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FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS

JOSEANE CARDOSO GOMES DE ALENCAR

OBTENÇÃO DE PECTINAS DE CASCAS DE UMBU (*Spondias tuberosa* L.): DESENVOLVIMENTO E OTIMIZAÇÃO DE PROCESSO DE EXTRAÇÃO ASSISTIDA POR ULTRASSOM DE ALTA INTENSIDADE

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Prof. Dr. Bruno Nicolau Paulino
Orientador

Profa. Drª. Alini Tinoco Fricks
Coorientador

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Dr. BRUNO NICOLAU PAULINO (ORIENTADOR)
Universidade Federal da Bahia (UFBA, BA)

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Universidade do Estado do Rio de Janeiro (UERJ, RJ)

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Drª. RAQUEL GUIDETTI VENDRUSCOLO (EXAMINADORA)
Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM, MG)

Dedico este trabalho,

À mãe que Deus me abençoou: Magnólia Alencar, à Camilo Valverde, à minha família e a todas as pessoas que fizeram parte dessa caminhada.

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RESUMO

Os resíduos e subprodutos da indústria de frutas representam uma fonte significativa de compostos valiosos com potencial para serem transformados em produtos de alto valor agregado. Entre esses compostos, as pectinas se destacam como biopolímeros de grande interesse industrial devido às suas propriedades gelificantes, espessantes e estabilizantes. Este estudo teve como objetivo investigar o potencial das cascas de umbu (*Spondias tuberosa* L.) como matéria-prima para a extração de pectinas utilizando tecnologia de ultrassom de alta intensidade e ácido orgânico. O foco principal foi desenvolver um método de extração ecologicamente correto que proporcionasse altos rendimentos e um grau de esterificação adequado para diferentes aplicações industriais. O Delineamento Composto Central (CCD) foi utilizado para otimizar o processo de extração de pectina, sendo três variáveis independentes (2^3) avaliadas: amplitude de ultrassom (%), razão sólido:líquido (SLR) e pH da solução de extração. Cinco níveis foram utilizados para cada variável, totalizando 19 experimentos. O rendimento de pectina e o grau de esterificação (DE) foram as respostas analisadas. A otimização do processo de extração de pectina das cascas de umbu através do CCD resultou em um alto rendimento de pectina próxima a 22% com um DE de 46%. As condições ótimas de extração foram: amplitude de ultrassom de 60%, SLR de 1:33 e pH 1,5. O processo de extração otimizado foi validado em escala laboratorial e o efeito de diferentes ácidos orgânicos (cítrico, oxálico, nítrico e clorídrico) no rendimento e DE da pectina foi avaliado. A validação do processo de extração em escala laboratorial confirmou a reprodutibilidade dos resultados obtidos na etapa de otimização. O uso de ácido cítrico resultou em um rendimento de pectina LMP (pectina de baixa esterificação) de cerca de 22%. Os ácidos oxálico, nítrico e clorídrico levaram à produção de pectinas HMP (pectinas de alta esterificação) com rendimentos em torno de 13%. As pectinas extraídas foram caracterizadas quanto à cor instrumental, utilizando um colorímetro, os resultados foram comparados com os de uma pectina cítrica comercial (CCP). As pectinas extraídas das cascas de umbu apresentaram diferenças significativas de cor em comparação com a pectina cítrica comercial, estas diferenças podem ser atribuídas à influência do tipo de matéria-prima e do método de extração na qualidade do produto final. Este estudo demonstra pela primeira vez a viabilidade da extração de pectinas de cascas de umbu utilizando tecnologia de ultrassom. As cascas de umbu se configuram como uma fonte promissora de pectinas LMP e HMP. A tecnologia de ultrassom provou ser um método verde eficiente para a obtenção de pectinas de qualidade alimentar com alto rendimento e qualidade. Este estudo contribuiu para o desenvolvimento de métodos de extração de pectinas mais eficientes e sustentáveis. A valorização das cascas de umbu como fonte de pectinas representa uma oportunidade para a indústria de alimentos e a caracterização das pectinas extraídas forneceu informações valiosas para o desenvolvimento de novos produtos e aplicações.

Palavras-chave: Tecnologia emergente. Polissacarídeos. Valorização de Subprodutos.

ABSTRACT

Fruit industry byproducts represent a significant source of valuable compounds with potential to be transformed into high-added value products. Among these compounds, pectins stand out as biopolymers of great industrial interest due to their gelling, thickening, and stabilizing properties. This study aimed to investigate the potential of umbu peels (*Spondias tuberosa* L.) as raw material for pectin extraction using high-intensity ultrasound technology and organic acid. The main focus was to develop an eco-friendly extraction method that would provide high yields and a degree of esterification suitable for different industrial applications. The Central Composite Design (CCD) was used to optimize the pectin extraction process, evaluating three independent variables (2^3): ultrasound amplitude (%), solid-to-liquid ratio (SLR), and pH of the extraction solution. Five levels were used for each variable, totaling 19 experiments. The pectin yield and degree of esterification (DE) were the responses analyzed. The optimization of the pectin extraction process from umbu peels using CCD resulted in a high pectin yield close to 22% with a DE of 46%. The optimum extraction conditions were: ultrasound amplitude of 60%, SLR of 1:33, and pH 1.5. The optimized extraction process was validated at a laboratory scale, and the effect of different organic acids (citric, oxalic, nitric, and hydrochloric) on the pectin yield and DE was evaluated. The validation of the extraction process at a laboratory scale confirmed the reproducibility of the results obtained in the optimization step. The use of citric acid resulted in a yield of LMP pectin (low esterification pectin) of around 22%. Oxalic, nitric, and hydrochloric acids led to the production of HMP pectins (high esterification pectins) with yields around 13%. The extracted pectins were characterized regarding instrumental color using a colorimeter; the results were compared to those of a commercial citrus pectin (CCP). The pectins extracted from the umbu peels showed significant color differences compared to the commercial citrus pectin. These differences can be attributed to the influence of the type of raw material and the extraction method on the quality of the final product. This study demonstrates for the first time the feasibility of extracting pectins from umbu peels using ultrasound technology. Umbu peels are a promising source of LMP and HMP pectins. Ultrasound technology has proven to be an efficient green method for obtaining food-grade pectins with high yield and quality. This study contributes to the development of more efficient and sustainable pectin extraction methods. The valorization of umbu peels as a source of pectins represents an opportunity for the food industry and for family farming. The characterization of the extracted pectins provides valuable information for the development of new products and applications.

Keywords*: Emerging Technologies. Polysaccharides. Byproduct Valorization.*

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Capítulo I

Umbu (*Spondias tuberosa* L.): importância econômica, social e potencial tecnológico

1 INTRODUÇÃO

O Brasil apresenta uma rica diversidade natural, abrigando seis biomas distintos. Entre eles, destaca-se a Caatinga, um bioma tropical semiárido exclusivo do território brasileiro. Essa região singular, que ocupa cerca de 11% do país, concentra-se principalmente no Nordeste, mas também se estende por áreas de outros estados. A Caatinga apresenta árvores baixas e arbustos, muitos dos quais com espinhos e folhas coriáceas, adaptações que lhes permitem enfrentar a aridez do clima. As comunidades tradicionais da Caatinga possuem um profundo conhecimento sobre o bioma e seus recursos, utilizando-os de forma sustentável (IBGE, 2019; Souza *et al*., 2024).

O umbuzeiro (*Spondias tuberosa* L.), espécie nativa da Caatinga e pertencente à família Anacardiaceae, destaca-se por seus frutos nutritivos, é uma planta que oferece múltiplas opções para seu aproveitamento. O umbu, quando maduro, possui um sabor cítrico e refrescante, perfeito para consumo in natura, sucos, doces, geleias, compotas, licores, vinagres e até mesmo cerveja. Esse fruto é rico em vitamina C, carotenoides, minerais e compostos bioativos com propriedades antioxidantes, anti-inflamatórias e antimicrobianas proporcionando diversos benefícios à saúde e é amplamente consumido na região Nordeste do Brasil. Dessa forma, além de seu valor nutricional, o umbu possui grande importância social e econômica para as populações do semiárido brasileiro, sendo uma planta que oferece múltiplas opções para seu aproveitamento (Anjos; Rybka, 2016; Dias *et al*., 2019; Galvão *et al*., 2011).

A pectina, um polissacarídeo natural abundante em frutas e vegetais, apresenta propriedades funcionais promissoras para diversas aplicações (Roy *et al*., 2023). Na indústria alimentícia, a pectina é utilizada como espessante, gelificante, emulsificante e estabilizador, contribuindo para a textura, sabor e estabilidade de produtos como geleias, doces, sorvetes e bebidas. Apesar de sua ampla utilização, a relação entre a estrutura molecular da pectina e suas propriedades funcionais ainda não é totalmente compreendida. Essa lacuna limita o desenvolvimento de novas aplicações para este polímero versátil. Pesquisas que explorem essa relação são essenciais para o desenvolvimento de novos produtos e aplicações da pectina em áreas como a indústria alimentícia, farmacêutica e cosmética (Roy *et al*., 2023; Kumar *et al*., 2023; Li *et al*., 2018; Wang *et al*., 2018).

Nas últimas décadas, diversos métodos de extração foram desenvolvidos para obter pectina de diferentes materiais vegetais. Entre os métodos mais utilizados estão a extração ácida, alcalina, por digestão enzimática, assistida por micro-ondas e ultrassom. É importante destacar que a escolha do método de extração impacta diretamente nas características da pectina obtida. A fonte vegetal e as condições experimentais durante o processo (temperatura, pH,

tempo de extração, entre outras) influenciam a estrutura e as propriedades da pectina final (Cui *et al*., 2021; Kumar *et al*., 2023; Roy *et al*., 2023).

Nesse contexto, diferentes métodos de extração geram pectinas com características estruturais distintas o que, por sua vez, impacta significativamente suas propriedades e funções. Através da seleção adequada do método de extração e do controle das condições experimentais, é possível obter pectinas com as características desejadas para aplicações específicas na indústria alimentícia, farmacêutica e cosmética (Cui *et al*., 2021; Picot-Allain *et al*., 2022).

A exploração de fontes diversificadas de pectina, aliada à pesquisa e desenvolvimento de métodos eficientes de extração, é fundamental para suprir a demanda crescente e garantir a sustentabilidade da produção. Essa diversificação abre caminho para a inovação na indústria alimentícia, farmacêutica e cosmética, impulsionando o desenvolvimento de novos produtos e aplicações para a pectina (Kumar *et al*., 2023; Wang *et al*., 2023).

Até o presente momento, não existem estudos realizados para investigar a potencialidade do fruto umbu, como fonte de polissacarídeos utilizando a tecnologia emergente ultrassom de alta intensidade, desenvolvimento e otimização de processo. E para abordar todos os aspectos essenciais teóricos e práticos, este trabalho foi estruturado e dividido nos seguintes capítulos:

Capítulo I: **Revisão bibliográfica:** Umbu (*Spondias tuberosa* L.): importância econômica, social e potencial tecnológico. Este capítulo teve como objetivo destacar o fruto nativo da Caatinga umbu como importante aliado para o desenvolvimento socioeconômico, além de destaca-lo como fonte de compostos com potencial tecnológico.

