zeta potential test showed the biggest difference in charge between pH 2,3,4 and 5, while intrinsic viscosity didn't show statistic difference at this range of pH, probably due the neutral sugar at GH composition. Moreover, the GH showed a pectin-like behavior during the gelling, as it formed a strong gel with 60% sucrose in pH lower than 3. Thus, GH is shown to be a polysaccharide with interesting gelling properties for applications.

Keywords: zeta potential, intrinsic viscosity, strong gel, pectin-like behavior.

1. Introduction

Jaboticaba *(Plinia Cauliflora)* is a fruit originating in the center-south region of Brazil, in the form of small berries, with a diameter of 3 to 4 cm, black peel and white pulp adhered to the seed. The fruit has a sweet and astringent flavor, due to its high content of sugars, acids and polyphenols (Abulquerque et al., 2020). During the processing of jaboticaba, the peel and the seed are generally neglected, representing up to 50% of the fruit *in natura* (Rosa et al., 2022). A good alternative for the use of this residue is the recovery of its bioactive and technological substances, for applications with industrial purposes. Fruit peels are plant cell walls that consist of a polysaccharide matrix, which protects the internal compartments of cells and performs

numerous functions in the development of the plant (Klaasse & Trindade, 2020). These polysaccharides may have interesting technological properties, as previously demonstrated by the investigation of the material from jaboticaba peels, showing functionalities in relation to antioxidant activity and emulsifying properties (Miranda et al., 2022).

In a previous work, the galactose-rich heteropolysaccharide (GH) was extracted from jaboticaba peels, under the optimized conditions and showed that the main chain consists of a (1→ 4) galactoside branched at carbon 3, containing galactose (67.21 %), glucose (15.78 %), arabinose (9.78 %), rhamnose (2.26 %) and traces of esterified and non-esterified uronic acids (Miranda et al., 2020). Based on this chemical composition, it is possible to observe some similarities with the chemical composition of pectin, assuming the possibility of similarity in the gelling mechanisms behavior, as functional properties of biopolymers are based on the macromolecular structure and the molecular interaction with the solvent.

Though, GH is a completely new material in the literature, with a research gap about their structure-functionality, been important to study and understand it. Moreover, it is known that water soluble polysaccharides are industrially important materials due to their rheological properties in aqueous systems, and should be studied as a form for quality control, texture evaluation, process design and determination of food structure (Alvarado & Aguilera, 2001). Understanding polysaccharides properties is crucial to forecast their potential industrial applications. Furthermore, these properties can be affected by several parameters, such as pH, salt concentration, temperature, polymer average molecular weight and shear rate (Torres et al., 2015).

In this way, the aim of this study is elucidating and understanding the acid-induced gelling behavior of the polysaccharide from jaboticaba peel with the hypothesis that the mechanisms are similar to pectin, in which the polysaccharide gel will be formed due to sucrose addition and pH reduction, since hydrogen bonds between the polymers can be formed.

2. Material and Methods

2.1. Plant material

The Jaboticaba (*Plinia Cauliflora*) fruits were obtained in a mature state from the Jabuticabal Farm & Winery in Nova Fátima, Hidrolândia-GO, Brazil. The fruits were pulped in an electric pulper (Itametal, Bonina 0.25 DF, Brazil) and the peels were manually separated from the seeds, quick-frozen in -80ºC freezer, lyophilized and stored at 25 °C.

2.2. Heteropolysaccharide extraction

The galactose-rich heteropolysaccharide (GH) was extracted according to the methodology described by Miranda et al. (2020). Briefly: 1 g (dry basis) of lyophilized peel (0.25 mm particle size), was suspended in 30 mL distilled water and the pH was adjusted to 1.0 with 70% (v/v) nitric acid. The mixture was heating until 90 °C, and kept under stirring for 30 min. The hot extract was filtered through filter paper (unifil no 1), cooled to 4 °C and the filtrate was precipitated with 2 volumes of ethanol (99.5% v/v). The system was mixed and left to rest at 4 °C for 24 h. The mixture was centrifuged at 10000 g for 15 min and the supernatant was removed. The solid material was dialyzed with a 14 kDa dialysis bag for 48 h to remove impurities. Finally, the GH was obtained by freeze-drying. The figure 1 show the extraction procedure.

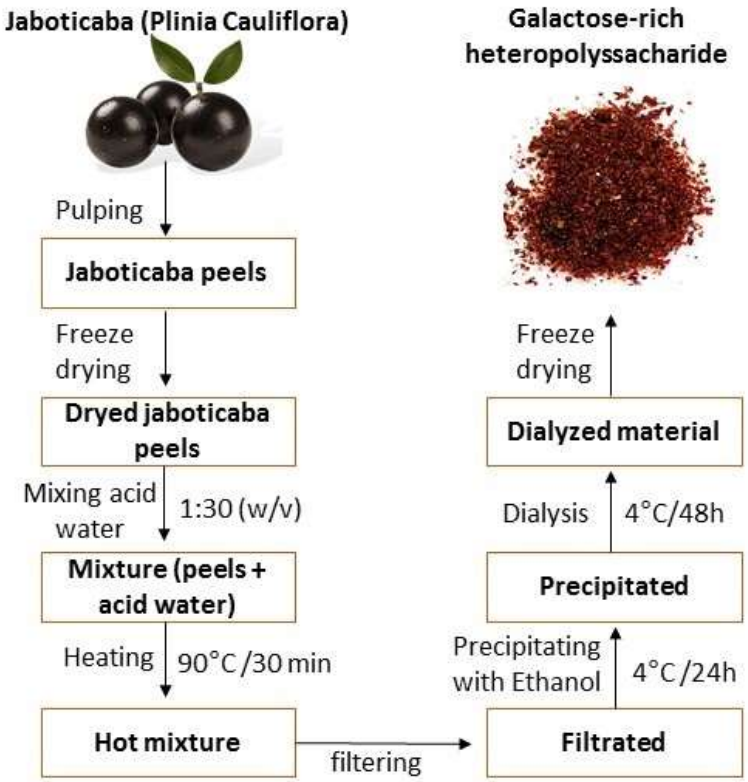


Figure 1. Flowchart of the extraction procedure from GH.

2.3 Zeta potential

The surface charge of the GH solution (1 g/L) was measured in ZetaSizer Nano ZS (Malvern Instruments, UK) at 25 °C and values of zeta potential was expressed in mV. The pH of the GH solution was adjusted from 2 to 10 with NaOH or HCl solutions (0.1M) and the zeta potential was measured in triplicate.

2.4 Intrinsic viscosity

The intrinsic viscosity was measured in solutions of GH from 0.2 to 1.2 g/L in 10mM NaCl solution, in different pH (2,3,4 and 5) with sucrose (60%) and without sucrose, using the rolling ball microviscometer (LOVIS 2000 M, Anton Paar GmbH, Germany). A glass capillary with a radius of 1.59 mm and a steel ball with a radius of 1.5 mm at an angle of 50° were used. The intrinsic viscosity was calculated using the Hunggins equation and the approach of Wolf (Wolf, 2007). In order to obtain the intrinsic viscosity according to Huggins, the reduced viscosity was fitted to equation (1) using a linear least square regression to obtain the intrinsic viscosity and the Huggins coefficient. The approach by Wolf (Wolf, 2007) calculates the intrinsic viscosity based on the relationship between the logarithmic relative viscosity and the concentration. Accordingly, the intrinsic viscosity can be calculated from the initial slope of this function as stated in equation (2). Each measurement was performed in triplicate.

$$\eta_{red} = \frac{\eta_{sp}}{c} = \frac{\eta_s - \eta_0}{\eta_0\, c} = [\eta] + k_H[\eta]^2 c \qquad (1)$$

$$[\eta] = \left(\frac{d(\ln\, \eta_{rel})}{dc}\right)_{c=0} \qquad (2)$$

2.5 Rheological measurements

All samples for rheological measurements were prepared using distilled water, with a fixed sucrose concentration of 60 wt% and 1 wt% of GH. The solutions were prepared separately, mechanically stirred at 95 ºC for 10 min, each one. After complete dissolution, the GH and Sucrose solutions were mixed and mechanically stirred for 5 min at 90ºC, with subsequent addition of water to compensate for evaporation. For gelation with GDL, the GH/sucrose solution were allowed to cool to room temperature (1h), and the required amount of GDL was added as solid powder. The mixture was then homogenized by brief (2 min) and centrifuged to avoid the bubbles (min). The sample was loaded into the rheometer at 20ºC.

Oscillatory measurements of storage modulus (G'), loss modulus (G'') were made using concentric cylinder system CC17 (Anton Paar GmbH, Graz, Austria) on a Physica MCR 102 rheometer (Anton Paar GmbH, Graz, Austria). After loading, all samples were coated around their periphery with light silicone oil, to minimize loss of water by evaporation. The gel formation during acidification with GDL were measured for 16 h at 20 °C at a strain of 0.5 % and a frequency of 1 rad s^{-1}. Afterward a frequency sweep (strain 0.5 %, frequency 0.01 – 100

rad s^{-1}) and an amplitude sweep (strain 0.1 – 1000 %, frequency rad s^{-1}) were performed at 20 °C. For temperature sweeps gels were prepared and measured as described without performing an amplitude sweep before the samples were heated from 20ºC to 90 ºC at 1ºC/min, with measurements again being made at 1 rad s^{-1}.

3. Results and discussion

3.1 Zeta potential

The zeta potential (*Zp*) reflected the stability of polysaccharides solutions, according to McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008 *Zp* could be defined as the electrical potential which exists at the hydrodynamic plane of shear surrounding a charged particle and is essentially the potential at the point in space where low molecular weight ions cease to move with the particle and remain within the surrounding solvent. Generally, when all the particles have a large positive or negative *Zp* (where the positivity and negativity is greater or lower than +30 mV and −30 mV), they will repel each other and the dispersion is stable (Wongsagonsup, Shobsngob, Oonkhanond, & Varavinit 2005).

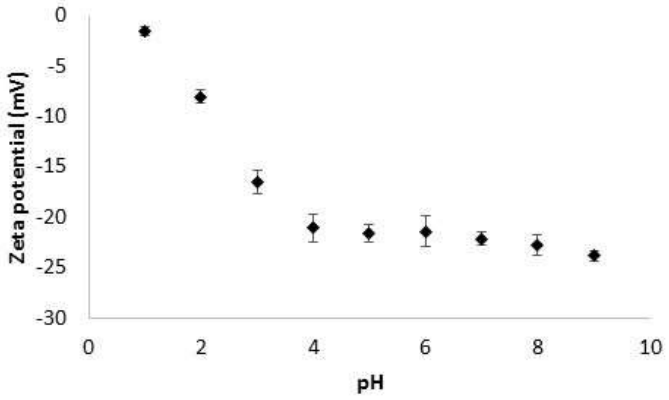


Figure 2. Zeta potential in different range of pH.

The GH solution showed a negative charge in all pH values evaluated (Figure 2). Zeta potential values were decreased according to pH values were increased, achieving values around -20 mV at pH values higher than 4. These typical weak acid ionisation behavior could be observed for other kinds of polymers, such as pectin, gum Arabic and alginate (Barbosa et al., 2019). The latter decrease in Zeta potential values may be related to a decrease in intermolecular interactions when the pH increases. Therefore, the decrease in the charge density leads to a decrease in the electrostatic repulsion between the polysaccharides, allowing other interactions to occur, such as hydrogen bonds or hydrophobic interactions. That is, the decrease

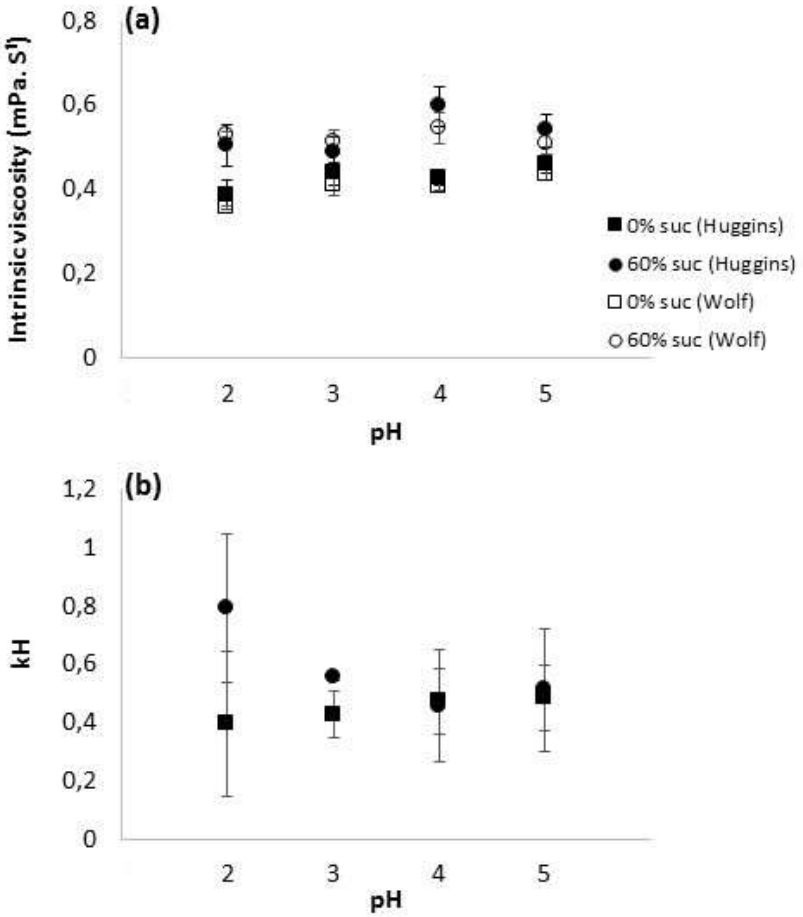
in the charge density of the GH solution can be caused by the reduction in the concentration of H+ ions, as reported by (Carneiro-da-Cunha et al., 2011; Khemakhem et al., 2018). On the other hand, the increases in the absolute value of the charge indicated the good stability of the polysaccharide solution, as the increase in the pH value causes an increasing protonation of the COO residues, leading the charge and zeta potential to tend to zero (Khemakhem et al., 2018).

A direct relationship exists between the charge of polysaccharide and the *Zp* of the polysaccharides. The GH were negatively charged across the entire pH range, which could suggest that GH possessed donated electron capacities, due the small percentual of uronic acids in their compositon (Miranda et al., 2020).

3.2 Intrinsic viscosity

The intrinsic viscosity describes the viscosity change of a solution due to the presents of a single polymer. (Gawkowska et al., 2018). It could be measure of the flow behavior of macromolecules and an indirect measure of their size and shape, indicating the specific hydrodynamic volume of individual polymer molecules without shear at infinite dilution (Bae, Oh, Lee, Yoo, & Lee, 2008).

The experimental data obtained in this work, give access to hydrodynamic volume using two methods (Figure 3a): the classical Huggins equation (Huggins, 1943) and the more recently model developed by Wolf (Wolf, 2007).The classical procedure for intrinsic viscosity determination, based on Huggins model, consists of the determination of viscosities of solutions of various concentrations, followed by extrapolation of specific viscosity to zero concentration (Pamies et al., 2008), however, this method works well for uncharged macromolecules and could be failed for polyelectrolytes, because of the pronounced non-linearity of the above dependence at high dilution resulting from the increasing electrostatic interactions, in this case, the Wolf method could be more precise, as the intrinsic viscosity is calculated based on the relationship of the logarithmic relative viscosity ηrel (Pa s) and the concentration of the solution (Wolf, 2007).



158

159 **Figure 3.** Variation of (a) Intrisic viscosity and (b) Coeficient of Huggins, in different ranges
160 of pH with: (□) 0% of sucrose and (●) 60% of sucrose.

161 The intrinsic viscosity of sample often can be related with its zeta potential, whereas
162 generally, the larger the absolute value of zeta potential is related with the higher the intrinsic
163 viscosity. However, in the case of GH solutions, as the main chain composition is formatted by
164 neutral sugar, the intrinsic viscosities difference between the different pH values was smaller.
165 This fact could be confirmed due the similarity between the results obtained by both methods
166 used, due, extrapolation procedures (Huggins model) are commonly used and work well with
167 solutions of uncharged polymers, but, for charged polymers, these relations often fail and
168 differences between results of Huggins and Wolf models could be observed (Wolf, 2007).
169 However, it was possible to observe the tendency of higher intrinsic viscosity when
170 sucrose is added, it could be explained because of the sugar concentration has an influence on
171 reducing the hydration of GH molecules and increasing the polymer-polymer interaction
172 (Gawkowska et al., 2018). According to Chen & Joslyn, (1967), in water solutions with sucrose
173 and pectin polysaccharides, sucrose can bind with water molecules, preventing its participation

in hydrogen bonds with pectin. This binding permits the pectin molecules to approach each other and to form cross-linked bonds. due to the formation of these cross-linking hydrogen bonds between the hydroxyl groups of sucrose and those of the pectin, could occur aggregate formation.

Moreover, the Huggins constant (K_H) is another result that could help to observe this fact According to Morris et al. (1981), K_H should lie between 0.3 and 0.8, while values of K_H higher than 1.0 are indicative of molecular aggregation. In pH 2 with sucrose we see a tendency of increase of the K_H, which might be the result of protonation of GH in pH 2, that caused a decrease on electrostatic repulsions (Torres et al., 2015), and the competition for water between GH and sucrose.

3.3 Rheological measurements

For the rheological study, the initial pH of the solution of 1% of GH and 60% of sucrose, was at 4.4 and no gelation was observed. Thus, it was necessary to add the pH reducer (GDL) in different concentrations to the gel formation, monitoring this over time, as can be seen at Figure 4.

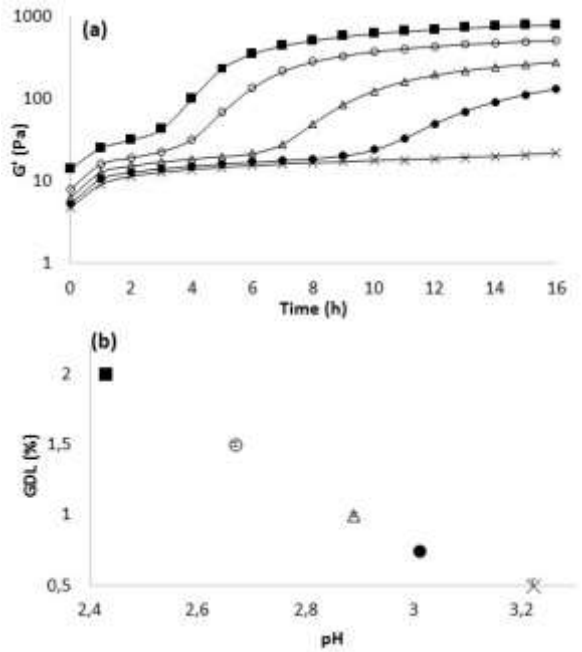


Figure 4. Variation of (a) G' and, measured at 1 rad s1 and 0.5% strain during holding at 20ºC, acidified with GDL (16 h at 20 ºC) in the following concentrations: 2% (■), 1.5% (○), 1% (Δ), 0.75% (●) and 0.5% (x) and (b) final the value of pH after 16h.

As expected, due the similarity in the composition of GH and pectin, the characteristics of the gel were similar to those found in the literature, where, the traditional use of pectin as a gelling agent (Rolin & De Vries, 1990) promotes the gelation induced by cooling in the presence of high concentrations of co-solute at acid pH (typically ~65 wt% sucrose at ~pH 3.0). The same behavior was observed for GH solution. The polymer with the high sucrose concentrations can occur intermolecular associations, when the acidic pH causes protonation of the carboxylic groups, decreases the electrostatic repulsion between the chains, consequently, promoting the formation of the gel. Moreover, the sucrose acts an important function at gel formation, reducing the water activity to stabilize junction zones by promoting hydrophobic interactions (Lara-Espinoza et al., 2018).

According to Gilsenan et al.,(2000), although protonation of a certain proportion of the carboxyl groups may be an important factor in stabilizing the intermolecular association of pectin chains, hydrogen bonds formed with ionized carboxyl groups as the receptors are stronger than those involving groups that carry only a partial negative charge (hydroxyl or protonated carboxyl) because the electrostatic attraction will be greater. Thus, more structured systems reflect the optimum balance between the facilitation of intermolecular association by charge suppression and the loss of enthalpic stability because of hydrogen bonding to ionized carboxyl groups, as the pH is reduced (Sato et al., 2008).

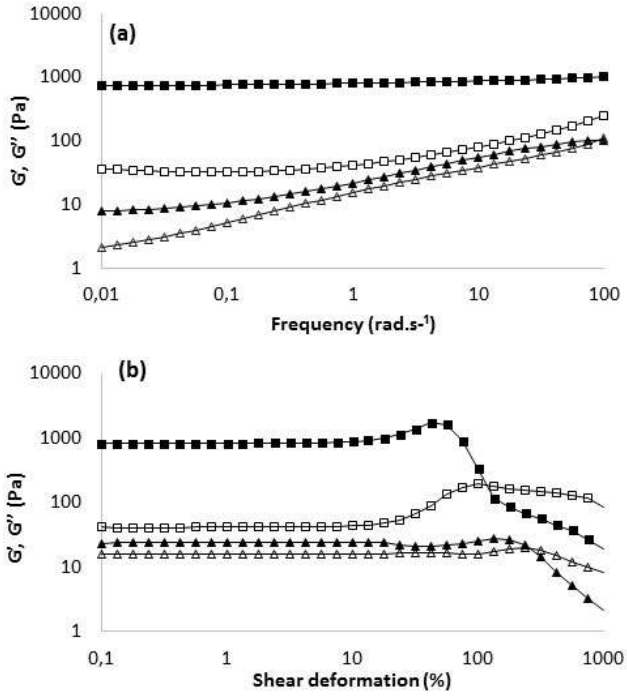


Figure 5. Variation of G' (filled symbols) and G'' (open symbols) with 2% of GDL (■) and 0.5% of GDL (▲) during (a) frequency variation and (b) shear deformation.

Typically, gels are classified into two main categories: strong and weak. According to Rosalina & Bhattacharya (2002), strong gels have the characteristics of true gels when under deformation conditions they present a typical behavior of viscoelastic solids, which, above a critical value of deformation, suffer rupture instead of yielding. Weak gels have intermediate rheological properties. Under low strain conditions, they resemble strong gels in their behavior, but as strain increases, the three-dimensional networks progressively break down into smaller clusters.

As observed in (Figure 5 a), all the samples exhibited the storage modulus (G') higher than the loss modulus (G"), denoting that the elastic character was always dominant, however, the sample with 2% of GDL showed a typically strong gel behavior, with $G' \gg G''$, constant G' value for the entire frequency range, showing a frequency-independence (Clark & Ross-Murphy, 1987). Moreover, the slope curve for G' was $R^2 = 0.035$, near to zero, the perfect behavior from strong gel (Gilsenan et al., 2000).

The variation at shear deformation (Figure 5b), showed that the strong gel with 2% of GDL was resistant until 7.61(%) of deformation, when it starts to change until 114.46 (%) where was the breaking point of the gel, the point of crossing between G' and G''. This is the yield point due to the breaking of the gel (Laurent & Boulenguer, 2003). For a weak gel-like, what is the case of the system wiht 0.5% of GDL, the values for deformation was later (33.73 % when it starts to change and 266,07 % the breaking point), which is expected due the lower interactions and resistance of the system.

In this way, could be observed for 0.5% GDL sample, G'' was constant over the entire range of strain, whereas G' decreased when the strain increased beyond the critical strain, but with constancy at low strain values. This kind of result shows a strain thinning behavior, which is typical behavior of most polymer gels (Roque et al., 2022) . On the other hand, the 2.0% GDL sample showed a constant G'' and G' only up to a certain strain amplitude followed by a dramatic decrease in G' and an overshoot in G'' as the strain increased beyond a certain critical value, what is expected for a weak strain overshoot (Flores et al., 2017), being a typical behavior of complex fluids such as biopolymer systems and emulsion (Stolz et al., 2021).