Capítulo II: **Artigo I:** Pectin and Pectic Oligosaccharides (POS): Recent advances for extraction, production, and its prebiotic potential. O artigo de revisão teve por objetivo apresentar as mais recentes tendências e avanços em relação às principais estratégias aplicadas à extração de pectina e produção de POS.

Capítulo II: **Artigo II:** Dual-objective optimization of ultrasound assisted organic acid extraction of pectin from umbu (*Spondias tuberosa* L.), a promising Brazilian native fruit from Caatinga biome. Este estudo teve como objetivo desenvolver e otimizar um processo ecologicamente correto para extração de pectinas das cascas deste fruto utilizando tecnologia de ultrassom e Ácido orgânico.

2 OBJETIVOS

- Desenvolver e otimizar processo de extração assistida por ultrassom de alta intensidade para obtenção de pectinas da casca do umbu (*Spondias tuberosa* L.).

2.2 Objetivos específicos

- Estabelecer o estado-da-arte sobre a extração de pectinas e obtenção de oligossacarídeos pécticos por métodos convencionais e por tecnologias emergentes através de revisão de escopo;
- Produzir, padronizar e caracterizar farinha da casca de umbu e resíduo insolúvel em álcool para extração de pectinas;
- Avaliar o efeito do tempo de sonicação no processo de extração de pectinas utilizando a tecnologia de ultrassom de alta intensidade;
- Desenvolver e otimizar processo de extração assistida por ultrassom para obtenção de pectinas através de Delineamento Composto Central Rotacional;
- Validar a otimização do processo de extração de pectinas;
- Avaliar a robustez do processo de extração utilizando ácidos inorgânicos (ácido nítrico e ácido clorídrico) e orgânicos (ácido cítrico e ácido oxálico);
- Caracterizar as pectinas obtidas em diferentes condições de extração através de ensaios físico-químicos, químicos e físicos;
- Compreender a influência dos parâmetros de extração sobre as características químicas e de qualidade das pectinas.

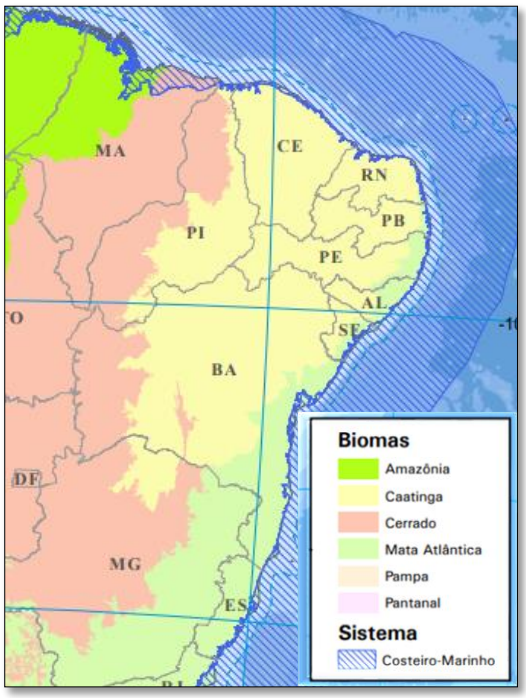
3 FUNDAMENTAÇÃO TEÓRICA

3.1 Bioma Caatinga

A Caatinga é um bioma dinâmico e heterogêneo exclusivamente brasileiro. No Nordeste, a Caatinga se faz presente em todos os estados, com exceção do Maranhão. No Ceará, por exemplo, o bioma cobre a totalidade do território, enquanto em outros estados, como Bahia e Pernambuco, ocupa áreas consideráveis. No estado de Minas Gerais, a Caatinga se manifesta em uma pequena porção no norte do estado. **(Figura 1)** (IBGE, 2019; Luna *et al*., 2022).

O bioma Caatinga, nome derivado do tupi-guarani que significa "mata branca", desponta como um bioma singular no cenário brasileiro. Apesar de sua localização em área semiárida, a Caatinga ostenta uma rica diversidade de paisagens, com flora e fauna que se adaptaram de forma admirável à escassez de água **(Figura 2).** Embora frequentemente subestimada, a Caatinga abriga uma surpreendente variedade de espécies endêmicas, ou seja, que não são encontradas em nenhum outro lugar do planeta. Essa rica biodiversidade se manifesta em diferentes tipos de vegetação, como as caatingas arbóreas, as caatingas arbustivas e os campos rupestres, cada um com suas características e belezas próprias (IBGE, 2019).

Figura 1. Mapa Bioma Caatinga



Fonte: IGBE, 2019**.**

Figura 2. Bioma Caatinga

Fonte: Autoria própria.

A Caatinga tem no umbuzeiro um importante aliado para o desenvolvimento socioeconômico da região. A época de colheita do umbu no varia de acordo com a região, mas

geralmente se concentra entre os meses de janeiro e abril e mobiliza milhares de famílias de agricultores. Novas formas de aproveitamento estão surgindo, como a fabricação de diversos produtos como cervejas, geleias, doces e entre outros, com o objetivo de ampliar a geração de renda para além da safra, impactando positivamente a vida das comunidades locais (Anjos; Rybka, 2016; Castro; Rybka, 2015). Dessa forma, a Caatinga, com sua rica biodiversidade e características únicas, representa um patrimônio natural de grande valor para o Brasil e nas últimas décadas é perceptível o crescente interesse na caracterização do potencial comercial e bioativo de matrizes vegetais oriundas desse bioma, incluindo plantas medicinais e frutas (Vieira *et al*., 2022; Luna *et al*., 2022).

3.2 Umbuzeiro e o fruto umbu (*Spondias Tuberosa* L.)

O umbuzeiro (*Spondias tuberosa*) é uma planta nativa do semiárido nordestino brasileiro e seus frutos conhecidos como umbu são muito valorizados e apreciados devido ao sabor agridoce e aroma característicos (Gouvêa *et al*., 2017). A árvore do umbuzeiro é uma espécie resistente a climas secos e é considerada um símbolo de resistência para o povo nordestino, tendo uma importância cultural considerável nos vários estados da região, em especial na Bahia **(Figura 3).** A fruta é colhida em grande quantidade, podendo ser comercializada in natura ou processada e pode ser empregado na produção de vários produtos. Esse fruto tem sido tradicionalmente utilizado para a produção de sucos, polpas congeladas, néctar, geleias, picolés, sorvetes, cervejas, entre outros (De Oliveira *et al*., 2021; De Oliveira *et al*., 2018; Vidigal *et al*., 2011; De Lima *et al*., 2018).

Figura 3. Umbuzeiro (A) flores e folhas (B)

Fonte: Autoria própria.

O umbuzeiro presenteia o bioma Caatinga com um fruto de características marcantes. O umbu, como é popularmente conhecido, possui diâmetro que varia entre 2 cm e 5 cm, massa entre 10 g e 20 g, e forma arredondada ou ovalada **(Figura 4**). Sua superfície lisa apresenta coloração verde a amarelada, enquanto a polpa madura se destaca pela textura mole, suculência e sabor doce. Apesar de seus benefícios à saúde, o umbu exige cuidados especiais após a colheita, devido à sua alta perecibilidade. A colheita no ponto ideal de maturação, o uso de embalagens adequadas e o armazenamento em temperaturas baixas são medidas essenciais para garantir a qualidade e a oferta do umbu no mercado consumidor (Freitas; Oliveira, 2021).

Figura 4. Umbu (A e B) e frutos em diferentes estágios de maturação (C)

Fonte: Autoria própria.

No Semiárido brasileiro, o umbu se destaca como um fruto de grande relevância cultural, social e econômica para os agricultores familiares. Apesar de suas diversas potencialidades, seu aproveitamento ainda é limitado pelo desconhecimento de seus benefícios e pela falta de tecnologias adequadas para o seu processamento. Em contrapartida, observa-se um crescente interesse por frutas pouco conhecidas e com alto teor de compostos bioativos, impulsionado pela demanda por produtos saudáveis e com sabores exóticos. Nesse contexto, o umbu surge como uma fruta promissora para pesquisas e desenvolvimento de produtos inovadores (Anjos; Rybka, 2016; Castro; Rybka, 2015). Nesse contexto, o umbu e configura como um importante ativo para a agricultura familiar, fortalecendo a identidade cultural e a autonomia das comunidades.

3.3 Aspectos nutricionais e potencial tecnológico do umbu

A composição bromatológica e físico-química do umbu pode variar de acordo com fatores como o estágio de maturação do fruto e as condições climáticas e geográficas de onde ele é cultivado (Narain *et al*., 1992). Alguns estudos descreveram a composição químico-bromatológica do umbu, tanto da casca quanto da polpa, e avaliaram suas propriedades nutricionais e funcionais (De Lima *et al*., 2018). Em termos de composição estudos anteriores reportaram 85-90% de umidade, 0.4% de proteínas, 0.9% de lipídeos, 8.0-15.0% de sólidos solúveis, pH entre 2.0-3.0, teor entre 10-40 mg/100g de vitamina C, fenóis totais variando de 10 a 90 mg GAE/100g e consideráveis teores de cálcio e fosforo foram reportadas, tornando esse fruto atrativo em relação a sua composição (Narain *et al*., 1992; Rufino *et al.*, 2010; Gondim *et al*., 2012).

Considerando que o aroma e sabor do umbu são bem característicos, Galvão *et al* (2011) investigaram a composição de compostos voláteis presentes na polpa desse fruto. Os autores reportaram um perfil de voláteis compostos por trinta e sete compostos orgânicos de baixo peso molecular. Os principais compostos voláteis que poderiam ser responsáveis pelo aroma característico da polpa do umbu maduro foram (Z)-ocimeno, metil pirazina, 2-butil-tiofeno, metil octanoato, 2-hexil furano, 2-octanol, (E) -2-ciclohexen-1-ona, 3-bromo-ciclohexeno, 1-heptanol, 2-nonanol e 1-octanol.

As amostras de polpa apresentaram maiores valores de ácido fenólico, enquanto as amostras de casca apresentaram maiores valores de taninos, o que condiz com os taninos serem geralmente mais expressivos nas cascas do que nas polpas de frutas. Os maiores teores de taninos condensados e hidrolisáveis foram determinados para as amostras de casca, semelhantes aos fenólicos extraíveis. As amostras de umbu maduro apresentaram maiores valores de taninos hidrolisáveis e menores de taninos condensados que as amostras semi-maduras. Além disso, foi reportado que as amostras obtidas da casca e polpa de umbu apresentaram maiores teores de fenólicos não extraíveis em comparação com frutas convencionalmente consumidas como maçã, pêssego, nectarina e quando comparados com subprodutos do pequi (Cangussu *et al*., 2021).