In order to have a better understanding of the molecular interactions and behavior of the GH gel, heating analyzes were developed to verify their behavior at high temperatures (Figure 6).

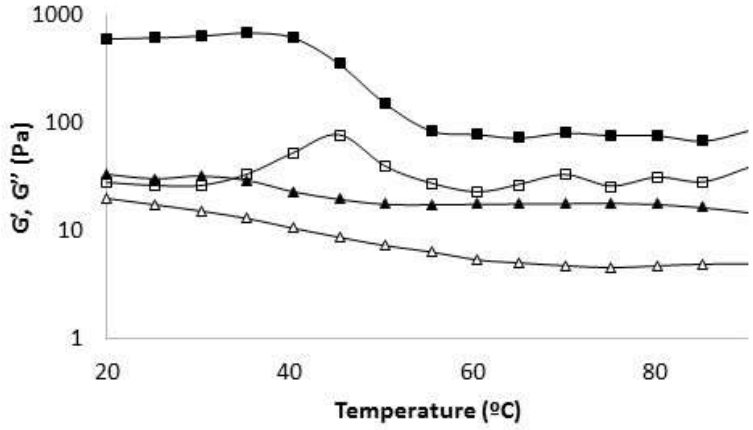


Figure 6. Variation of G' (filled symbols) and G'' (open symbols) with 2% of GDL (■) and 0.5% of GDL (▲) during heating at 1 ºC/min.

Usually, pectin gels are no thermally reversible as they do not melt during heating. This thermal hysteresis is attributed to the presence of large, aggregated assemblies that stabilize the structures formed during cooling (Morris et al., 1980). It is suggested that two types of interactions stabilising the gel, one is stabilised by increasing temperature and the other is destabilised. The first type corresponds to hydrophobic interactions, and the second type corresponds to hydrogen bonding and other types of thermally-labile non-covalent interactions (Zioga et al., 2022).

Thus, for conventional biopolymer networks, the sharp reduction in G' during thermal dissociation is often accompanied by the increase in G'', which arises from transient formation of a large fraction of network fragments, before these also dissociate. Moreover, a small increase in G' were observed over roughly the same temperature range as the sharp decreases seen under conditions more favorable to association of the GH chains. It could means that, the decreases come, not from insufficient hydrophobic association, but from excessive association of chains into large aggregates, leading to collapse of network structure (Agoub et al., 2009).

The same behavior was seen by (Zioga et al., 2022) during the heating of pectin gels with 65% sucrose at pH 3.0, whereas, this behavior was attributed to the presence of large, aggregated assemblies that stabilize the structures formed during cooling, making this kind of gel no thermally reversible.

4. Conclusion

The zeta potential showed different charge at different pH, however, the intrinsic viscosity did not change significantly at different ranges. Furthermore, a Kh lower than 0.8 was observed,

demonstrating the absence of aggregation in the solution. GH showed interesting properties for gel formation, with behavior similar to that of pectin, as it was observed in the rheological experiments the formation of gel at pH lower than 3 in the presence of 60% of sucrose and when heated, the gel showed a long time to break . Thus, GH is shown to be a gelling agent with high potential for application.

REFERENCES

Agoub, A. A., Giannouli, P., & Morris, E. R. (2009). Gelation of high methoxy pectin by acidification with d-glucono-δ-lactone (GDL) at room temperature. *Carbohydrate Polymers*, *75*(2), 269–281. https://doi.org/10.1016/j.carbpol.2008.07.016

Barbosa, J. A. C., Abdelsadig, M. S. E., Conway, B. R., & Merchant, H. A. (2019). Using zeta potential to study the ionisation behaviour of polymers employed in modified-release dosage forms and estimating their pKa. *International Journal of Pharmaceutics: X*, *1*, 100024. https://doi.org/10.1016/j.ijpx.2019.100024

Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., & Vicente, A. A. (2011). Influence of concentration, ionic strength and pH on zeta potential and mean hydrodynamic diameter of edible polysaccharide solutions envisaged for multinanolayered films production. *Carbohydrate Polymers*, *85*(3), 522–528. https://doi.org/10.1016/j.carbpol.2011.03.001

Chen, T.-S., & Joslyn, M. A. (1967). The effect of sugars on viscosity of pectin solutions: I. Comparison of corn sirup with sucrose solutions. *Journal of Colloid and Interface Science*, *23*(3), 399–406. https://doi.org/10.1016/0021-9797(67)90184-1

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Biopolymers*, 57–192. https://doi.org/10.1007/BFb0023332

Flores, S. L., Descallar, F. B. A., Matsukawa, S., & Bacabac, R. G. (2017). Dynamic rheological properties of mixed carrageenan gels under large strains. *Journal of Biorheology*, *31*(2), 35–39. https://doi.org/10.17106/jbr.31.35

Gabriel da Rosa, R., Sganzerla, W. G., Barroso, T. L. C. T., Buller, L. S., Berni, M. D., & Forster-Carneiro, T. (2022). Sustainable production of bioactive compounds from jabuticaba (Myrciaria cauliflora): A bibliometric analysis of scientific research over the last 21 years. *Sustainable Chemistry and Pharmacy*, *27*, 100656. https://doi.org/10.1016/j.scp.2022.100656

Gawkowska, D., Cybulska, J., & Zdunek, A. (2018). Structure-Related Gelling of Pectins and Linking with Other Natural Compounds: A Review. *Polymers*, *10*(7), 762. https://doi.org/10.3390/polym10070762

Gilsenan, P. M., Richardson, R. K., & Morris, E. R. (2000). Thermally reversible acid-induced gelation of low-methoxy pectin. *Carbohydrate Polymers*, *41*(4), 339–349. https://doi.org/10.1016/S0144-8617(99)00119-8

Huggins, M. L. (1943). Molecular Weights of High Polymers. *INDUSTRIAL AND ENGINEERING CHEMISTRY*, *35*(9), 7.

Khemakhem, I., Abdelhedi, O., Trigui, I., Ayadi, M. A., & Bouaziz, M. (2018). Structural, antioxidant and antibacterial activities of polysaccharides extracted from olive leaves. *International Journal of Biological Macromolecules*, *106*, 425–432. https://doi.org/10.1016/j.ijbiomac.2017.08.037

Lara-Espinoza, C., Carvajal-Millán, E., Balandrán-Quintana, R., López-Franco, Y., & Rascón-Chu, A. (2018). Pectin and Pectin-Based Composite Materials: Beyond Food Texture. *Molecules*, *23*(4), 4. https://doi.org/10.3390/molecules23040942

Laurent, M. A., & Boulenguer, P. (2003). Stabilization mechanism of acid dairy drinks (ADD) induced by pectin. *Food Hydrocolloids*, *17*(4), 445–454. https://doi.org/10.1016/S0268-005X(03)00028-6

Morris, E. R., Gidley, M. J., Murray, E. J., Powell, D. A., & Rees, D. A. (1980). Characterization of pectin gelation under conditions of low water activity, by circular dichroism, competitive inhibition and mechanical properties. *International Journal of Biological Macromolecules*, *2*(5), 327–330. https://doi.org/10.1016/0141-8130(80)90058-6

Pamies, R., Hernández Cifre, J. G., del Carmen López Martínez, M., & García de la Torre, J. (2008). Determination of intrinsic viscosities of macromolecules and nanoparticles. Comparison of single-point and dilution procedures. *Colloid and Polymer Science*, *286*(11), 1223–1231. https://doi.org/10.1007/s00396-008-1902-2

Rolin, C., & De Vries, J. (1990). Pectin. In P. Harris (Ed.), *Food Gels* (pp. 401–434). Springer Netherlands. https://doi.org/10.1007/978-94-009-0755-3_10

Roque, A. M., Montinola, D., Geonzon, L., Matsukawa, S., Lobarbio, C. F. Y., Taboada, E. B., & Bacabac, R. G. (2022). Rheological elucidation of the viscoelastic properties and network interaction of mixed high-methoxyl pectin and kappa-carrageenan gels. *Food Hydrocolloids*, *129*, 107647. https://doi.org/10.1016/j.foodhyd.2022.107647

Rosalina, I., & Bhattacharya, M. (2002). Dynamic rheological measurements and analysis of starch gels. *Carbohydrate Polymers*, *48*(2), 191–202. https://doi.org/10.1016/S0144-8617(01)00235-1

Sato, A. C. K., Oliveira, P. R., & Cunha, R. L. (2008). Rheology of Mixed Pectin Solutions. *Food Biophysics*, *3*(1), 100. https://doi.org/10.1007/s11483-008-9058-7

Stolz, J., Oguzlu, H., Khalili, Z., & Boluk, Y. (2021). Exploring the gelation of aqueous cellulose nanocrystals (CNCs)-hydroxyethyl cellulose (HEC) mixtures. *Rheologica Acta*, *60*(9), 483–495. https://doi.org/10.1007/s00397-021-01285-1

Torres, C. A. V., Ferreira, A. R. V., Freitas, F., Reis, M. A. M., Coelhoso, I., Sousa, I., & Alves, V. D. (2015). Rheological studies of the fucose-rich exopolysaccharide FucoPol. *International*

Journal of Biological Macromolecules, *79*, 611–617. https://doi.org/10.1016/j.ijbiomac.2015.05.029

Wolf, B. A. (2007). Polyelectrolytes Revisited: Reliable Determination of Intrinsic Viscosities. *Macromolecular Rapid Communications*, *28*(2), 164–170. https://doi.org/10.1002/marc.200600650

Zioga, M., Tsouko, E., Maina, S., Koutinas, A., Mandala, I., & Evageliou, V. (2022). Physicochemical and rheological characteristics of pectin extracted from renewable orange peel employing conventional and green technologies. *Food Hydrocolloids*, *132*, 107887. https://doi.org/10.1016/j.foodhyd.2022.107887

CAPÍTULO VI

Artigo 5: Artigo **submetido** na revista *Food Chemistry* em 20 de janeiro de 2023.

CHARACTERIZATION OF THE STARCH EXTRACTED FROM JABOTICABA (*Plinia cauliflora)* SEEDS

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Abstract

The aim of this work was the fine characterization of the granular, thermal and molecular properties of the novel starch from jaboticaba seeds. An aqueous/acid extraction was carried out (yield 22.65±0.63%), resulting in a fine and slightly beige powder. The jaboticaba starch showed small, smooth and irregular shape granules, with size between 6.1 and 9.6 μm. It was found that this is a starch with high content of AM (34.50±0.90%), intermediate AP chain length, with a larger proportion of B1-chains (51%), followed by A-chains (26%) and comparatively low Mw (5.3·106 g·mol-1), confirming that it is a Cc-type starch, as suggested in the X-ray diffractogram. Thermal investigation (DSC) showed a low temperature for gelatinization (To at 66.4±0.46 °C), but high temperature range (ΔT_{gel} =14.1±0.52 °C). According to the results, jaboticaba starch proved to be a promising material for future studies and versatile applications.

Keywords: Unconventional starch, granular properties, molecular characterization, thermal properties.

1. INTRODUCTION

Jaboticaba (*Plinia cauliflora*) is a spherical berry from south-center region of Brazil, with a black-purple peel and a white, sweet, viscous pulp, that holds one to four seeds (Benvenutti et al., 2021). The unprocessed fruit is highly appreciated for its sensory properties, but has a short shelf-life (3-7 days), which make the production of jellies, sweets, juices and alcoholic beverages more popular as its raw consumption. However, industrial processing produces peels and seeds as by-products, which account for up to 50% of the total weight of the jaboticaba fruits (Rosa et al., 2022). Hence, processes that convert jaboticaba by-products into valuable resources, could improve its economic value. In a recent study, galactose-rich polysaccharides with interesting rheological properties (Miranda et al., 2020) and anthocyanins with high antioxidant activity (Miranda et al., 2022) were extracted from jaboticaba peels. Another promising residue are the seeds as a considerable fraction of the fruit weight, which contain starch in their composition.

Starch is a versatile biopolymer with countless applications, low price, and abundant availability for use in many industries including food, textile, pharmaceutical and cosmetics (Tarique et al., 2021). Chemically, starch is composed of two different polysaccharide fractions, amylose (AM) and amylopectin (AP). AM is the polysaccharide fraction mostly linear, consisting of α-1,4-glycosidic linked anhydroglucose units (AGU). AP is the branched polysaccharide fraction with α-1,4-glycosidic and α-1,6-glycosidic linkages at the branching points (Ulbrich et al., 2022). Despite the simplicity of its chemical composition, starches can vary in their physicochemical properties as function of the relative amount of their constitutive polymers, their molar masses, their branching degrees and their chain length distribution. Thus, depending on their chemical composition and characteristics, starches obtained from unconventional sources may present attractive properties and technological advantages over the common starches (Makroo et al., 2021). Additionally, establishing new starch sources using waste or by-products from food production chains, meets the global demand for sustainability and the circular economy.

In this sense, the objective of this work was to extract and to characterize the new starch obtained from jaboticaba seeds in terms of minor components, granular morphology, thermal properties, and molecular characteristics. The thorough knowledge about the properties of this novel starch, gives a new perspective of this material for future studies and applications.

2. MATERIAL AND METHODS

2.1 Starch containing material and starch extraction

P. cauliflora (jaboticaba) fruits were obtained from Jabuticabal Farm & Winery, Hidrolândia-GO, Brazil. The fruits were pulped in an electric pulper (Itametal, Bonina 0.25DF, Brazil), and the peel was separated from the seeds before freezing/storage at -18 ºC. This project was registered in the National System of Management of Genetic Heritage (SisGen) of the Brazilian Ministry of Environment (register nºA95EFEC).

The seeds were soaked in distilled water at 4 ºC for 24 h and the seed coat was manually removed. The seeds were crushed in a blender and ground with water (1:2 w/v). The slurry was filtered and washed with distilled water until the turbidity disappeared. The starch was rinsed with citric acid (0.1% w/v) and dried under vacuum at 20±2 °C. The starch yield was calculated as percentage (dry basis).

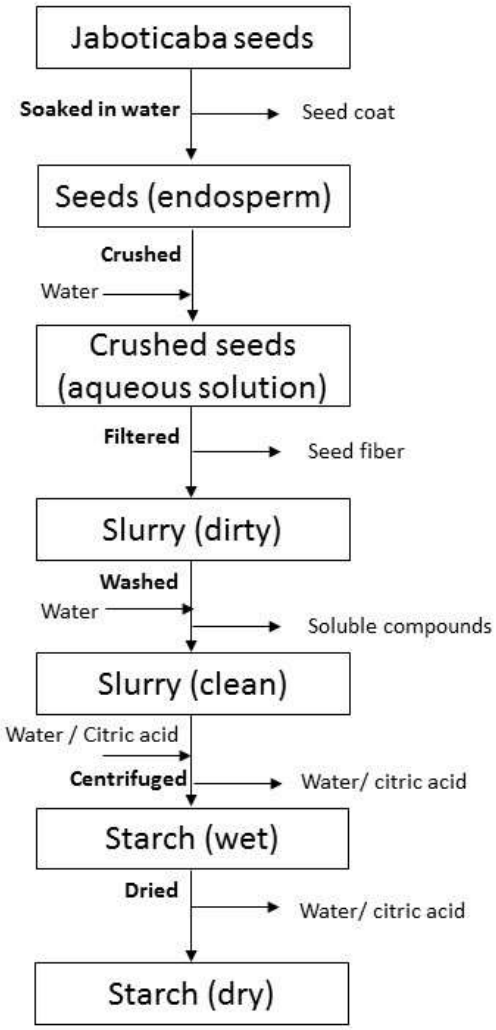


Figure 1. Flowchart of the starch extraction from *P. cauliflora* seeds.

2.2 Minor components and color measurement.

The protein content was determined by the micro-Kjeldahl method (AOAC, 2012) using a nitrogen conversion factor of 6.25. The phenolic compounds were determined according to (Waterhouse, 2002), with the Folin-Ciocalteau reagent, using three 1:10 (w/v) extracts of the starch (water, ethanol and ether). The quantification of the phenolic compounds was performed using a gallic acid standard curve (R^2=0.99) as reference and the results were expressed in gallic acid equivalent (GAE) per g of sample. The color of the starch powder was measured in a ColorQuest XE colorimeter (Hunter Lab, Reston, USA) equipped with diffuse light in the reflection mode in the CIE L* a* b* color scale.

2.3 Analysis of the starch morphology

2.3.1 Light microscopy (LM) techniques

The starch was suspended in water, placed between the slide and coverslip and sealed with nail polish, to prevent moisture loss during the analysis (Cai et al., 2014). The micrographs were taken using a light microscope (LM; AxioScope A1 equipped with a camera AxioCam ICm1, Carl Zeiss, Germany) under normal (LM) and polarized light (LM-pol). The magnification was set to x250 and x500. The LM images were processed using the blue ZEN Software (Carl Zeiss, Jena, Germany).

2.3.2 Scanning electron microscopy (SEM)

The granule morphology was analyzed by scanning electron microscopy (SEM) using a secondary electron detector with 15 kV acceleration (JEOL, JSM 6610, USA). The sample was uniformly dispersed on aluminum stubs with conductive double-sided adhesive and then sputter-coated with gold under vacuum.

2.3.3 Determination of granule size and granule size distribution

2.3.3.1 Size based on LM images

The post-processing of the microscopic images was performed using ImageJ 1.53c including the calculation of the particle size (Wayn Rasband, National Institutes of Health, USA). After scaling and thresholding, the number of starch granules, the circularity and the size, were determined. The calculation of the circularity followed the equation:

$$Circularity = 4\pi * area * perimeter^2 \quad \text{(Eq. 2)}$$

In this case, the factor of 1.0 represents a perfect circle. To exclude irrelevant data (noise and non-starchy material), the considered particle size was set to 25-2000 μm^2 and the circularity was set to values between 0.74 and 1.0. In order to evaluate an adequate number of starch granules, a number of grid images (5x5) were taken and analyzed. The equivalent diameter of the three-dimensional starch granules was calculated assuming a rotationally symmetric (longitudinal axis) granule. The relative particle size distribution curve was calculated and the medians ($x_{50.0}$) were determined from the cumulative distribution.

2.3.3.2 Laser particle analysis (LPA)

The determination of the starch granule size was performed in a laser particle sizer (LPS; Analysette 22, Fritsch GmbH, Idar-Oberstein, Germany). The measurements of the aqueous suspension of the granules were performed at 20ºC±2 and in triplicate. The relative particle size distribution curve was calculated and the median ($x_{50.0}$) was determined from the cumulative distribution.

2.4 Molecular characterization

2.4.1 Amilose content

The AM content was determined by spectroscopy, according to the method of American Association of Cereal Chemistry (AACC, 2000). Doing so, the AM content was calculated by inserting the absorbance value at 620 nm into the function of the calibration curve, which was determined with pure AM (Sigma-Aldrich) in the range of 0.01-0.07 mg mL^{-1}(R^2=0.99).

2.4.2 Characterization using Size exclusion chromatography (SEC) techniques

2.4.2.1 Preparation of a molecularly dispersed starch solution

A starch solution was prepared by heating an aqueous dispersion of 2.5% (w/w) in an autoclave (Model I, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) to 145 °C under continuous stirring (300 min^{-1}) for 30 min and subsequent high-shear-treatment using an Ultra-Turrax T25 (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 24.000 min^{-1} for 2 min at approximately 80±5°C. An aliquot of the dispersion was diluted 1:10 (v/v) in DMSO (2.5 mg·mL^{-1}), and the stabilized solution was passed through a 5µm PTFE filter (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) before analysis (SEC-MALS-DRI, no enzymatic treatment).

2.4.2.2 Specific enzymatic degradation

Two different starch degrading enzymes were used, a pullulanase (PUL; solution, Promozyme®D2, 200 $U \cdot mL^{-1}$, Novozymes A/S, Bagsvaerd, Denmark) and a β-amylase (β-AMY; solution, Secura, 5000 $BAMU \cdot g^{-1}$, Novozymes A/S, Bagsvaerd, Denmark).

A volume of 10 mL of the freshly prepared aqueous starch solution (2.5 % w/w) was tempered to 40°C and the respective volume of the enzyme solution was added (PUL: 187 µL, β-AMY: 100 µL). The dispersions were gently stirred at 40 °C for 20 min (enzymatic digestion) and subsequently heated to 95 °C and tempered for 20 min (termination of the reaction). Aliquots (0.5 mL) of the solutions (PUL: debranched starch; β-AMY: β-limit dextrins [β-LD]) were diluted 1:10 (w/v) in DMSO and passed through 5 µm PTFE syringe filter before analysis. The remaining aqueous solution after treatment with β-AMY (containing β-LD) was subsequently tempered at 40 °C and debranched (PUL: 187 µL) by processing according to the previous description (β-AMY-PUL: containing debranched β-LDs) including stabilization in DMSO. Enzyme blanks (E-β-AMY and E-β-AMY-PUL) were prepared according to the above description with starch substituted by water. In total, six SEC measurements were performed (starch [no enzymatic treatment], debranched [PUL], β-LDs [β-AMY], debranched β-LDs [β-AMY-PUL], E-β-AMY and E-β-AMY-PUL).

2.4.2.3 Analysis by means of SEC-MALS-DRI and SEC-cal-DRI techniques

The molecular characterization was carried out by means of SEC-MALS-DRI. The separation was executed with an SEC-3010 module (WGE Dr. Bures GmbH & Co. KG, Dallgow-Döberitz, Germany) including degasser, pump and auto sampler connected to a MALS detector and a differential refractive index (DRI) detector according to the description elsewhere (Ulbrich et al., 2022) . During the sample run on the SEC-MALS-DRI system, the data from the MALS and DRI detectors were collected and processed using ParSEC Enhanced V5.61 chromatography software to give the concentration of the eluted solution and the molar mass at each retention volume (M_i). The basis for the molecular characterization by means of SEC-MALS-DRI has been described elsewhere (Podzimek, 2011; Wyatt, 1993).

The separation system was additionally calibrated (SEC-cal-DRI) using a set of 10 pullulan standards (800k, 400k, 200k, 110k, 50k, 22k, 10k, 6k, 1.3k and 342; PSS Polymer Standards Service GmbH, Mainz, Germany) as well as Glc with a molar mass range between 180 and 805000 $g \cdot mol^{-1}$ (Ulbrich et al., 2022). The calibration related to the DP was calculated from the M_i divided by 162 (M AGU). The weight average DP (DP_w) was calculated from the M_w divided by 162.

2.4.2.4 Processing of the chromatograms and mathematical peak separation

The SEC-chromatograms of the enzyme blanks E-β-AMY and E-β-AMY-PUL were subtracted from the SEC-chromatograms of β-AMY (β-LDs) and β-AMY-PUL (debranched β-LDs), and the resulting calculated chromatograms as well as the SEC-chromatogram of the debranched starch (PUL) were advanced analyzed using peak separation and analysis software PeakFit® Version 4.12 (Ulbrich et al., 2016). Based on the fitted SEC-chromatograms, single peaks (chromatograms) representing different fractions were identified and calculated. The chromatogram originating from the enzyme solution was subtracted (in the case of PUL, debranched starch), and the relative chromatogram area of each separated fraction was taken as the relative amount (PUL, β-AMY, β-AMY-PUL). The values of M_w and DP_w were calculated by means of the correspondent separated chromatogram and the molar mass curve (fit) from the MALS-detector (SEC-MALS-DRI; AM fraction and β-LD fraction) or the standard calibration curve (SEC-cal-DRI; AP BC fraction and β-LD BC fraction), respectively (Ulbrich et al., 2019, 2020). The applied method was indicated properly.