Diferentes estudos voltados à investigação das atividades biológicas do umbu foram reportados nos últimos anos, principalmente focados no estudo da atividade antioxidante, antiviral, anti-inflamatória e antimicrobiana. Outras atividades como a inibição da acetilcolinesterase e as atividades anticâncer também tem sido relatadas (Zeraik *et al*., 2016; Guedes *et al*., 2020). Esses trabalhos em sua maioria limitaram-se a estudar o potencial de uso

da polpa de umbu ou folhas do umbuzeiro e há poucos estudos sobre a aplicação das sementes e da casca, que podem ser considerados subprodutos/resíduos agroindustriais da cadeia produtiva dessa fruta e que permanecem sem aplicabilidade definida.

Entre os produtos elaborados com as partes comestíveis do umbu, a polpa congelada e a geleia são os principais comercializados na região nordeste. A polpa do umbu tem valor comercial e seus parâmetros físico-químicos de qualidade são estabelecidos na Instrução Normativa N°37, de 01 de outubro de 2018 que estabelece os parâmetros analíticos de suco e de polpa de frutas e a listagem das frutas e demais quesitos complementares aos padrões de identidade e qualidade já fixados pelo Ministério da Agricultura, Pecuária e Abastecimento através da IN MAPA n° 49 de 26 de setembro de 2018. Segundo essa normativa a polpa de umbu é produto definido no art. 19 do Decreto n ° 6.871, de 2009, obtido da parte comestível do umbu, através de processo tecnológico adequado. Em termos de composição a polpa de umbu deve apresentar como parâmetros mínimos: 8,5 de sólidos solúveis em °Brix, a 20°C, 9,0 de sólidos totais (g/100g), 2,4 de pH, 1,4 de acidez total expressa em ácido cítrico (g/100g), 2,4 de açúcares totais e 12,9 de ácido ascórbico (mg/100g).

Um estudo recente realizou a investigação da composição química e características físico-químicas da polpa e casca de umbu em diferentes estágios de maturação onde revela que durante o processamento dos frutos de umbu para a obtenção de polpa pode haver a separação da casca e posterior descarte dessa fração. Assim, a casca pode ser considerada um subproduto da cadeia produtiva de polpa de umbu e estudos que busquem investigar e atribuir finalidade para essa fração dos frutos de umbu são considerados de interesse comercial e econômico. Considerando a viscosidade inerente da polpa e o fato dessa juntamente com a casca serem utilizadas na fabricação de geleia de umbu, depreende-se que esse fruto contenha quantidade considerável de pectinas. Nesse sentido, escassos são os estudos que descreveram de forma consistente a extração e quantificação das pectinas da polpa e particularmente da casca de umbu. Segundo estudo de Cangussu *et al*. (2021) o teor de pectinas extraídas utilizando o método de extração assistida por micro-ondas foi de em torno de 9% para a polpa madura, 14,5% para a polpa semi-madura, enquanto para a casca madura e semi-madura os teores de pectina observados foram de 16,7 e 20,4%, respectivamente. Entretanto, os autores não trouxeram informações pormenorizadas acerca da caracterização e potencialidades das pectinas do umbu. Portanto, a casca de umbu pode ser considerada uma fonte promissora de pectinas, que podem ser destinadas tanto para o uso como aditivo espessante e gelificante em formulações alimentícias e cosméticas.

3.4 Importância econômica e social do umbu

No Nordeste brasileiro, o extrativismo vegetal se configura como uma das principais fontes de renda para os pequenos agricultores. Dentre as espécies exploradas, o umbuzeiro se destaca pela sua versatilidade e pelas diversas possibilidades de aproveitamento. Dessa forma, o umbuzeiro é caracterizado como uma planta multifacetada, com potencial para gerar benefícios socioeconômicos para a região Nordeste. As técnicas de processamento adequadas permitem a criação de diversos produtos, impulsionando a geração de renda e emprego, a inclusão social e a segurança alimentar. Além disso, o umbuzeiro contribui para a agregação de valor à produção local, impactando positivamente os indicadores socioeconômicos da região (Anjos; Rybka, 2016).

Com base em dados do Instituto Brasileiro de Geografia e Estatística (IBGE) de 2021 a produção de umbu na região nordeste foi de 12,8 mil toneladas com valor de produção estimado de 17,6 milhões de reais. Segundo esses dados, o estado da Bahia é o principal estado produtor de umbu, movimentando em torno de 8,4 milhões de reais. Os dados estão disponíveis no site do IBGE, em https://www.ibge.gov.br/explica/producao-agropecuaria/umbu/br. Portanto, esse levantamento demonstra a importância econômica do umbu para o estado da Bahia e de outros estados a região Nordeste, sendo desejável iniciativas em prol do fortalecimento da cadeia produtiva desse fruto. Nesse contexto, a exploração do umbu pode ser caracterizada como uma relevante fonte de renda para os produtores rurais do nordeste brasileiro. Portanto, pesquisas científicas voltadas à valorização do umbu como produto regional contribui para a preservação do meio ambiente, uma vez que incentiva a manutenção das áreas de plantio e a conservação da biodiversidade local (IGBE, 2021).

O umbu é muito perecível, o que limita a comercialização in natura para mercados consumidores distantes das áreas de produção. Por consequência, as pessoas envolvidas na coleta e nos meios tradicionais de vendas nas feiras livres e ruas de cidades da região, obtém rendimentos pouco expressivos (Castro; Rybka, 2015). A cadeia produtiva do umbu na Bahia antes estava limitada à pequenos produtores da agricultura familiar sendo encontrados principalmente em pequenas feiras urbanas. Atualmente, percebe-se o fortalecimento dessa cadeia produtiva com o aumento de cooperativas de pequenos produtores e estabelecimento de processos produtivos padronizados e organizados em diferentes regiões do estado da Bahia. Nesse contexto, a Cooperativa Agropecuária Familiar de Canudos, Uauá e Curaçá (COOPERCUC), nasceu da união de 44 pessoas, sendo 24 mulheres e 20 homens que desejavam organizar sua produção e comercialização de umbu. Segundo o site dessa

cooperativa (https://coopercuc.com.br/nossa-historia/) em 1986, vinte mulheres se reuniam para preparar, de forma artesanal, produtos do umbu. Posteriormente criou- se o grupo Unidos do Sertão, que agregava cerca de 30 comunidades, envolvendo mais de 100 pessoas. A produção do grupo era levada para comercializar nas feiras dos municípios. O município de Uauá recebeu a primeira barraca de venda dos produtos à base de umbu. O trabalho dessas famílias recebeu um aporte financeiro em 1999, com a aprovação do Programa de Convivência com o Semiárido (PROCUC). O recurso possibilitou ampliar o número de pessoas e comunidades envolvidas no trabalho de beneficiamento e comercialização **(Figura 5 e 6).**

Figura 5. Produtores da Agricultura familiar (A e B)

Fonte: https://coopercuc.com.br/, 2024.

Figura 6. Produtos produzidos pela cadeia produtiva da COOPERCUC
Cerveja Artesanal de umbu (A), umbuzada (B) e doce de umbu (C)

Fonte: https://coopercuc.com.br/, 2024.

Dessa forma percebe-se o impacto social e econômico do umbu para o Estado da Bahia, justificando assim a proposta de estudos que visem contribuir para a melhoria e valorização da cadeia produtiva deste fruto. Sob o ponto de vista científico, a investigação das propriedades nutricionais e funcionais do umbu têm sido as abordagens mais utilizadas (De

Lima *et al*., 2018). Já estudos voltados ao desenvolvimento de produtos, de forma padronizada, controlada e avaliando os aspectos químicos do fruto e dos produtos produzidos são escassos. Adicionalmente, estudos focados no aproveitamento de subprodutos da cadeia produtiva do umbu, como por exemplo o aproveitamento da casca não tem sido encontrado na forma de artigos científicos nas diferentes bases de dados.

3.5 Agroindústria e aproveitamento de resíduos e subprodutos

Em relação a produção agrícola, a fruticultura se consolida como um setor vital e figura entre um dos setores mais importantes com impactos socioeconômicos e ambientais significativos. O Brasil é o terceiro maior produtor de frutas do mundo com cerca de 40 milhões de toneladas ao ano. A maior parte desta produção é voltada para o mercado consumidor interno, sendo que em torno 2,5% é exportada. Nesse contexto, a região Nordeste se destaca, ocupando a segunda colocação entre as regiões produtoras de frutas do país com produção de aproximadamente 8 milhões de toneladas por ano, ficando atrás apenas da região sudeste que produz em torno de 20 milhões de toneladas por ano (Vidal, 2021).

O setor industrial de polpas de frutas que é responsável pela produção de bebidas não alcóolicas como sucos e néctares necessita de tecnologias de processamento que garantam produtos com qualidade nutricional e com parâmetros físico-químicos e microbiológicos adequados e que estejam alinhados às expectativas dos consumidores (Hernández-Hernández *et al*., 2019). Além disso, esse setor é responsável pela geração de grandes quantidades de resíduos e subprodutos, sendo o manejo adequado destes um desafio para a área de alimentos. Estudos de aproveitamento de resíduos e subprodutos agroindustriais está em constante crescimento e o desenvolvimento de processos para a produção de compostos de valor agregado a partir desses substratos é uma alternativa atraente e promissora (Kaur *et al*., 2023).

Entre os compostos extraídos a partir de subprodutos e resíduos agroindústrias oriundas da cadeia produtiva de frutas utilizando tecnologias emergentes, os polissacarídeos têm despertado o interesse devido ao seu valor agregado e diversidade de aplicações industriais (Cui *et al*., 2021). Nesse contexto, a aplicação de tecnologias emergentes no aproveitamento de resíduos e subprodutos agroindustriais para a geração de novos produtos ou compostos de interesse industrial é uma tendência, que nos últimos dez anos se estabeleceu de forma robusta e vem a cada dia agregando conceitos de sustentabilidade, redução de gasto energético, menor geração de resíduos, aproveitamento total de substratos, entre outros que estão alinhados com os princípios da Química Verde (Galanakis, 2012; Donn *et al*., 2022; Liu, 2023; Méndez-Carmona *et al*., 2022; Rocha *et al.*, 2022).