2.4.3 Characterization using HPAEC-PAD chromatography

2.4.3.1 Separation of Amylose from Amylopectin

AM and AP were separated according to the methodology of (Mua & Jackson, 1998). The AM leaching was carried out by incubating an aqueous starch suspension (4 $mg \cdot mL^{-1}$) at 60 ºC for 1 h with gentle stirring. Then, the suspension was centrifuged (1430 x g for 5 min) and the AM present in the supernatant was removed. The procedure was repeated twice. After removal of AM, the AP residue was dried at 25 ºC.

2.4.3.2 Amylopectin enzymatic debranching

AP was debranched according to methodology described by (Tako & Hizukuri, 2000), using isoamylase (iso-AMY; EC3.2.1.68, Sigma Aldrich, São Paulo, Brazil). An amount of 10 mg of the AP was solubilized in 988 µL acetate buffer (50 $mmol \cdot L^{-1}$, pH 3.5), heated at 100 ºC for 5 min and subsequently cooled to 25 ºC. Then, 6 µL of iso-AMY solution (0.6 U) was added to the reaction and the mixture was incubated for 15 h at 45 ºC. Finally, the dispersion was centrifuged (1430 x g, 5 min) and filtered (PTFE syringe filter 0.45 µm), and the filtrated solution analyzed.

2.4.3.3 Amylopectin chain length distribution

The debranched AP sample was analyzed by high-performance anion-exchange chromatography with pulsed amperometry detection (HPAEC-PAD) on a Dionex ICS-5000 chromatographic system, using a CarboPac PA-100 (4×250 mm) column. The sample was eluted in a gradient of sodium acetate in sodium hydroxide 150 mmol·L^{-1}, as follows: 0 - 10 min, 5 mmol·L^{-1}; 10.1 - 20 min, 15 mmol·L^{-1}; 20.1 - 95 min, 25 mmol·L^{-1}; 95.1 - 120 min, 165 mmol·L^{-1}; 120.1 - 130 min, 500 mmol·L^{-1}; 130.1 - 145 min, 5 mmol·L^{-1}. The eluent flow was 1 cm^3·min^{-1}. The temperature was kept constant at 30 ºC. The applied PAD potentials followed the manufacturer recommendations. The degree of polymerization (DP) was identified under the same chromatographic conditions using malto-oligosaccharides (Sigma Aldrich) as authentic standards (Pollock, 1982).

2.5 Starch granule properties

2.5.1 X-ray diffraction (XRD) analysis

The XRD measurement was performed in a XR diffractometer (D8 Discover, Bruker AXS). The diffraction pattern of the starch sample was collected with Cu Kα radiation at 40 kV and 40 mA. The sample was scanned at the diffraction angle (2θ) from 3° to 80° at a scanning rate of 0.01°. The XR diffractogram was postprocessed using peak the separation in the software PeakFit® Version 4.12. The XR diffractogram was used based on the original data with 2θ ranging from 3° to 80° (i), and alternatively with reduced data between 7° and 60° (ii), and 7° and 41.5° (iii), respectively.

Each diffractogram (i-iii) was smoothed and a baseline was set (area XR diffractogram), and the amorphous background (AB) was calculated subsequently. The starch's crystallinity [%] was calculated based on the following equation:

$$Crystallinity\ (\%) = 1 - \frac{area\ AB}{area\ XR\ diffractogram} * 100\% \quad \text{(Eq. 3)}$$

2.5.2 Thermal analysis by differential scanning calorimetry (DSC)

The thermal transition (gelatinization) properties were examined using differential scanning calorimetry (DSC; 214 Phoenix equipped with an intercooler, Netzsch, Selb, Germany) according to (Ulbrich et al., 2014) with modifications. Starch (5.5-6.0 mg, dry matter) was weighted in aluminum pans (Netzsch, Selb, Germany), suspended in water (about

20 µL water, ratio about 1:3.5), and the crucibles were hermetically sealed. The crucibles were stored at 20±2 °C for at least 2 h before measurement (equilibration). A pan filled with air was used as reference. The scanning rate was 10 $K \cdot min^{-1}$, and the cycle was performed as follows: the initial temperature was kept at 20 °C for 2 min (isotherm), ramped to 140 °C (heating), maintained at 140 °C for 2 min (isotherm), followed by cooling to 20 °C.

The obtained thermograms were evaluated (Netzsch Proteus thermal analysis-Version 7.1.0 software) in terms of gelatinization onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), gelatinization range (ΔT_{gel}; T_c-T_o), and swelling enthalpy (ΔH_{gel}). The DSC experiment were performed at least in quadruplicate, and the arithmetic mean as well as the corresponding standard deviation were calculated.

3. RESULTS

3.1 Starch extraction, minor components, and color

The extraction of starch from *Plinia cauliflora* (jaboticaba) seeds resulted in a fine, and slightly beige color powder. The yield was 22.65±0.63% (dry basis), a significant value considering that jaboticaba seeds are residues discharged during fruit processing, representing about 30% of the total fruit weight (Fidelis et al., 2021). Thus, the extraction of this unconventional starch is an excellent opportunity to increase the added value for the jaboticabas's industrial processing residue.

Although present in low concentration, compounds such as proteins, lipids and phenolics may influence the properties of the starch (Makroo et al., 2021). The starch from jabotica seeds seems to be highly pure, presenting low quantity of proteins (1.19% ± 0.11) as contaminant. Despite the low content of phenolics present either in aqueous (0.58 ± 0.02), ethanolic (0.27 ± 0.01) or ethereal extract (0.07 ± 0.02), the jaboticaba starch presented a slightly beige color, as indicated by the values of a* 1.92 ± 0.03, b* 10.82 ± 0.17 and L* 92.27 ± 0.24. Aside from their alteration in color, phenolic compounds in starches have been related to some functional properties, such as, emulsifying and antioxidant activity, which are highly desirable in some products formulations (Giuberti et al., 2020).

3.2 Starch granule morphology

3.2.1 Microscopy, granule size and particle size distribution

The morphology, size and surface properties of the starch granule are closely related to the starch functionalities (Makroo et al., 2021). The optical microscopy (LM, LM-pol) showed

a large variety of size (Figure 2A and B), which was confirmed by the SEM and LPA analysis (Figure 2C and D). The use of the LM-pol allowed observing the presence of the Maltese cross, a feature of the starch granules derived from its semi-crystalline structure (Figure 2B). Starch granules present a varied degree of birefringence, mainly as a function of their AP composition, since AP is the main responsible for the granule's crystallinity.

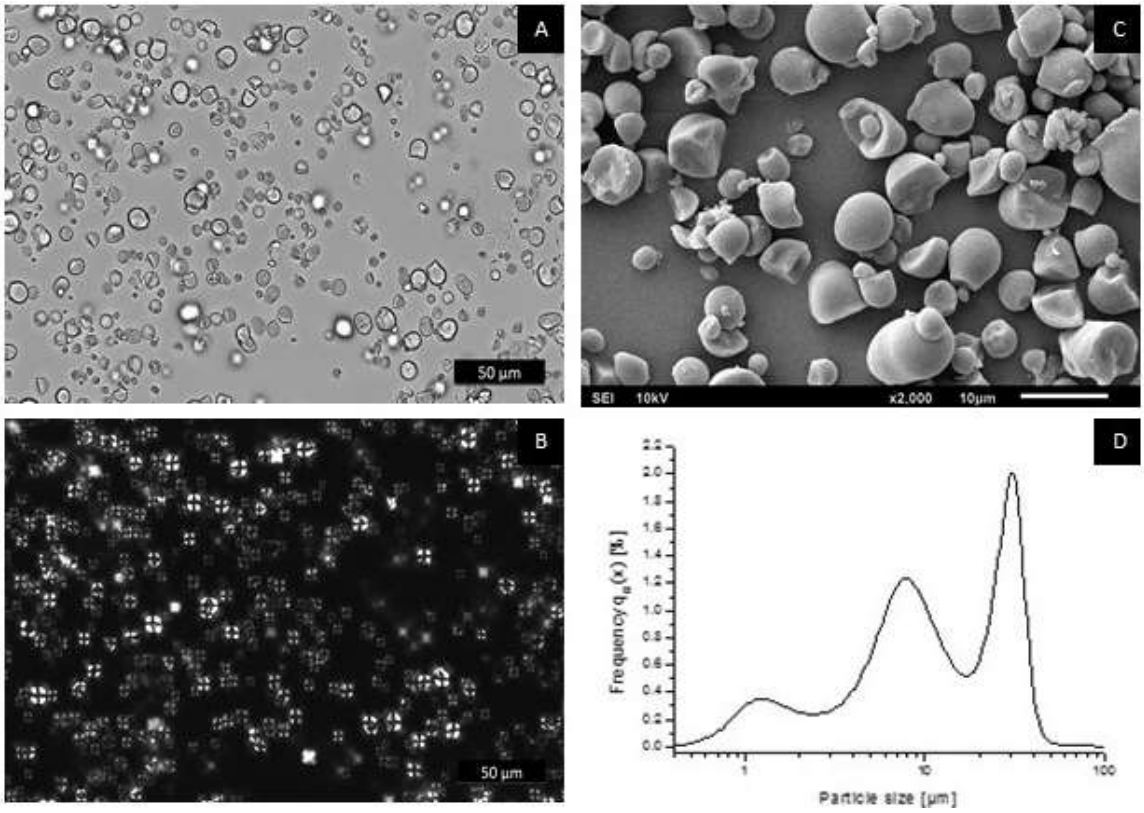


Figure 2. Micrographs from jaboticaba starch. A: Light microscopy (LM); B: Polarized light microscopy (LM-Pol); C: Scanning electron microscopy (SEM) and D: Laser particle analysis (LPA).

Starch granules from different botanical sources can show different shape, particle size (2-100 µm) and particle size distribution (unimodal, bimodal, or trimodal) (Junejo et al., 2022). Jaboticaba starch showed the presence of clean, smooth and irregular shaped granules (Figure 2C), resembling the starch extracted from *Solanun lycocarpum* fruit, which is composed by subunits joint together in the granule (Pascoal et al., 2013).

Two methods were used to determine the granule size. Based on LPA, the median of the granule size was 9.6 µm, whereas, based on the ImageJ software (LM images), it was 6.1 µm. Both results were compatible to a classification of small-granules (<10 µm), a characteristic expected for seed starches, since the higher the superficial area, the higher is the release of

glucose for attending the energy requirements of the plant. Small-granule starches are frequently used by industry for fat replacement in frozen desserts, as carrier material for flavors, as thickeners or stabilizers in cosmetics, for printing paper and plastic sheets (Irani et al., 2017). The versatility of the small granules enables high absorption of water, which is important when the starches are applied in products.

The jaboticaba starch showed a trimodal particle size distribution (Figure 2D), with three peaks at approximately 1 μm, 9 μm, and 30 μm, which can be explained by the granule morphology observed in the SEM micrograph (Figure 2C). Apparently, starch is composed of small spherical granules (smallest fraction) in the initial phase of granule formation, that are later organized into hemispherical subunits that fit together to form the final granule.