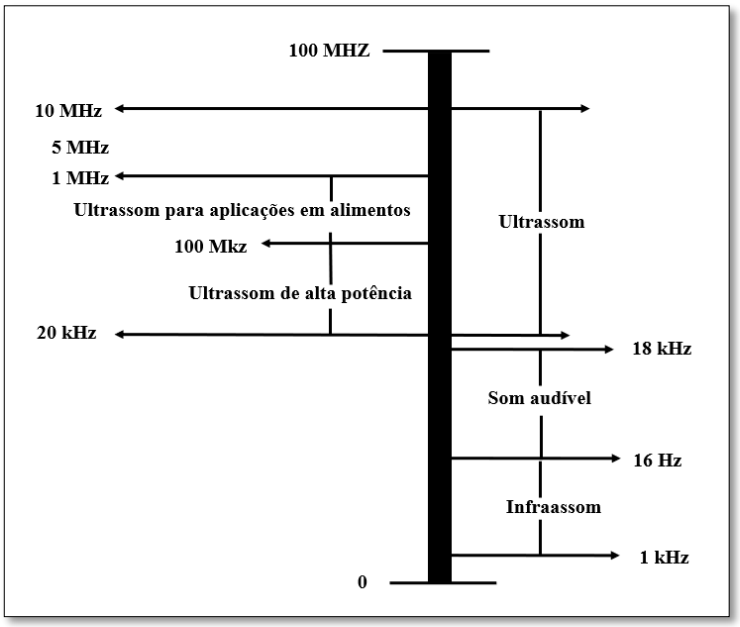
3.6 Tecnologia de Ultrassom pra obtenção de compostos

Entre as tecnologias emergentes, vários estudos têm demonstrado a viabilidade da aplicação da tecnologia de ultrassom de alta intensidade para a obtenção de compostos com potencial funcional e de interesse alimentar a partir de resíduos (Barrales *et al*., 2018; Oliveira *et al*., 2022). Essa tecnologia apresenta vantagens significativas quando comparado aos tratamentos térmicos convencionais incluindo menores custos de produção, preservação da qualidade nutricional dos alimentos, melhoria dos atributos sensoriais e das propriedades físicas do produto (Balthazar *et al*., 2019). Entretanto, vale destacar que os efeitos do processamento ultrassônico nos alimentos dependem da intensidade acústica aplicada e dos parâmetros de processamento, que devem ser calculados com precisão para alcançar o efeito desejado (Monteiro *et al*., 2020).

O ultrassom compreende ondas sonoras com uma faixa de frequência que varia entre 20 kHz a 10 MHz **(Figura 7).** Baseado nas suas aplicações e dependendo da frequência e quantidade de energia gerada pelo campo acústico são propostas subdivisões, sendo que o agrupamento em dois tipos é o mais aceito, a saber: a) ultrassom de baixas frequências (20 kHz–100 kHz), que têm sido propostas para o processamento de alimentos, e b) ultrassom de diagnóstico de baixa intensidade que é usado em medições físicas, principalmente para uso médico e diagnóstico, que é caracterizado por altas frequências (5 MHz–10 MHz) (Gallo *et al*., 2018; Guimarães *et al*., 2021). O ultrassom de baixa frequência e alta intensidade tem se mostrado promissor para aplicação em estudos de processamento de matrizes alimentícia, modificação estrutural de macronutrientes e em processos extrativos de compostos de valor agregado, incluindo pectinas.

Além disso, a divisão mais observada na literatura para essa tecnologia é a que categoriza em ultrassom de alta intensidade (*High-Intensity Ultrasound*, HIUS) que compreende frequências entre 16-100 kHz e intensidades maiores que 1 W/cm^2 e ultrassom de baixa intensidade (*Low-Intensity Ultrasound*, LIU) que trata dos processos com frequências maiores que 100 kHz e intensidades menores que 1 W/cm^2 (Guimarães *et al*., 2019). O HIUS Entretanto, vale destacar que os efeitos do processamento ultrassônico nos alimentos dependem da intensidade acústica aplicada e dos parâmetros de processamento, que devem ser calculados com precisão para alcançar o efeito desejado (Monteiro *et al*., 2020; Scudino *et al*., 2020).

Figura 7. Ultrassom: faixa de frequência das ondas sonoras



Fonte: Adaptado de Cheng e*t al*. (2015).

A cavitação gerada pelo ultrassom aumenta a permeabilidade da matriz vegetal, facilitando a extração da pectina e a ação das ondas ultrassônicas acelera a ruptura das células vegetais, liberando a pectina de forma mais rápida e eficiente. A técnica exige menos energia e solvente para alcançar um alto rendimento de pectina, tornando-a mais sustentável. A otimização desse processo leva à redução de custos de produção onde ácidos como o ácido cítrico, considerados ecologicamente corretos, podem ser utilizados na extração por ultrassom (Chemat *et al*., 2011; Lepilova *et al*., 2023).

4 CONSIDERAÇÕES FINAIS

O umbu é amplamente consumido na região Nordeste do Brasil, contribuindo para a segurança alimentar e a identidade cultural local. Além de seu valor nutricional, o umbuzeiro possui grande importância social e econômica para as populações do semiárido brasileiro, gerando renda e promovendo o desenvolvimento regional. No entanto, o potencial desse fruto ainda é subaproveitado, em parte pela falta de aplicação de tecnologias adequadas de processamento e conservação. A crescente consciência ambiental e as demandas dos consumidores por produtos sustentáveis e ecologicamente corretos impulsionam a busca por alternativas inovadoras na indústria alimentícia. Nesse contexto, o ultrassom surge como uma tecnologia promissora para a extração de pectina do umbu. Essa tecnologia oferece diversas vantagens em relação aos métodos tradicionais, como maior eficiência, menor tempo de processamento, menor consumo de energia e menor geração de resíduos. A valorização do umbuzeiro e do umbu, por meio da aplicação de tecnologias inovadoras como a extração de pectina por ultrassom, representa uma oportunidade para o desenvolvimento sustentável da Caatinga. Essa iniciativa pode gerar benefícios sociais, econômicos e ambientais para a região, além de contribuir para a diversificação da indústria alimentícia e a criação de produtos inovadores.

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Capítulo II

Manuscrito: Pectin and Pectic Oligosaccharides (POS): Recent advances for extraction, production, and its prebiotic potential

Pectin and Pectic Oligosaccharides (POS): Recent advances for extraction, production, and its prebiotic potential

Joseane Cardoso Gomes de Alencar[a], Klycia Fidelis Cerqueira e Silva[b], Miriam Dupas Hubinger[b], Carmen Lúcia de Oliveira Petkowicz[c], Bruno Nicolau Paulino[a]

[a]Faculty of Pharmacy, Federal University of Bahia, Campus Ondina, Salvador, Bahia, Brazil.
[b]School of Food Engineering, University of Campinas, UNICAMP, Campinas, São Paulo, Brazil.
[c]Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Paraná, Brazil

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***Corresponding author:** *Bruno Nicolau Paulino (*Department of Bromatological Analysis, Federal University of Bahia, Barão de 15 Jeremoabo Street, 147, 40.170-115 Salvador, Bahia, Brazil) *E-mail:* bruno.nicolau@ufba.br.

ABSTRACT

Background: Pectin is complex heteropolysaccharide widely used as food additive in industry, it is consumed as dietary fiber in human diet and play an important role in the maintenance of health. It has numerous industrial uses, mainly in the food sector and can be obtained from different plant sources through chemical, enzymatic and emerging methods. Furthermore, the controlled degradation of pectin can lead to the production of pectin oligosaccharides (POS), oligomers that have stood out due to their multifaceted profile of biological activities, mainly prebiotic effects. Scope and approach: In this review are highlighted the recent advances in pectin extraction and production of POS using conventional methods and emerging technologies. A critical approach based on the analysis and discussion of the most relevant studies for obtaining these substances, in addition to main approaches employed in the fractionation and purification are provided. Furthermore, the influence of chemical features in the biological activities, focusing on the prebiotic effects, are analyzed as well as and the effects of these molecules in gut microbiota and related microorganisms are discussed. Key findings and conclusions: The use of emerging technologies, mainly ultrasound, and microwaves, has established itself as an alternative for pectin extraction with advantages such as shorter extraction time and high yields, when compared to the conventional acid extraction method. In contrast, POS production is still driven mainly by enzymatic and chemical methods, with the use of emerging technologies being a growing path. A great effort has been made to understand the influence of the structural features of pectins and POS on the biological profile of these compounds. A solid set of information about the effects of prebiotics and microbiota modulators is found in the literature, but the consolidation of pectins and POS as prebiotics depends on robust studies in in vivo models, notably in humans, so that these effects observed in vitro are properly proven and these compounds are established on the market as an alternative to classic prebiotics.

Keywords: Pectin; pectic oligosaccharides; prebiotics; emerging technologies; pectin extraction; POS production.

1 INTRODUCTION

According to International Scientific Association for Probiotics and Prebiotics (ISAPP), the term prebiotics refer to substrates that are selectively utilized by host microorganisms conferring health benefits (Gibson et al., 2017). The most studied and widely accepted prebiotics are those of carbohydrate nature, including fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), human milk oligosaccharides (HMO), inulin and lactulose, which are found in different food matrices such as plants and human milk, applied as food supplements/nutraceuticals, and are produced from carbohydrate-based substrates through chemical, enzymatic, and microbial processes (Mano et al., 2018; Wang et al., 2020; Bujna et al., 2022; Awasthi et al., 2022).

Different authors have proposed expanding this concept to include other non carbohydrate compounds, applications to body sites other than the gastrointestinal tract, and diverse categories other than food (Cunningham et al., 2021). This conceptual expansion has been envisioned by ISAPP as the future of the prebiotics field, fostering the quest for new prebiotics substances while encouraging further research to consolidate scientific evidence regarding the health benefits of emerging prebiotic as xylo-oligosaccharides (XOS), pectic-oligosaccharides (POS), malto-oligosaccharides (MOS), soya-oligossacharides (SOS), gentio-oligosaccharides (GnOS), polydextrose (PDX) and pectin (Wang et al., 2020; Devi et al., 2023; Sun et al., 2023). Currently, GOS, FOS, and inulin are the most widely known and consumed prebiotic substances in the global prebiotic market. According to Grand View Research Inc., the global prebiotics market size was valued at USD 6.05 billion in 2021 and with an expected 14.9% CAGR from 2022 to 2030. In terms of applications, the food and beverage industry represent more than 82% as the largest share of the prebiotic market, followed by animal feed and dietary supplements segments (Grand view Research., 2020).

In recent years, there has been a notable surge in research focusing on the prebiotic potential of pectins and oligosaccharides derived from their hydrolysis, named as pectic oligosaccharides (POS) (Calvete-Torre et al., 2022; Foti et al., 2022). Given that pectin is a readily available polysaccharide found in numerous cost-effective plant substrates, such as agricultural wastes and by-products, there is a growing interest in broadening its applications beyond its conventional uses as a gelling agent and thickener in the food industry and cosmetics (Kumar et al., 2023; Reichembach & Petkowicz, 2021). Hence, pectic substances have considered as potential substrates in biotechnological processes, which may or may not be associated with the utilization of emerging technologies for prebiotic production (Gonçalves et al., 2023).

Therefore, this review article aims to delve into the most recent trends and advances regarding the main strategies applied to extraction of pectin and POS production. Initially, fundamental concepts about the chemistry and sources of pectin will be presented, followed by sections dedicated to exploring and discussing the novel technologies used to extraction of this polysaccharide and to produce pectic prebiotics, with a particular emphasis on POS production. Additionally, the article will present the main approaches for separation or purification of these prebiotic substances, as well as the recent findings concerning the functional properties of pectin and POS, with a focus on the effects related to modulation of gut microbiota and prebiotic potential.

2 CHEMISTRY AND BIOCHEMISTRY OF PECTINS

2.1 Definition and structure

Plant cells are surrounded by an extracellular matrix referred as cell wall. It is primarily made up of polysaccharides which are grouped into three classes, cellulose, hemicellulose, and pectin. Thus, pectins are found in all land plants in amounts that can range from 2% to 35% of the dry mass of the cell wall (Mohnen, 2008). In plants, pectins are involved in cell adhesion, morphogenesis, defense against pathogen and mechanical properties of tissues (Mohnen, 2008; Lin *et* al, 2022; Haas et al., 2020). Being a component of fruits and vegetables, pectins are also present in our daily diet as dietary fibers.

The term pectin describes a group of acidic polysaccharides, namely homogalacturonana (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II) and xylogalacturonan (XG), which are believed to be interlinked in the plant cell wall. They share the presence of α-(1→4)-linked piranosidic galacturonic acid (GalA) units which is responsible for the acidic nature of the pectins. Apiogalacturonan, also a pectin, has been found only in some aquatic plants, being abundant in duckweed (Sowinski et al., 2019). Moreover, the structural description of pectin reveals a composition featuring alternating “smooth” and “hairy” regions throughout the molecule (Roman-Benn et al., 2023). The smooth regions consist of homogalacturonans (HG), which create the linear backbone of the molecule. In contrast, the hairy regions are composed of rhamnogalacturonans (RG-I and RG-II), with RG-I being the primary branched structure within the pectin molecule (**Figure 1**).

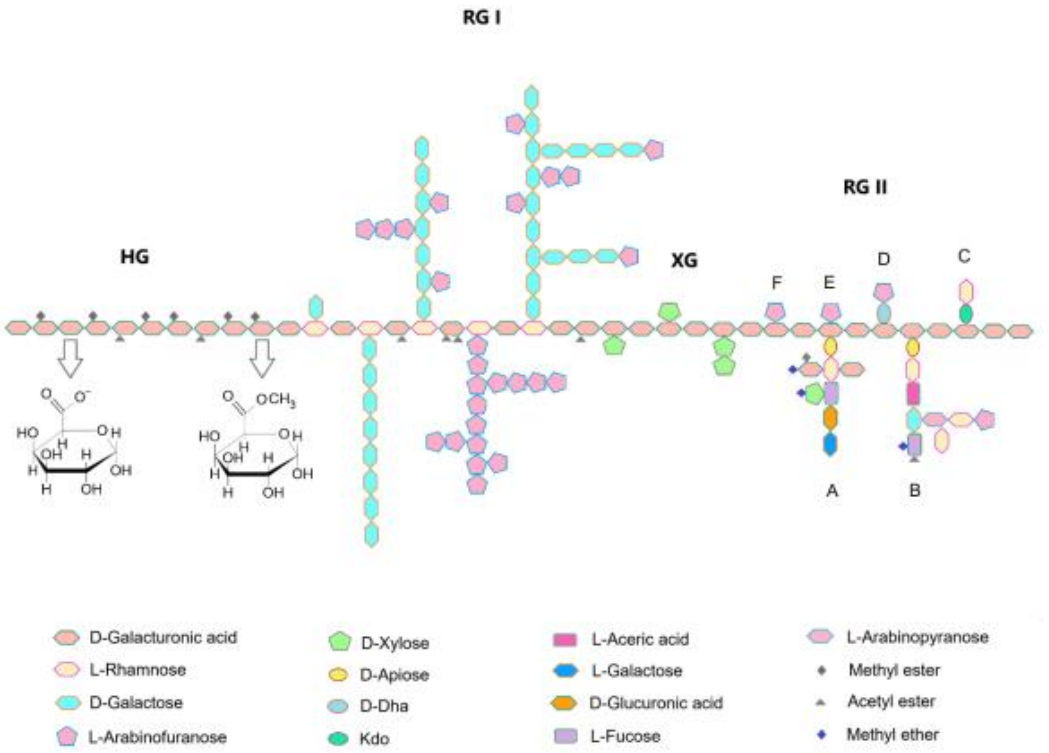

Figure 1. Schematic representation of pectin structure.

Although the relative amount and fine structure of each class of pectin depends on the botanical origin, plant tissue and developmental stage, the HG has been identified as the most abundant one. The HG is a homopolymer of α-(1→4)-linked D-galacturonic acid residues. The GalA units are partially methyl-esterified at C-6 carboxyl and may also be acetylated at O-2 and/or O-3 (Atmodjo et al, 2013; Mohnen, 2008). The proportion of methyl-esterified carboxyl groups in relation to the total carboxylic groups is referred as the degree of methyl-esterification (DM) or sometimes degree of esterification (DE).

RG I is the second most abundant pectin. The factors related to the plant source and extraction techniques that affect the yield and composition of RG I was recently reviewed by Kaczmarska *et al* (2023). The RG I has a main chain of repeating units of →4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→, where typically 20-80% of the rhamnosyl (Rha) units are substituted at O-4 with linear or branched oligo or polysaccharides (Voragen et al., 2009; Kaczmarska et al., 2022). No methyl-esterification has been identified in RG I. In the pectin from leaves of birch, the HMBC and ROESY NMR analyses confirmed that methyl-esterified GalA units were only present in HG and not in RG-I (Golovchenko et al., 2022). However, the GalA residues of RG I can be acetylated at O-2 and/or O-3 (Shahin et al., 2023).

The side chains of RG I are mainly arabinans, galactans and arabinogalactans. However, monosaccharides such as, fucose, glucuronic acid and 4-O-methyl-glucuronic acid, have also

been found in minor amounts in some side chains. Side chains of single Gal units have also been found. Around 40 different structures have been identified as RG I side chains. It is the most structurally diverse pectin (Albersheim *et al*., 2011; Atmodjo, Hao & Mohnen, 2013; Mohnen, 2008; Shahin et al., 2023).

Arabinans and galactans are branched and linear homopolysaccharides, respectively. Arabinans have a main chain of furanosidic units of L-arabinose (Ara) α-(1→5) linked to which arabinosyl residues, arabinobiose or short arabinan chains are attached at O-2 or O-3. On the other hand, galactans are linear chains of β-(1→4)-linked piranosidic D-galactose (Gal) units. Arabinogalactans are found in two structurally different types: type arabinogalactan (AG I) and type II arabinogalactan (AG II). AG I has a backbone of β-2 (1→4)-linked galactan substituted at O-3 by single L-Araf units and/or short α-(1→5)-arabinan chains. In AG II, a β-(1→3)-D-galactan backbone is attached to short β-(1→6)-galactan chains which carry additional branches of short arabinan chains or L-Ara units α-(1→3) and/or α-(1→6) linked.

RG II is recognized as the most complex and structurally conserved component of pectin, but it represents a very minor part of pectin. Despite the name "rhamnogalacturonan", the RG II is a substituted homogalacturonan. Thus, the main chain is a galacturonan where some GalA units are methyl-esterified (O'Neil et al., 2020). It has only 9-11 GalA to which five different side chains (A, B, C, D and E/F) are attached at O-2 or O-3. These side chains contain 11 different monosaccharides including some rare sugars, such as: the ketoses Kdo and Dha; the branched-chain monosaccharide apiose (Api); and the only branched acid deoxy monosaccharide identified in nature, aceric acid (AceA). Modification of monosaccharides by methyl-esterification, methyl-etherification and acetylation is also observed (Albersheim et al., 2011). The side chain A is an octasaccharide, side chain B has 6-9 monomers and side-chains C and D are disaccharides. Side chains A and B are linked at O-2 to the galacturonan backbone and side chains C and D are O-3 linked. The side chain E/F consist of a single α-L-Araf residue also attached at O-3. The identification depends on the GalA from main chain has another side chain at O-2 or not (Ropartz and Ralet, 2020; Shahin et al., 2023). RG II exist as dimers where two RG-II molecules are crosslinked by a borate diester covalente bond between the C-2 and C-3 of one apiosyl residue of each side chain A (O'Neill et al., 2020). Like RG II, XGA account for only a small proportion of pectin.

XGA is also a substituted galacturonan, in which single pyranosidic β-D-xylose units or disaccharides are attached to O-3 of some GalA. For the XGA from pea pectin, the proportion of substitution was found to be 63 % (Noguchi et al., 2020).

2.2 Pectin types

Depending on the DM, pectins are classified as high-methoxyl (HM pectin; DM 50% or higher) or low-methoxyl (LM pectin; DM lower than 50%). Thus, the DM defines the pectin net charge. The DM as well as the pattern of distribution of unesterified and methyl-esterified residues along the chain (random or in blocks) deeply impact the pectin properties and functionalities (Einhorn-Stoll *et al*., 2021). One of the most important aspects that is determined by the DM is the gelling mechanism of pectin. LM pectins are able to form gel in the presence of divalent cations, usually Ca^{2+}. On the other hand, HM pectins require high concentration of soluble solids, usually sucrose, and low pH (Funami and Nakauma, 2023). Commercial HM pectins are often labelled as rapid set or slow set according to the time for gelation at a specified temperature. Rapid set pectins have higher DM and gel faster at a higher temperature than slow set pectins (Brejnholt, 2009).

The carboxyl groups of GalA from HG can also be partly amidated, resulting in a third type of pectin, the amidated pectin, which is not a natural component of pectins. The amidated pectin is obtained by treatment with ammonia that convert some methyl esterified carboxylate groups into amides (Feng *et al*., 2023).

2.3 Conventional and unconventional sources of pectin

Pectin is used a safe food additive (INS N°. 440/E440) with gelling, thickening and stabilizing properties. For this purpose, a minimum 65% GalA calculated on the ash-free and dried basis is legally required. Thus, food grade pectins are predominantly homogalacturonans. The main industrial producers of pectins are Cargill, CP Kelco, Herbstreith & Fox, DSM Andre Pectin, CEAMSA, IFF and JRS Silvateam (IPPA, 2023). Most of commercially available pectin is extracted from apple pomace and citrus peel (from lime, lemon, and orange), wastes from juice production (Morris and Binhamad, 2020). Apple pomace is composed of peel, pulp, and seeds and accounts for about 25% of the processed apple (Zacharof, 2017). Apple pomace contains approximately 10-15% of pectin on a dry basis (Vidović et al., 2020). Citrus peel represents between 50% to 70% (w/w) of processed fruits, depending on the technology of processing and fruit type (Zema et al., 2018). The pectin content of citrus peel is 20-30% (Ruano et al., 2020). The consumption of pectin has steadily growing in the last years due to the increasing demand for low-calorie and safe food products and the broad range of new applications recognized for pectins (Ciriminna et al., 2022). Pectin is the most consumed non-caloric hydrocolloid from plant origin and third revenue in the food hydrocolloids market, only behind gelatin and starches (Seisun and Zalesny, 2021), motivating the search for unconventional sources. Intense research

has been done to identify novel raw material for pectin production. Different plant wastes have been investigated, such as, jackfruit waste (Begum et al., 2021), dragon fruit peels (Costa *et al*., 2022), stalk from sun-dried figs (Çavdaroğlu & Yemenicioğlu, 2022), almond hulls (Najari et al., 2022), onion skin (Benito-Román et al., 2022), lavender industrial by-products (Marovska et al., 2022), mango peels (Karim et al., 2022; Thakare et al., 2023). Information on other agroindustrialwastes that have been investigated for pectin extraction as well as the extraction conditions, yields and composition of the polysaccharides can be found in recent reviews (Gerschenson et al., 2021; Reichembach and Petkowicz, 2021; Sabater et al., 2022; Sarangi et al., 2023; Kumar et al., 2023; Ling et al., 2023).