3.3 Molecular properties

3.3.1 Amylose content

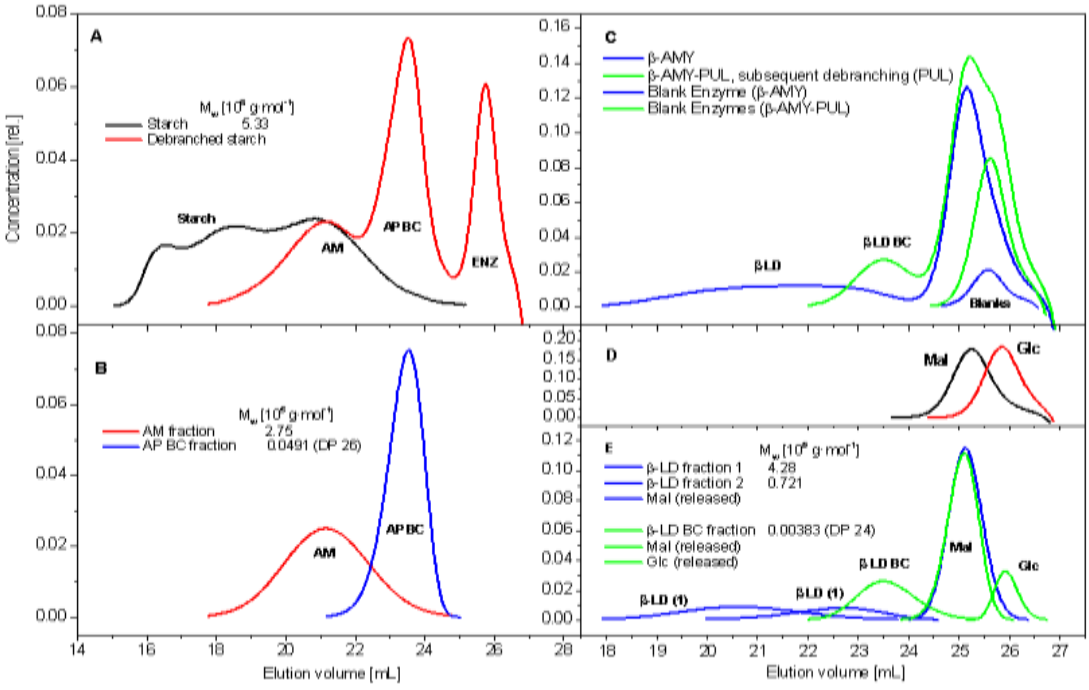
The AM content is an important factor that affects the chemical and technological properties of starch. Typically, the ratio of AM to AP is around 20-30:80-70% (w/w) in starches obtained from conventional sources such as potato, rice and corn (Tejavathi et al., 2020). Jaboticaba starch showed a high AM content (34.50±0.90%). High content of AM has been frequently related to resistant starches. Furthermore, a high content of AM may predispose starches to interact with other components in the food matrix, such as essential oils, fatty acids and flavoring molecules. Moreover, AM can act as an encapsulant that contributes to an increase of shelf life in food products (Zhao et al., 2018). Recently, starch with a high AM content was reported as preferred for film formation, due to its excellent mechanical resistance (Onyeaka et al., 2022). In this way, for applications as resistant starch, pharmaceutical materials (nanoparticles for delivery drugs) and biodegradable plastics, starches with high AM content is preferably used (Faisal et al., 2022).

3.3.2 Size exclusion chromatography (SEC)

Figure 3A shows the SEC-chromatogram of the starch (*Plinia cauliflora*) and after complete debranching using PUL. The SEC-chromatogram exhibits a unusual trimodal distribution, which probably indicates three different structures (branched and non-branched).

In contrast to normal starches (e.g. corn, wheat, potato) having M_w values of 20 to $40 \cdot 10^6$ g·mol^{-1} (Ulbrich et al., 2016; Ulbrich et al., 2015), a M_w of $5.3 \cdot 10^6$ g·mol^{-1} (SEC-MALS-DRI) was calculated, which is comparatively low. Similarly, low M_w values can be found in varieties of e.g. corn starches having significantly enhanced AM contents (high AM corn starch

686 genotypes with about 55-70 % w/w AM). This is tantamount to the lower AP content,
687 represented by the SEC-chromatogram area between 5 and 17 mL elution volume (1st peak;
688 Figure 3A). Regarding such high AM starches, the existence of an intermediate fraction (IM)
689 with a branched structure is commonly accepted (Ulbrich et al. 2022). Hence, the SEC-
690 chromatogram area between 17 and 20 mL could arise from such an IM (2nd peak; Figure 3A),
691 which is of branched nature. However, the 3rd peak between 20 and 22 mL represents mainly
692 the AM fraction of the starch (non-branched). The SEC-chromatogram of the debranched starch
693 underlines the assumption of fraction 3 (3rd peak of the non-debranched starch) consisting
694 mostly of AM, since it was found to be superposable to the high molar mass non-branched
695 fraction assigned to (debranched) AM. The cleavage of the α-1,6-glycosidic linkages effect a
696 good separation of the AM fraction from the comparatively low molar mass fraction of the AP
697 branch chains (AP BC), which were eluted distinct sequentially (AM: about 20-22 mL, AP BC:
698 23-24 mL, ENZ: about 26 mL; indicated in Figure 3A). Based on the separated chromatograms
699 (deconvolution) of the AM and the AP BC fraction, the M_w (SEC-MALS-DRI) and the DP_w
700 (SEC-cal-DRI), were calculated, respectively (Figure 3B). The M_w of the AM fraction of about
701 $2.75 \cdot 10^5$ g·mol^{-1} corresponds well with data reported for other starches or AM, respectively
702 (Radosta et al., 2001; Vorwerg et al., 2002), and the average chain length of the AP BC (DP_w)
703 was estimated to 26, which is a level typical for different kinds of starch (Hizukuri, 1986).
704



705 **Figure 3.** SEC-chromatograms of jaboticaba seed starch. A: starch and debranched starch
706 (PUL); B: chromatograms of the AM fraction and AP BC fraction, calculated via mathematical

peak separation (deconvolution) based on the debranched starch (A); C: starch digested by means of β-AMY and subsequently debranched (β-AMY-PUL); D: Maltose and Glucose, for reference; E: chromatograms of the β-LDs fractions (1 and 2), the β-LD BC fraction, and the Mal/Glc released, calculated via deconvolution based on the chromatograms in C (β-AMY and β-AMY-PUL).

Aiming detailed information about the starch's branched structure fractions (AP, IM), the starch was digested using β-AMY to produce β-LDs in a first step (release of Mal), which were debranched using PUL subsequently in a second step generating the β-LDs BC fraction. Figure 3C shows the SEC chromatograms after specific degradation using β-AMY (β-LDs), and advanced debranched using PUL (β-AMY-PUL; β-LDs BC fraction), as well as the corresponding ENZ blanks. Since the ENZ solutions contain low molar mass substances as stabilizers (e.g. sorbitol, glycerol, sucrose, Glc), this contribution to the chromatogram was considered by subtracting the blanks from the respective chromatograms (not shown). From the SEC-chromatogram of the starch digested with β-AMY a bimodal distribution of the β-LDs fraction was estimated (Figure 3C). After deconvolution, two different SEC-chromatograms reflecting accordingly two β-LDs fractions, and a 3rd fraction reflecting the Mal released, were calculated (Figure 3E). Two different fractions of β-LDs, having a M_w of $4.3 \cdot 10^5$ (high molar mass β-LD fraction) and $7.2 \cdot 10^4$ g·mol^{-1} (low molar mass β-LD fraction), respectively, could fortify the existence of two different fractions of branched polymer molecules. Debranching of the β-LDs resulted the β-LD BC fraction (DP_w about 24) and a low molar mass fraction consisting most likely of the Glc (Figure 3E). Since the relative portion of the Mal (β-AMY) didn't change remarkedly after debranching (β-AMY-PUL), the β-LDs were assumed to consist mostly of the β-LD BC fraction as well as Glc residues linked via α-1,6-glycosidic bonds. The differentiation between Mal and Glc is demonstrated by the separate measurements of the pure substances (Figure 3D; pullulan standard 342 and Glc).

Because the SEC-chromatogram area corresponds to the carbohydrate concentration, an estimation of the relative composition of the investigated starch was possible. About 60% of the starch sample was of branched nature (AP fraction, IM fraction), and about 40% were assigned to the AM fraction. Moreover, about 31-32% were found to be β-LDs (18.5% high molar mass β-LD fraction, about 12.6% low molar mass β-LD fraction), which suggests that nearly half of the branched structure fraction was prone to degradation to Mal (mostly A-chains). The comparable low molar mass of the β-LDs fractions indicate a low molar mass of

both, the AP as well as the assumed IM. Since the AM fraction of the starch contains branches (α-1,6-linkages) likewise, the complete digestion of the AM chains to Mal is unlikely.

3.3.3 Amylopectin Chain length distribution

In this study, malto-oligosaccharides with a DP of 3-6 were used for the determination of the retention times and for the identification of these compounds in the samples subjected to co-chromatography, which allowed the identification of the malto-oligosaccharide peaks (DP 3-6) in the debranched AP sample (Figure 4B). The maximum chain length observed for debranched AP was DP 82 (Figure 4A), a value close to that observed for potato (*Solanum tuberosum*), and for other plants from Cerrado bioma, such as the fruit of *Solanum lycocarpum* (DP 86) and the underground steam of *Trimezia juncifolia* (DP 81).

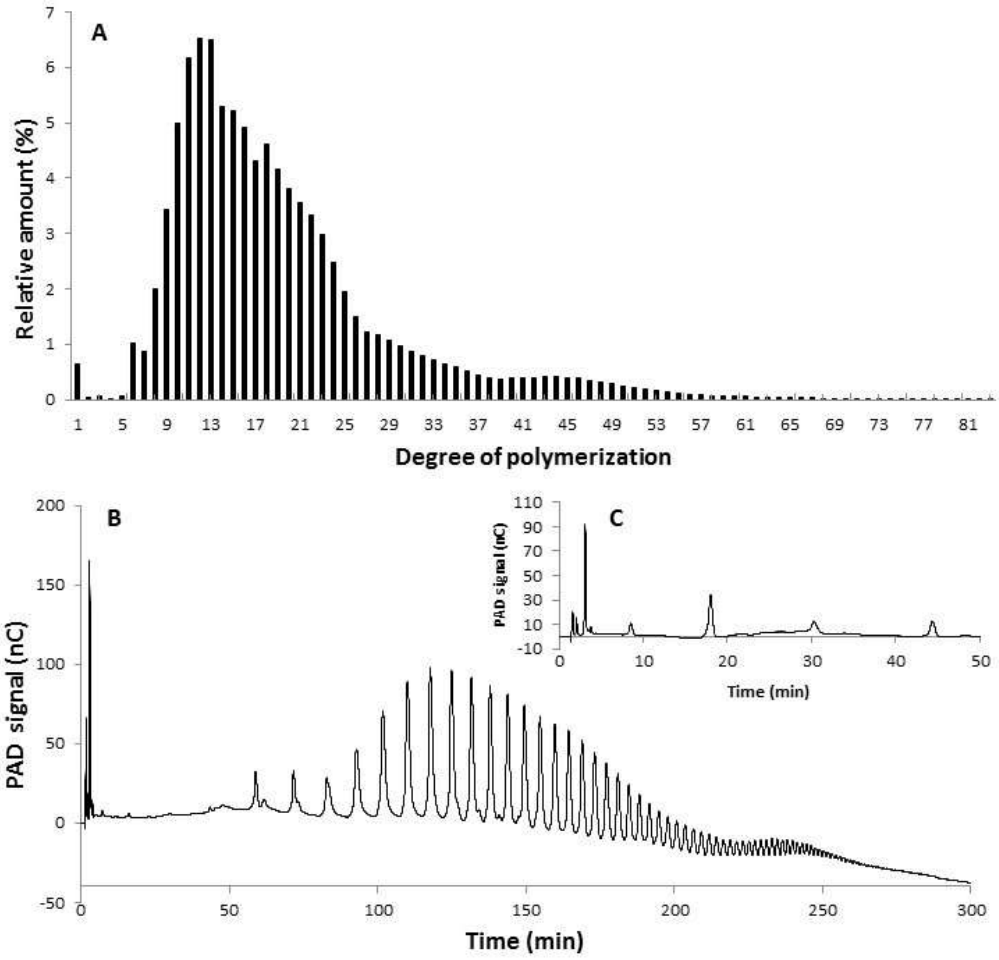


Figure 4. A) Chain length distribution of iso-AMY-debranched AP from *P. cauliflora* seed starch. B) HPAEC-PAD of iso-AMY-debranched AP from *Plinia cauliflora* starch. C) Samples were evaluated in co-chromatography with standard malto-oligosaccharides. Inserts represent the retention time of malto-oligosaccharides with DP ranging from 1 to 5.