Despite the large number of studies that propose new sources of pectins, many raw material provide pectic polysaccharides, not commercial pectins, as they contain less than 65% GalA. In addition, other structural features may be a constraint to the industrial application of pectin, such as those that prevent gelation. Low molar mass, high contente of neutral monosaccharides and high acetyl content are associated with poor gelling properties. Sugar beet pectin is well known for its high content of neutral sugars and acetyl groups which result in poor gelling ability but on the other hand is associated with interesting emulsifying and stabilizing effects (Archut et al., 2023; Chen et al., 2018).

Recently, a recent study described the chemical characteristics in terms of galacturonic acid (GalA), degree of methyl-esterification (DE) and weight-average molecular mass (Mw) of pectic polysaccharides extracted from different conventional and Unconventional plant sources (Zhao *et al*., 2024). The results indicated that the GalA content was highest in pectins extracted from apple peels (73.1%) and hawthorn (72.9%), followed by apple pulp (69.7%) and peaches (63.8%), while the lowest concentration was observed in pectins from tomato (58.7%), broccoli (57.9%), strawberry (56.9%), carrot (55.9%) and pumpkin (54.8%), respectively. In terms of DE values, four pectins exhibited DE values greater than 70%, including hawthorn (80.3%), followed by broccoli (75.4%), pumpkin (73.6%), and carrot (73.5%). Meanwhile, five presented DE values less than 64%, such as apple pulp (63.4%), peach (59.5%), apple peel (56.8%), strawberry (51.8%), and tomato (50.2%). These results show that some non-conventional sources may contain pectins with characteristics like or even better than commercial citrus pectin, demonstrating the importance of an adequate chemical characterization of the extracted substances.

In **table 1** are showed the chemical, physical and technological properties of pectins extracted from conventional and unconventional sources as well as information about the extraction method employed and yield achieved.

272 **Table 1.** Unconventional vegetal species used as substrates for pectin extraction and its general characteristics

Unconventional substrates	Pectin content	Extraction method	Chemical features	Properties and applications	Reference
Elephant Apple and Pomelo peel	Pomelo IP Yield (10.84±0.19%) Elephant Apple (6.37±0.10%) Pomelo OP Yield (2.97±0.11%).	Acid hydrolysis and sodium-hexa-meta-phosphate extraction	Moisture (%) 6.98±0.08 to 9.04±0.07. Ash (%) 3.07±0.06 to 7.12±0.04. Equivalent weight 785.3±5.13 to 987.1±5.57. Methoxyl content (%) 5.02±0.04 to 7.47±0.02 %. Anhydrouronic acid (%) 52.89±0.33 to 57.89±0.22 %. Degree of esterification (DE) 51.61 ±0.38 to 75.56±0.09%.	The extracted pectin from Pomelo IP has met the criteria for use as an additive in various food and pharmaceutical industries, indicating its excellence as an alternative source of commercial pectin.	Rahman et al. (2023).
Okra (*Abelmoschus esculentus* L.)	PB Yield (19.6±4.0%, 15.8±1.0% and 11.9±1.5% for 0.5 mm, 1 mm and 2 mm particle sizes, whereas that of the CAS counterparts were 32.7±8.1%, 25.6±0.8% and 35.6±5.5%	Different extraction solvents: Phosphate buffer (PB) and citric acid solution (CAS)	Ash contents (0.1-1.3 %). Total carbohydrate content (58-95%) PB and CAS (56-59%). Protein (2,0- 14,3) PB and CAS (9,5-24,5%	Such extracts can be used as natural food-grade emulsifiers or thickeners and emulsion stabilizers suggesting that they could be a promising source of texture modifiers for complex food matrices.	Afotey et al. (2023).
Watermelon (*Citrullus lanatus*) peel	Yield (%) 18.1% pH 2.0 and 0.74% pH 5.0	Aqueous acetic acid	Moisture content (%) 8.42 Ash content (%) 5.10 Equivalent weight (mg\mol) 983.90 Methoxy content (%) 7.30 Degree of esterification (%) 57.30	Watermelon peel can be an alternative source for pectin production with reasonable pectin yield and pectin quality.	Mamiru and Gonfa (2023).

			Anhydrouronic acid (% AUA) 72.36. Peel Moisture % (9.52 ± 0.64) Ash % (1.55 ± 0.11) Equivalent weight (mg/mol) 476.19 ± 0.81 Methoxyl Content (%) 3.41 ± 0.33, Total Anhydrouronic Acid content (%) 44.79 ± 0.41 Degree of Esterification (%) 43.30 ± 0.70.		
Jackfruit (*Artocarpus heterophyllus*) peel, core and tandem	Yield (%) core (35.13 ± 0.49 %) tandem (28.21 ± 0.34 %) peel (25.35 ± 0.21 %).	Acid extraction	Core Moisture % (10.70 ± 0.27) Ash % (0.90 ± 0.07), Equivalent weight (mg/mol) 454.54 ± 0.50, Methoxyl Content (%) 3.10 ± 0.29, Total Anhydrouronic Acid content (%) 45.76 ± 0.71, Degree of Esterification (%) 38.46 ± 0.55 Tandem Moisture % (10.94 ± 0.43) Ash % (1.30 ± 0.17), Equivalent weight (mg/mol) 555.56 ± 1.23, Methoxyl Content (%) 3.87 ± 0.17, Total Anhydrouronic Acid content (%) 40.48 ± 0.45, Degree of Esterification (%) 54.34 ± 0.84	This study confirmed the pectin extracted from jackfruit by-products was of good quality with promising application in modifying and improving the thickening properties of vegetable soups	Islam et al. (2023).
Durian (*Durio zibethinus*) peel	Yield (%) 6,02 a 10,96%	Acid-heating extraction	Anhydrouronic Acid (AUA %) 49,88 a 77,35 Degree of	T50% (◦C) 265.92 ± 1.65, DTGmax (◦C) 232.62 ± 0.06,	Jong et al. (2023).

			esterification (DE) 18,99±0,36, Mv (kDa) 42.12 ± 0.41	Total mass loss at 600 ◦C (%) 73.83 ± 0.66, Mass residue at 600 ◦C (%) 26.17 ± 0.66, [η] (mL/g) 148.74 ± 1.18. Durian rind is a potential source of low methoxyl pectin, which could be a capable thickener for low-calorie foods and beverages	
Mango species (*Mangifera indica* L.) peel	Yields (27.62% for Bet-CA and 30.01% for ChCl-MaA) than conventional HCl (13.17%)	Ultrasound treatment co-friendly deep eutectic solvents (DESs) etaína-ácido cítrico (Bet-CA) e cloreto de colina-ácido málico (ChCl-MaA) and conventional extraction HCl	Degree of esterification (DE) (55.10–65.55%) of HCl, (75.05%– 83.43%) Bet-CA, ChCl-MaA (71.17%–87.06%). Molecular weight-average (Mw), number-average molecular weight (Mn) Bet-CA Mw (781.665 kDa) and Mn (715.553 kDa), ChCl-MaA Mw (641.474 kDa) and Mn (386.679 kDa) and Mw (549.382 kDa) and Mn (286.034 kDa)	Which provides theoretical basis for the functional application of mango peel pectins in the food and pharmaceutical industry.	Chen et al. (2022).
Strawberries (*Fragaria* × *ananassa*), blackberries (*Rubus fruti cosus* L.), *raspberries* (*Rubus idaeus* L.), *redcurrant (Ribes rubrum*)	yields between 8.8 and 12.4% for redcurrant and raspberry	Enzyme-assisted with cellulase, citric acid ultrasound-assisted and enzyme-ultrasound-assisted treatment and conventional citric acid extraction	Regarding the structural characteristics of pectins, enzymatically extracted pectins from redcurrant and strawberry exhibited the highest levels of galacturonic acid (≥73%) whereas, in general, this	Of particular relevance is the consideration of the by-roducts derived from the industries of preservation of berries as an alternative of the conventional sources of pectin that open new	Almagro et al. (2021).

			monosaccharide was found from 51 to 69% in the rest of samples	routes of commercialization of bioactive natural compounds.	
Persimmon fruit (*Diospyros kaki Thunb*. var. Rojo brillante) peel, pulp, whole fruit	Yields 0.2 to 6.27%	Conventional citric acid extraction	Galacturonic acid (GalA) 78 a 84,9%, methoxylation degree (DM) 4 a 12 %	Techno-functional assessment (zeta potential, particle size, apparent viscosity, gelation) showed the suitability of the persimmon pectins for a broad range of industrial applications.	Almagro et al. (2021).
Chayote (*Sechium edule*)	Yields 3,33% a 6,78%	Ultrasound-assisted extraction (UAE)	Ash (%) 3.19 ± 0.53, Moisture (%) 11.30, Protein (%) 3.61 ± 0.14, Phenols (mg/g) 7.18 ± 0.48, DE (%) 17.60, Gal A (%) 57.25, Mw (g/mol) 2.47 × 10, Mn (g/mol) 1.29 × 10, Mw/Mn 1.91	It may be used as a gelling agent and preservative in the production of jam or as a viscosity enhancer in the production of various beverages.	Ke et al. (2020).
Sunflower (*Helianthus annuus* L.) by-produc	Yields 8,71%-10,95%	Ultrasoundassisted extraction (UAE)	Moisture (%) 9.06 ± 0.03, Ash (%) 1.43 ± 0.05, Protein (%) 1.25 ± 0.13, Carbohydrate (%) 89.58 ± 2.94, TPC (mg GAE/g pectin) 8.11 ± 0.01, DE (%) 34.06 ± 0.05, Monosaccharides composition (%) Galacturonic acid 72.94, Galactose 9.85, Arabinose 8.01, Rhamnose 2.86, Fructose ND, Xylose ND, Glucose 5.25	Sunflower by-product should be considered as an additional resource along with commercial resources and SFBP can be used as a high quality pectin sample with good functional and technological properties in pharmaceutical or food industries	Ezzati et al. (2020).
Coffee (*Coffea arabica* L.) pulp	Yield (%) (14.6± 0.6)	Conventional extraction: Acid extraction using HCl	Moisture (%) 13.0 ± 1.2, Protein (g/100 g) 1.4 ± 0.1, Phenolics	Pectin from Coffea arabica pulp could be used in regular or low-	Reichembach and Petkowicz (2020).

			(g/100 g) 0.70 ± 0.03, Ashes (g/100 g) 3.2 ± 1.0, GalA (%) 81.2 ± 2.6, DM (%) 63.2 ± 0.8,	calorie preparations that require gel formation at high contents of dissolved solids and low pH, such as confectionary jellies and jams. It probably could also be used in acidified dairy drinks and yogurts	
Gabiroba (*Campomanesia xanthocarpa* Berg) pulp	Yield (%) 0.76 (11.4g/1.500g)	Conventional extraction: Hot water extraction	Crude pectin (GW) composition: Arabinose (54.5%), galacturonic acid (33.5%), galactose (7.6%), and rhamnose (1.6%).	Apparent viscosity values of GW 1%, 3% and 5% pectin solutions were 0.02, 0.13, and 0.28 Pa, respectively.	Barbieri et al. (2019).

IP: inner peel, OP: outer peel; Eq. W: Equivalent weight, MeO: Methoxyl, AUA: Anhydrouronic acid, DE: Degree of esterification, PB: Phosphate buffer, CAS: citric acid solution, Mv: Molecular weight, DESs: eco-friendly deep eutectic solvents, Bet-CA: betaine-citric acid, ChCl-MaA: choline chloride-malic acid, HCl: hydrochloric acid, Mw: weight-average, Mn: Molecular weight, UAE: ultrasound-assisted extraction.

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3. The main technologies for pectin extraction and POS production

The extraction of pectins and subsequently production of POS can be involving several steps, since the selection and preparation of the vegetal matrix, application of different methods for extracting (conventional or novel approaches), purification and chemical characterization as well as evaluation of the potential applications in terms of biological activities (**Figure 2**). In the next sub items, the main advances, and trends in the extraction of pectins and the production of POS will be discussed in detail.

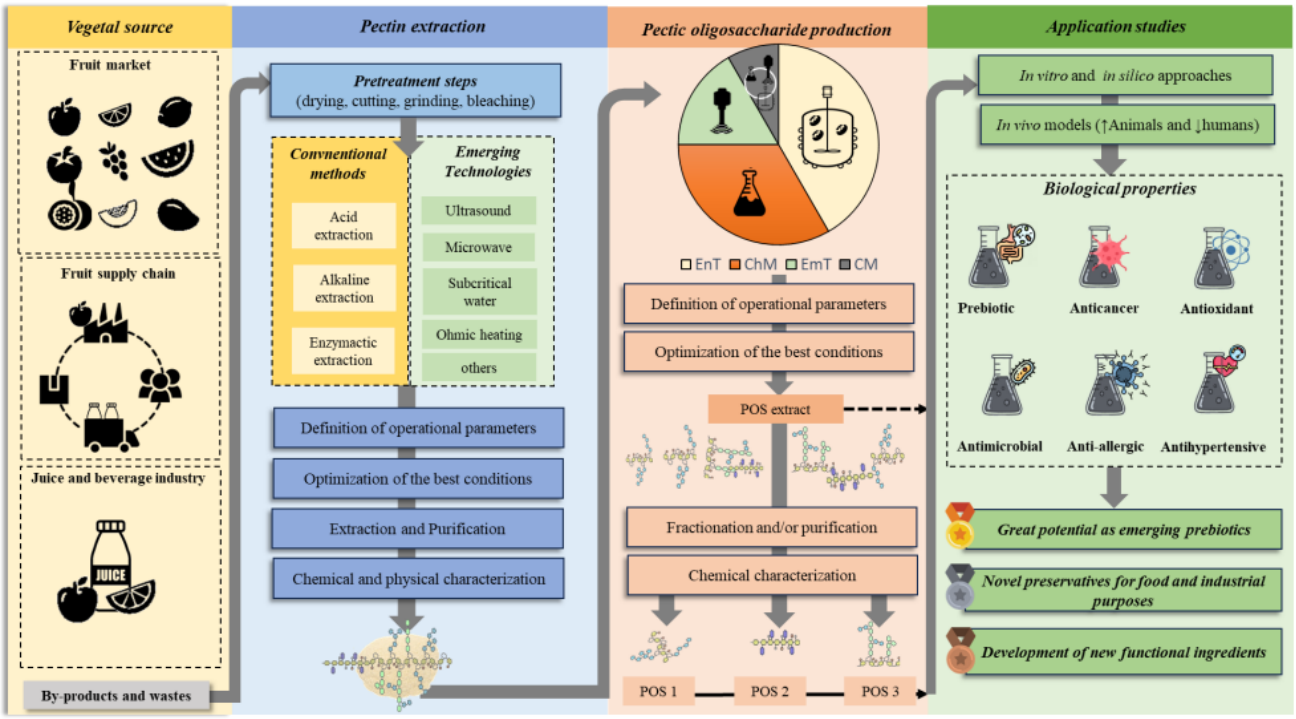

Figure 2. General overview of the main steps involved in pectin extraction and POS production using different technologies, including enzyme technology (EnT), chemical methods (ChM), emerging technologies (EmT) and combined methods (CM).

3.1 Conventional methods for pectin extraction

Polysaccharides can be released from plant cell wall by sequential extraction with different aqueous solvents. Pectins have been traditionally defined by their extractability from the plant cell with chelating agents, such as ethylenediaminetetraacetic acid (EDTA), 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA) and ammonium oxalate, which release pectins that are cross-linked by calcium ions. Other solvents that have been used to extract pectins include hot water (Liu et al., 2023a), hot buffer (Andreani et al., 2021), dilute cold alkali (Patova et al., 2023) and hot dilute acid (Muñoz-Almagro et al., 2021). Depending on the solvent and extraction procedure, pectins with different composition, molecular size and fine structure are obtained.

The conventional method to produce commercial pectins use hot diluted acid. In these conditions, pectin undergoes, depolymerization, removal of neutral sugars side chains and loss of ester substituents. Long times of extraction, high temperatures and low pH favour high pectin yields, but are detrimental to the physicochemical properties. Thus, the exact extraction conditions are adjusted for each raw material to obtain pectins with suitable properties.

The waste material (apple pomace or citrus peel), often dry, is treated with a mineral acid, usually nitric acid pH 1-3 at 50-90°C for 3-12h (Rolin, 2002). The typical solid:liquid ratios are reported to be 1:15 for apple pomace and 1:35 for citrus peel. The pectin extract is separated from the insoluble part by filtration and the pH is raised to 3-4 to prevent depolymerisation and demethylation. The cleaned extract is usually concentrated to 3-4% pectin content. Then, the pectin is recovered from the extract by precipitation with an alcohol followed by filtration. Methanol, ethanol, and isopropanol are permitted to be used to precipitate pectin, but isopropanol is the most used. Irrespective to the alcohol used for precipitation, it is always recovered by distillation. The pectin precipitate is subjected to washing with acidified alcohol, dried and milled to a fine powder which is typically an HM pectin. To produce LM pectins, prior to separation and drying, the suspension of pectin in alcohol is subjected to controlled acid or alkali treatment to obtain the desired DM. If the de-esterification is carried out using ammonia, methyl ester groups are replaced by amide groups, producing amidated pectins (Brejnholt, 2009; Rolin, 2002; Voragen et al., 1995).

Due to the variations in the raw material that result in differences in the gelling power of pectins from batch to batch, pectins from individual lots are blended and standardized to a uniform jelly grade by addition of sucrose (Brejnholt, 2009; Voragen et al., 1995). Although, commercial pectins are produced by conventional hot-acid extraction, several new greener extraction approaches have been proposed.

3.2 Emerging technologies applied to pectin extraction

Beyond the conventional approach mentioned above, new methods for pectin extraction have been proposed in recent years, which often involve the use of emerging technologies (Gavahian et al., 2021). These technologies can be classified into thermal methods (e.g., Ohmic heating, Microwave) and non-thermal methods (Ultrasound, Pulsed electric field, High pressure). Each of these technologies operates through a specific mechanism, typically resulting in enhanced mass transfer during the pectin extraction process by disrupting plant tissues and increasing the contact surface area between the plant substrate and the surrounding solvent. It is also possible to combine these technologies with each other or with pectinolytic enzymes. As a result,

emerging methods for pectin extraction encompass a wide range of techniques, such as Ultrasound-assisted extraction (UAE), Ultrasound- and enzyme-assisted extraction (UEAE), Subcritical water extraction (SWE), Microwave-assisted extraction (MAE), Ultrasound- and microwave-assisted extraction (UMAE), Radio-frequency assisted extraction (RFAE), Hydrothermal extraction (HE), Moderate electric field extraction (MEFE), Ohmic heating-assisted extraction (OHAE), Ultrasound-assisted ohmic heating extraction (UAOHE), and others (Reichembach & Petkowicz, 2021; Roy et al., 2023).

These technologies have demonstrated several advantages, such as reduced extraction time for pectins, higher yields when compared to traditional methods, and lower waste generation. Furthermore, statistical tools for experimental design and process optimization have also been employed to determine the best extraction parameters, with the Box-Behnken design (BBD), Factorial design (FD), and Central Composite design (CCD) being the most used for this purpose (Ke et al., 2020; Jong et al., 2023; Kamal et al., 2023). Some these experimental designs allow the application of the Response Surface Methodology (RSM), which results in the establishment of optimal conditions for each type of substrate from which pectins are intended to be extracted. The independent variables most used in these experimental designs include pH, type of acid, solid: extracting solution ratio, temperature, time, and variables linked to the technology used such as nominal power (W), frequency (kHz), amplitude (%), energy density (J/cm3), pressure (MPa), among others.

While alignment with the sustainability aspects and potential of these new pectin extraction methods is prevalent, few studies undertake techno-economic evaluations of emerging technologies, comparing them with conventional methods (Sucheta et al., 2020; Roman-Benn et al., 2023). Consequently, it is challenging to locate studies that focus on the assessment of factors such as infrastructure investments, energy consumption, industrial-scale feasibility, waste generation analysis, as the majority of studies center on emerging technologies developed at the laboratory scale. On the other hand, it is worth highlighting that many studies have evaluated the impact of the emerging technology used on the structural and functional characteristics of pectins. In this context, it has been demonstrated that it is essential to control the intensity of energy applied during extraction, as in the UAE and MAE, since very intense processes lead to a reduction in extraction yield due to the degradation of the pectin chains (Karbuz & Tugrul, 2020).

Although several studies have explored the extraction of pectins from new sources, it is clear that interest in citrus varieties as sources of these polysaccharides still remains strong. Several studies have described interesting results for the application of ultrasound and other

technologies to obtain pectins from different citrus substrates. In general, higher pectin yields are observed using UAE when compared to conventional acid extraction. Panwar et al. (2023) developed an optimized ultrasound-assisted extraction process for obtaining pectins from Citrus limetta peels, utilizing a Box-Behnken Design (BBD). They achieved a maximum pectin yield of 28.82% after 24 minutes of sonication at 40°C, 37% amplitude, and a pH of 1.9. This approach demonstrated significant advantages over conventional acid extraction, even under optimized conditions, where the pectin yield ranged from 3.97% to 22% (Panwar et al., 2022). Recently, a pectin yield of around 27% was achieved using an ultrasound-assisted enzymatic extraction method and orange peel as substrate (Bosch & Malgas, 2023). This approach consisted of two steps, the first being conducted in an ultrasonic bath (40 kHz, 300 W) programmed at 80 °C for 30 min, then 1mL of Celluclast (64.7 mg/mL) was added and incubated at 50 °C and 70 rpm for 4 h. This yield was higher than that obtained with acid extraction (22%), with the method being carried out at 80 °C, pH 1.5, in water bath for 4h. Furthermore, conventional extraction resulted in pectins with higher molecular weight and DE compared to its ultrasound-assisted counterpart, which possibly caused degradation of pectins during the extraction process. This study demonstrates that UAE leads to higher yields but has a notable impact on the structure of the obtained pectins. Thus, the effects of the extraction method must always be considered since they can significantly influence the structure and, consequently, the functionality of the pectins.