The chain length distribution of the AP branches is a quite important feature influencing the physicochemical properties of the starch granules. According to the cluster model, AP branches can be categorized in short A-chains with DP 3-6 without branching points, and B-chains, that can be sub-categorized in B_1-chains (DP 13-24), B_2-chains (DP 25-36) and B_3-chains (DP >37) and C-chains which carry the reducing-end group but is otherwise similar to the B-chains (Bertoft, 2017; Hanashiro et al., 1996). As shown in Table 2, the AP from jaboticaba starch had a larger proportion of B_1-chains (51%), followed by A-chains (26%). According to (Shi et al., 2018) the ratio between the short (S) and long (L) chains are a good indicator of the AP branching. The higher the ratio S/L, the higher the branching degree of the amylopectin molecule. The S/L ratio of jaboticaba AP was 4.05, indicating a high branching degree, as demonstrated in the SEC chromatography.

Table 1. Crystal type and AP branch length distributions of *Plinia cauliflora* and other starches.

Source	Highest DP*	Crystal type	Relative area (%)				Reference
			DP 6-12 (A-chain)	DP 13-24 (B_1-chain)	DP 25-36 (B_2-chain)	DP ≥37 (B_3-chain)	
Plinia cauliflora	**82**	**C**	**26**	**51**	**12**	**7**	**This work**
Lotus seeds	75	C	18	45	19	18	Wang et al. (2021)
Ipomoea batatas	70	A	21	47	17	15	Hanashiro et al. (1996)
Solanum tuberosum	81	B	18	48	15	18	Hanashiro et al. (1996)
Trimezia juncifolia	81	C	10	55	12	12	Almeida et al. (2019)
Solanum lycocarpum	86	C	27	48	12	13	Almeida et al. (2020)

Additionally, the relative chain length distribution of starches has been used to predict the crystal type polymorph. The A-type polymorph of those starches has a high proportion of the A and B1-chains. The B-type polymorph is present in starches with lower amount of the A and B1-chains and higher proportions of the B2 and B3-chains (Hanashiro et al., 1996). As shown in Table 2, the AP from jaboticaba starch had a larger proportion of B_1-chains (51%), followed by A-chains (26%), therefore resembling a A-type polymorph. However, the presence

of a significative number of intermediary chain length (IM) branches of AP and the high AM content, as shown in the SEC chromatography, must be considered when examining the starch crystalline structure.

3.4 Starch granule properties

3.4.1 X-ray diffraction (XRD)

Much of the information about starch granule crystallinity can be acquired from X-ray diffraction, where starches are classified as A-, B-, C- and V-type. According to the data obtained (Figure 5A), the jaboticaba starch can be classified as Cc-type, which is a blend of A- and B- types. The peaks at 2θ = 15.11◦, 17.02◦, and 23.19◦ are characteristic of A-type pattern, whereas the peak at 2θ = 5.69◦ is characteristic of B-type pattern (He & Wei, 2017). In this way, due to the presence of the singlet at 2θ ≅ 17º and 23º, and a small peak at 5.6º, the C_C-type could be identified. Moreover, this is in agreement with the results from AP chain length distribution, due the a significative number of IM chain lengths, since normally, the A- type is assigned to those starches with high level of short chains, and the B-type is attributed to starches with higher amount of long chains (Lagunes-Delgado et al., 2022).

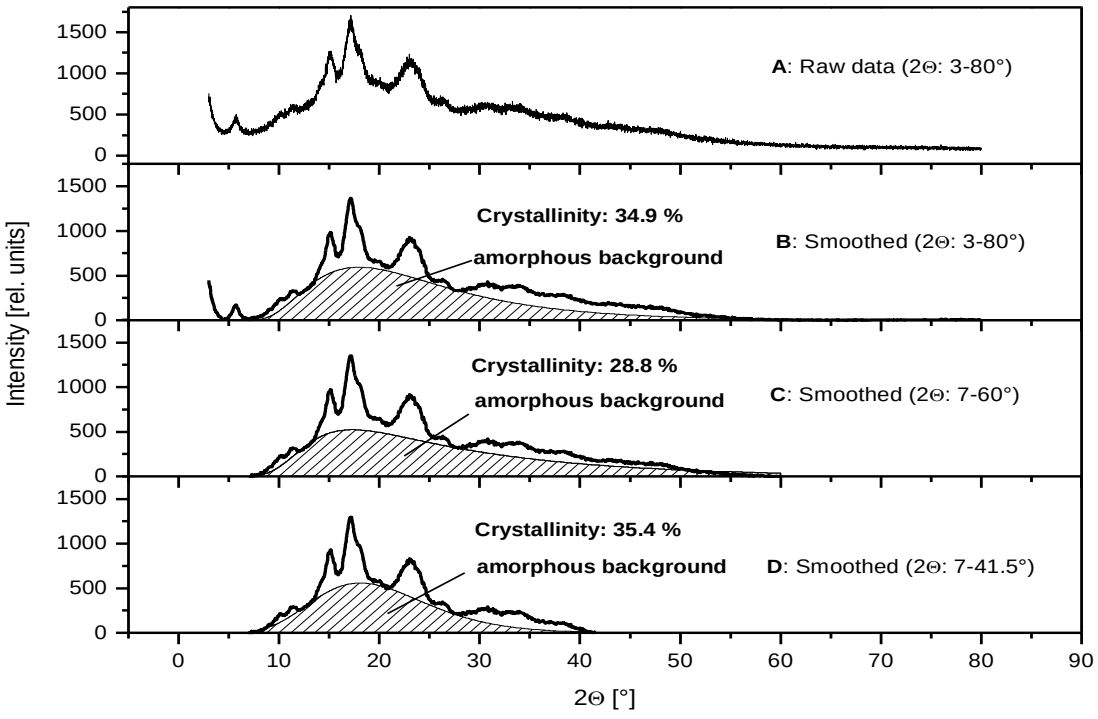


Figure 5. X-ray diffractogram of jaboticaba seed starch. A: original measurement (not processed), B-D: processed (baseline set and smoothed, indication of the amorphous

798 background); B: 2θ ranging from 3° to 80°; C: 2θ ranging from 7° to 60°; D: 2θ ranging from
799 7° to 41.5°.

800 Starch crystallinity can be attributed to well-ordered structures of AP within the
801 granules. The method of fitting a smoothed curve under the main minima of diffractograms can
802 be used to quantify the crystallinity of the starch, as shown in Figure 5 B-D. As jaboticaba
803 starch is new in the literature, three different 2θ ranges were used adjusting the y axis for the
804 calculation of the crystallinity. According to (Dome et al., 2020), the degree of crystallinity in
805 native starches is normally between 15 and 45%. In this study, the range variation was between
806 28.8 and 35.4% (Figure 5B-D), which is the expected range for an intermediate type of starch
807 (C-type).

808 **3.4.2 Thermal analysis by differential scanning calorimetry (DSC)**

809 The thermal characteristics of the jaboticaba starch were accessed by DSC. The
810 endothermic curve of jaboticaba starch showed the T_o at 66.4±0.46 °C, a T_p at 73.5±0.5 °C and
811 a T_c at 80.5±0.76 °C. The temperature range of the thermal transition (ΔT_{gel}) was 14.1±0.52 °C
812 and the ΔH_{gel} was 9.1±1.19 $J \cdot g^{-1}$.

813 Understanding the thermal properties of starches is important for defining their
814 potential applications, as these factors are directly correlated with the palatability, structure and
815 texture of starch-based products (Chakraborty et al., 2022). In general, T_o and T_p, and therefore
816 ΔT_{gel} are influenced by the molecular structure of the crystalline region and the percentage of
817 A-chain present in AP (Elmi Sharlina et al., 2017). The value observed for ΔT_{gel} (14.1±0.52
818 °C) is high, reflecting the presence of high number of short chains (A and B1) in the crystalline
819 regions. For comparison, the ΔT_{gel} for potato starch is 3.8 °C (Alvani et al., 2011). On the other
820 hand, the value of ΔH_{gel} is related to the AM fraction. Starches with a high AM content in the
821 amorphous region demand lower energy, consequently, lower gelatinization temperature to
822 melt. According to (Peng et al., 2022), there is a negative correlation between DSC results and
823 AM. The increase in AM content is related to a decrease of ΔH_{gel}, due to the enthalpy change
824 associated with the energy required to the dissociation of the double-helical crystalline
825 structures of the starches. Thus, it is likely that the high AM content of jaboticaba starch
826 explains the lower results of ΔH_{gel} compared to common starches, such as corn (13.04 $J \cdot g^{-1}$),
827 potato (15.92 $J \cdot g^{-1}$) and rice (11.63 $J \cdot g^{-1}$) (Wang & Wang, 2001).

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830

Conclusions

A novel starch with a small size (<10 μm), smooth and irregular granular shape, high AM content (34%) and classified as a C_C-type polymorph was obtained from jaboticaba seeds. The molecular characterization showed the presence of intermediary AP chain length and low M_w ($5.3 \cdot 10^6$ $g \cdot mol^{-1}$), while the thermal investigation suggested a low temperature for gelatinization (T_o = 66.4±0.46 °C) but a high temperature range (ΔT_{gel} =14.1±0.52 °C). The results indicate that this is a promising material for future studies and versatile applications for food industries, as well as pharmaceutical.

References

AACC (2000). Approved methods of the American Association of Cereal Chemistry, in 365 Method 61-032000, AACC: St. Paul.

Almeida, V. O., Batista, K. A., Di-Medeiros, M. C. B., Moraes, M. G., & Fernandes, K. F. (2019). Effect of drought stress on the morphological and physicochemical properties of starches from Trimezia juncifolia. *Carbohydrate Polymers*, *212*, 304–311. https://doi.org/10.1016/j.carbpol.2019.02.015

Almeida, V. O., Pascoal, A. M., Ferreira, M. C., Morais, R. R., Moraes, M. G., Batista, K. A., & Fernandes, K. F. (2020). Molecular structure of amylopectin/amylose from Solanum lycocarpum starch after enzymatic hydrolysis. *Food Hydrocolloids*, *100*, 105203. https://doi.org/10.1016/j.foodhyd.2019.105203

Alvani, K., Qi, X., Tester, R. F., & Snape, C. E. (2011). Physico-chemical properties of potato starches. *Food Chemistry*, *125*(3), 958–965. https://doi.org/10.1016/j.foodchem.2010.09.088

Benvenutti, L., Zielinski, A. A. F., & Ferreira, S. R. S. (2021). Jaboticaba (Myrtaceae cauliflora) fruit and its by-products: Alternative sources for new foods and functional components. *Trends in Food Science & Technology*, *112*, 118–136. https://doi.org/10.1016/j.tifs.2021.03.044

Bertoft, E. (2017). Understanding Starch Structure: Recent Progress. *Agronomy*, *7*(3), 56. https://doi.org/10.3390/agronomy7030056

Cai, C., Huang, J., Zhao, L., Liu, Q., Zhang, C., & Wei, C. (2014). Heterogeneous Structure and Spatial Distribution in Endosperm of High-Amylose Rice Starch Granules with Different Morphologies. *Journal of Agricultural and Food Chemistry*, *62*(41), 10143–10152. https://doi.org/10.1021/jf502341q

Chakraborty, I., N, P., Mal, S. S., Paul, U. C., Rahman, Md. H., & Mazumder, N. (2022). An Insight into the Gelatinization Properties Influencing the Modified Starches Used in Food Industry: A review. *Food and Bioprocess Technology*, *15*(6), 1195–1223. https://doi.org/10.1007/s11947-022-02761-z

Dome, K., Podgorbunskikh, E., Bychkov, A., & Lomovsky, O. (2020). Changes in the Crystallinity Degree of Starch Having Different Types of Crystal Structure after Mechanical Pretreatment. *Polymers*, *12*(3), 641. https://doi.org/10.3390/polym12030641

Elmi Sharlina, M. S., Yaacob, W. A., Lazim, A. M., Fazry, S., Lim, S. J., Abdullah, S., Noordin, A., & Kumaran, M. (2017). Physicochemical Properties of Starch from Dioscorea pyrifolia tubers. *Food Chemistry*, *220*, 225–232. https://doi.org/10.1016/j.foodchem.2016.09.196

Faisal, M., Kou, T., Zhong, Y., & Blennow, A. (2022). High Amylose-Based Bio Composites: Structures, Functions and Applications. *Polymers*, *14*(6), 1235. https://doi.org/10.3390/polym14061235

Fidelis, M., Santos, J. S., Escher, G. B., Rocha, R. S., Cruz, A. G., Cruz, T. M., Marques, M. B., Nunes, J. B., do Carmo, M. A. V., de Almeida, L. A., Kaneshima, T., Azevedo, L., & Granato, D. (2021). Polyphenols of jabuticaba [Myrciaria jaboticaba (Vell.) O.Berg] seeds incorporated in a yogurt model exert antioxidant activity and modulate gut microbiota of 1,2-dimethylhydrazine-induced colon cancer in rats. *Food Chemistry*, *334*, 127565. https://doi.org/10.1016/j.foodchem.2020.127565

Gabriel da Rosa, R., Sganzerla, W. G., Barroso, T. L. C. T., Buller, L. S., Berni, M. D., & Forster-Carneiro, T. (2022). Sustainable production of bioactive compounds from jabuticaba (Myrciaria cauliflora): A bibliometric analysis of scientific research over the last 21 years. *Sustainable Chemistry and Pharmacy*, *27*, 100656. https://doi.org/10.1016/j.scp.2022.100656

Giuberti, G., Rocchetti, G., & Lucini, L. (2020). Interactions between phenolic compounds, amylolytic enzymes and starch: An updated overview. *Current Opinion in Food Science*, *31*, 102–113. https://doi.org/10.1016/j.cofs.2020.04.003

Hanashiro, I., Abe, J., & Hizukuri, S. (1996). A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. *Carbohydrate Research*, *283*, 151–159. https://doi.org/10.1016/0008-6215(95)00408-4

He, W., & Wei, C. (2017). Progress in C-type starches from different plant sources. *Food Hydrocolloids*, *73*, 162–175. https://doi.org/10.1016/j.foodhyd.2017.07.003

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research*, *147*(2), 342–347. https://doi.org/10.1016/S0008-6215(00)90643-8

Irani, M., Abdel-Aal, E.-S. M., Razavi, S. M. A., Hucl, P., & Patterson, C. A. (2017). Thermal and Functional Properties of Hairless Canary Seed (Phalaris canariensis L.) Starch in Comparison with Wheat Starch. *Cereal Chemistry*, *94*(2), 341–348. https://doi.org/10.1094/CCHEM-04-16-0083-R

Junejo, S. A., Flanagan, B. M., Zhang, B., & Dhital, S. (2022). Starch structure and nutritional functionality – Past revelations and future prospects. *Carbohydrate Polymers*, *277*, 118837. https://doi.org/10.1016/j.carbpol.2021.118837

Lagunes-Delgado, C., Agama-Acevedo, E., Patiño-Rodríguez, O., Martinez, M. M., & Bello-Pérez, L. A. (2022). Recovery of mango starch from unripe mango juice. *LWT*, *153*(112514). https://doi.org/10.1016/j.lwt.2021.112514

Makroo, H. A., Naqash, S., Saxena, J., Sharma, S., Majid, D., & Dar, B. N. (2021). Recovery and characteristics of starches from unconventional sources and their potential applications: A review. *Applied Food Research*, *1*(1), 100001. https://doi.org/10.1016/j.afres.2021.100001

Miranda, B. M., Cruz, M. V., de Campos, I. T. N., Fernandes, K. F., & Silva, F. A. (2022). A Halochromic Film Containing *Plinia cauliflora* Peel Anthocyanins Loaded into a Cashew

912 Gum Polysaccharide-Polyvinyl Alcohol Matrix. *Waste and Biomass Valorization*.
913 https://doi.org/10.1007/s12649-022-01688-y

914 Miranda, B. M., Di-Medeiros, M. C. B., Batista, K. A., Carbonero, E. R., Fernandes, K. F., &
915 Silva, F. A. (2020). A galactose-rich heteropolysaccharide extracted from "jaboticaba" (*Plinia*
916 *cauliflora*) peels. *Carbohydrate Polymers*, *249*, 116821.
917 https://doi.org/10.1016/j.carbpol.2020.116821

918 Mua, J. P., & Jackson, D. S. (1998). Retrogradation and Gel Textural Attributes of Corn
919 Starch Amylose and Amylopectin Fractions. *Journal of Cereal Science*, *27*(2), 157–166.
920 https://doi.org/10.1006/jcrs.1997.0161

921 Onyeaka, H., Obileke, K., Makaka, G., & Nwokolo, N. (2022). Current Research and
922 Applications of Starch-Based Biodegradable Films for Food Packaging. *Polymers*, *14*(6),
923 1126. https://doi.org/10.3390/polym14061126

924 Pascoal, A. M., Di-Medeiros, M. C. B., Batista, K. A., Leles, M. I. G., Lião, L. M., &
925 Fernandes, K. F. (2013). Extraction and chemical characterization of starch from S.
926 lycocarpum fruits. *Carbohydrate Polymers*, *98*(2), 1304–1310.
927 https://doi.org/10.1016/j.carbpol.2013.08.009

928 Peng, M., Yin, L., Dong, J., Shen, R., & Zhu, Y. (2022). Physicochemical characteristics and
929 in vitro digestibility of starches from colored quinoa (Chenopodium quinoa) varieties. *Journal*
930 *of Food Science*, *87*(5), 2147–2158. https://doi.org/10.1111/1750-3841.16126

931 Podzimek, S. (2011). *Light Scattering, Size Exclusion Chromatography and Asymmetric Flow*
932 *Field Flow Fractionation: Powerful Tools for the Characterization of Polymers, Proteins and*
933 *Nanoparticles*. John Wiley & Sons.

934 Pollock, C. J. (1982). Oligosaccharide intermediates of fructan synthesis in Lolium
935 temulentum. *Phytochemistry*, *21*(10), 2461–2465. https://doi.org/10.1016/0031-
936 9422(82)85241-2

937 Radosta, S., Haberer, M., & Vorwerg, W. (2001). Molecular Characteristics of Amylose and
938 Starch in Dimethyl Sulfoxide. *Biomacromolecules*, *2*(3), 970–978.
939 https://doi.org/10.1021/bm0100662

940 Shi, K., Gu, X., Lu, W., & Lu, D. (2018). Effects of weak-light stress during grain filling on
941 the physicochemical properties of normal maize starch. *Carbohydrate Polymers*, *202*, 47–55.
942 https://doi.org/10.1016/j.carbpol.2018.08.114

943 Tako, M., & Hizukuri, S. (2000). Molecular origin for the thermal stability of Koshihikari rice
944 amylopectin. *Food Research International*, *33*(1), 35–40. https://doi.org/10.1016/S0963-
945 9969(00)00021-1

946 Tarique, J., Sapuan, S. M., Khalina, A., Sherwani, S. F. K., Yusuf, J., & Ilyas, R. A. (2021).
947 Recent developments in sustainable arrowroot (Maranta arundinacea Linn) starch
948 biopolymers, fibres, biopolymer composites and their potential industrial applications: A

review. *Journal of Materials Research and Technology*, *13*, 1191–1219. https://doi.org/10.1016/j.jmrt.2021.05.047

Tejavathi, D. H., Sujatha, B. S., & Karigar, C. S. (2020). Physicochemical properties of starch obtained from Curcuma karnatakensis—A new botanical source for high amylose content. *Heliyon*, *6*(1), e03169. https://doi.org/10.1016/j.heliyon.2020.e03169

Ulbrich, M., Bai, Y., & Flöter, E. (2020). The supporting effect of ultrasound on the acid hydrolysis of granular potato starch. *Carbohydrate Polymers*, *230*, 115633. https://doi.org/10.1016/j.carbpol.2019.115633

Ulbrich, M., Beresnewa-Seekamp, T., Walther, W., & Flöter, E. (2016). Acid-thinned corn starch—Impact of modification parameters on molecular characteristics and functional properties. *Starch - Stärke*, *68*(5–6), 399–409. https://doi.org/10.1002/star.201500210

Ulbrich, M., Daler, J. M., & Flöter, E. (2019). Acid hydrolysis of corn starch genotypes. I. Impact on morphological and molecular properties. *Carbohydrate Polymers*, *219*, 172–180. https://doi.org/10.1016/j.carbpol.2019.05.010

Ulbrich, M., Lampl, V., & Flöter, E. (2016). Impact of modification temperature on the properties of acid-thinned potato starch. *Starch - Stärke*, *68*(9–10), 885–899. https://doi.org/10.1002/star.201500365

Ulbrich, M., Natan, C., & Flöter, E. (2014). Acid modification of wheat, potato, and pea starch applying gentle conditions—Impacts on starch properties. *Starch - Stärke*, *66*(9–10), 903–913. https://doi.org/10.1002/star.201400089

Ulbrich, M., Scholz, F., & Flöter, E. (2022). High Amylose Corn Starch Gels – A Molecular Investigation of the Network Constituting Polymers. *Starch - Stärke*, *74*(5–6), 2200032. https://doi.org/10.1002/star.202200032

Ulbrich, M., Wiesner, I., & Flöter, E. (2015). Molecular characterization of acid-thinned wheat, potato and pea starches and correlation to gel properties. *Starch - Stärke*, *67*(5–6), 424–437. https://doi.org/10.1002/star.201400233

Vorwerg, W., Radosta, S., & Leibnitz, E. (2002). Study of a preparative-scale process for the production of amylose. *Carbohydrate Polymers*, *47*(2), 181–189. https://doi.org/10.1016/S0144-8617(01)00164-3

Wang, J., Jiang, X., Guo, Z., Zheng, B., & Zhang, Y. (2021). Long-term retrogradation behavior of lotus seed starch-chlorogenic acid mixtures after microwave treatment. *Food Hydrocolloids*, *121*, 106994. https://doi.org/10.1016/j.foodhyd.2021.106994

Wang, L., & Wang, Y.-J. (2001). Structures and Physicochemical Properties of Acid-Thinned Corn, Potato and Rice Starches. *Starch - Stärke*, *53*(11), 570–576. https://doi.org/10.1002/1521-379X(200111)53:11<570::AID-STAR570>3.0.CO;2-S

984 Waterhouse, A. L. (2002). Determination of Total Phenolics. *Current Protocols in Food*
985 *Analytical Chemistry*, *6*(1), I1.1.1-I1.1.8. https://doi.org/10.1002/0471142913.fai0101s06

986 Wyatt, P. J. (1993). Light scattering and the absolute characterization of macromolecules.
987 *Analytica Chimica Acta*, *272*(1), 1–40. https://doi.org/10.1016/0003-2670(93)80373-S

988 Zhao, X., Andersson, M., & Andersson, R. (2018). Resistant starch and other dietary fiber
989 components in tubers from a high-amylose potato. *Food Chemistry*, *251*, 58–63.
990 https://doi.org/10.1016/j.foodchem.2018.01.028

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CONSIDERAÇÕES FINAIS

Os resíduos de jaboticaba podem ser considerados excelentes fontes para obtenção de compostos bioativos, assim como polissacarídeos com perspectiva para aplicação industrial. A antocianina, um dos principais compostos bioativos da casca de jaboticaba, teve alto rendimento de extração e apresentou excelente funcionalidade para aplicação em filmes pH sensível, com possibilidade de elaboração de uma embalagem inteligente. O polissacarídeo, também extraído da casca de jaboticaba, pode ser considerado um polissacarídeo rico em galactose com comportamento péctico. Tal material mostrou excelente característica antioxidante, emulsificante e capaz de produzir um gel semelhante a pectina. Já o amido obtido da semente de jaboticaba, apresenta-se como uma alternativa para amidos não convencionais com propriedades interessantes do ponto de vista industrial. Desta forma, é notória a importância da utilização de resíduos agroindustriais para obtenção de novos produtos, principalmente com o foco em uma economia circular e mais sustentável.