The extraction of pectins from finger citron (*Citrus medica* L. var. *sarcodactylis Swingle*) pomace employing UAE, MAE and conventional extraction showed can be influenced by pH and temperature (Yu et al., 2021a). For both conventional acid and alkaline extractions, the pectin yields (14.7% to approximately 19%) were directly proportional to the increase in temperature (60°C to 80°C), while an inverse relationship was observed for the molecular weight (Mw) of obtained pectins. Similar behavior was observed for acid and alkaline UAE and for acid MAE where yields increased from 12.6- 15.1% to 24.1-24.9% and 14.8% to 17.9%, respectively, as the temperature increased from 60°C to 80°C. For alkaline MAE, as the temperature increased the pectin yield decreased from 27.1% to 17.6%. These findings support the conclusions of previous studies that have highlighted the significant influence of pH and temperature on pectin extraction processes. The enhanced yields can be attributed to the disruption of plant cells at higher temperatures, facilitating the rapid separation and dissolution of pectin. It is worth noting that an acidic pH often results in better solubility and higher extraction yields, as it promotes the release of pectin from the plant matrix, whereas an alkaline pH, in some cases, may lead to degradation and lower yields due to the breakdown of pectin

molecules depending on temperature and energy intensity from the technology employed**.** This last situation can be considered when the direct hydrolysis of pectins is desired to directly obtain pectic oligosaccharides.

In contrast, some recent studies have shown that UAE does not always provide higher pectin yields, warning that the type of vegetable substrate and the instrumental parameters used are important factors to be considered. According to Spinei and Oroian (2023), UAE did not prove to be a more efficient method for extracting pectin from grape pomace of Vitis vinifera var. Fetească Neagră (FN) and Vitis vinifera var. Rară Neagră (RN) when compared to conventional acid extraction (CE) and MAE. In this study, UAE was conducted at a pH of 1.8, using a pulsed mode (1 second on and 1 second off) for 60 minutes at 100% amplitude (25 kHz, ultrasonic power of 200 W), while conventional extraction and MAE were performed at a pH of 1.9, at 90°C for 164 minutes, and pH 1.8 with 560 W for 120 seconds, respectively. The results indicated that UAE yielded approximately 8.83% and 8.94% for FN and RN, respectively, while CE resulted in yields of 9.96% (FN) and 11.08% (RN), and MAE produced yields of 9.03% (FN) and 11.23% (RN). Similarly, UAE was not the most appropriate method for extracting pectins from black carrots (*Daucus carota* L. ssp. *sativus var. atrorubens* Alef.) pomace (Sucheta *et al*., 2020). The conditions employed for CE were 110°C for 90 min, UAE (37 kHz, 550 W) at 70 °C for 30 min MAE at 110 °C for 5 min, all treatment were carried out at pH 2.5. The pectin yields achieved ranged to 8% using UAE to 17% and 22% using MAE and CE, respectively. Although these authors describe that UAE did not result in higher pectin yields, it should be noted that the comparison would be more appropriate if the same extraction time were applied for the different methods.

Another approach recently described for pectin extraction consists in the association of ultrasound treatment with moderate external pressure (usually from 0 to 300 kPa), resulting in the process named manosonication (MSE) (Hu et al., 2020; Hu et al., 2021). The alkaline mediated MSE was successfully applied for RG-I pectin recovery from different citrus peel wastes, including Eureka lemon (Citrus limon Burm.; LEPp), Guanxi pomelo (C. maxima Osbeck; POPp), navel orange (C.sinensis var.; ORPp), and grapefruit peels (C. paradisi Macf.; GRPp). The extraction parameters were 200 kPa, 25 °C, 25% amplitude, 1.14 W/mL, 20 min sonication time. The yields obtained and the GalA content in the pectins from these different substrates varied between 21.7% and 59.7% for LEPp, 20.17% and 55.1% for POPp, 20.81% and 32.4% for ORPp and 17.10% and 48.75% for GRPp, respectively (Hu et al., 2021). Previously, an alkaline MSE process was optimized using a Box-Behnken design for extraction

of RG-I pectin from citrus peels of C. unshiu Marc and a maximum extraction yield of 25.5% was obtained with sonication at 42 °C, 40% amplitude, and 250 kPa for 20 min (Hu et al., 2020). Another trend recently observed is the development of methods for simultaneous extraction of pectins and other compounds of industrial interest such as pigments, phenolic compounds, and essential oils from the same substrate (Huo *et al*., 2023; Thakare et al., 2023). This approach meets aspects of sustainable processes and biorefinery, in addition to being considered innovative when the proposal involves the use of emerging technologies (Guandalini *et al*., 2019; Villamil-Galindo & Piagentini, 2022).

Recently, an optimized MAE method was described for simultaneous recovery of pectin and phenolics from sour cherry (*Prunus cerasus* L.) pomace (SCP) (Housseini et al., 2020). This study employed a BBD with four variables in three levels, using microwave power, irradiation time, pH and LSR (liquid to solid ratio) as independent variables and pectin extraction yield and TPC (total phenolic content) extraction yield dependente variables. Under optimal conditions, including microwave power of 800 W, irradiation time of 300 s, pH of 1.00 and LSR of 20 v/w, the maximum yield of pectin and phenolic compounds achieved were 14.65% and 14.36%, respectively. Similarly, an optimized MAE method was developed by Khodaiyan & Parastouei (2020) for extraction of pectin and phenolics from black mulberry (Morus nigra L.) pomace (BMP) using the same independent and dependent variables. Under optimized conditions, including microwave power of 700 W, irradiation time of 300 s, pH of 1.42, and LSR of 20 mL/g, highest pectin (10.95%) and TPC (12.11%) yields were achieved. Although there are similarities in the extraction parameters, the pectins obtained from BMP and SCP differed in terms of molecular weight, DE, and GalA content. The BMP had a higher molecular weight (620.489 kDa) when compared to SCP pectin (472.977 kDa). Moreover, lower values of DE (62.1%) and GalA (70.15%) were observed to BMP pectin when compared to SCP pectin (DE and GalA of 68.3% and 72.8%, respectively).

A BBD with three factors (LSR, extraction/hydrodistillation time and voltage gradient) at three levels was employed to optimization of ohmic heating assisted extraction/hydrodistillation (OHAE/H) method for recovery of pectin and essential oil from lemon waste (Tunç & Odabaş, 2021). Under optimized conditions (8.7:1 LSR, 58.4 min extraction/hydrodistillation time and 14.2 V/cm voltage gradient), the pectin and essential oil yields were 16.5% and 3.62%, respectively. These yields were higher than those achieved by the conventional heating extraction/hydrodistillation, where the yield of pectin and essential oil were 15.4% and 2.4%, respectively. On the other hand, Karanicola *et al.* (2021) reported the optimization of a UAE method using a CCD for extracting pectin and essential oil from orange peel waste. The results

showed that under optimized conditions (5.75% solid loading, 1.21% acid concentration and 34.2 min) the maximum production yields of 0.12% w/w essential oils, 45% w/w pectin, were achieved. Thus, the integrated extraction of pectins and other value-added compounds from low cost substrates has grown in recent years, demonstrating that the development of biorefinery processes can be considered a promising approach and in some cases the use of emerging technologies can contribute to a satisfactory improvement in the extraction process (Boukroufa et al., 2015; Guandalini et al., 2019).

3.3 The main approaches for POS production

3.3.1 Enzyme technology

Processes for enzymatic production of POS from pectin or pectin rich-extracts are based to use of different enzymes, mainly pectinases, able to promotes the depolymerization and/or degradation of the pectic polysaccharide chain to short-chain oligosaccharides. Considering the chemical composition and complexity of the structure of pectins (see section 2.1) it is possible to expect that several enzymes can also be applied to the hydrolysis of this polysaccharide aiming at the production of POS. The investigation of pectinolytic enzymes spans from the search for novel microorganisms capable of producing them for biocatalytic applications to the characterization of the array of pectinases produced by diverse species within the intestinal microbiota (Hao et al., 2022; Elshahed et al., 2021). The last approach aims to gain insights into the metabolism of pectic substances within the context of prebiotic activity (Ndeh et al., 2017).
Pectinases constitute a broader group of related enzymes employed for degradation of pectic substances and are commonly found in both microbes and plants. According to Rehman et al. (2021), the term 'pectinase' would generically comprise the group of enzymes that catalyze the hydrolysis of the pectin structure through hydrolysis, trans elimination and de-esterification reactions. These enzymes can be applied for different industrial purposes such as in oil extraction, textile industry, juice processing, coffee and tea processing, seed germination, cellulose degradation, and animal feed processing (Kaur et al., 2023; Liu et al., 2023).
In previous review, Jayani, Saxena and Gupta (2005), proposed the division of pectinolytic enzymes into three groups, including I) protopectinases, II) esterases, and III) depolymerases. The first group comprises enzymes that degrade the insoluble protopectin and give rise to highly polymerized soluble pectin, being in this case most appropriate for processes of pectin extraction. The second group comprise the enzymes that catalyze the de-esterification of pectin by removal of methoxy acetyl groups linked to GalA moieties, while the third group catalyze

the cleavage of the α-(1→4)-glycosidic bonds in the GalA moieties through hydrolysis (polygalacturonases, PG) or β-elimination (pectin lyases, PL).

The knowledge of the biochemical and physical properties of pectinolytic enzymes is essential for designing a suitable hydrolysis bioprocess. In this context, parameters such as temperature, pH, substrate concentration, reaction time and requirement for ions in the reaction medium are important and must be strictly defined and controlled so that POS production is achieved (Wongkaew et al., 2021; Yu et al., 2021a; Yu et al., 2021b). Furthermore, characteristics of the pectin used as a substrate must be considered because depending on the predominance of "smooth" or "hairy" regions in the structure or the percentage of esterified methoxyl and acetyl groups, different combinations of pectinolytic enzymes will be required. The "smooth" region of pectin, predominantly composed of HG, if highly esterified, may require the de-esterification action of pectin methyl esterases and pectin acetyl esterases for subsequent action of depolymerases such as polygalacturonases. Since the hairy region of pectins is composed of RG-I, RG-II and XG, and other glycosidic bonds involving other monosaccharide residues are present in these structures, it can be expected that other enzymes are necessary for the hydrolysis and consequent production of oligosaccharides. Examples of enzymes employed to degradation of "hairy" region include rhamnolacturonases, arabinogalacturonases, galactosidases and xylogalacturonases, which can be used individually or in conjunction with other enzymes. Therefore, the choice of the enzyme(s) is a crucial step in designing a POS production process, requiring a robust chemical characterization of the pectin that will be used as a substrate.

Due to the different mechanisms, and sites of action these enzymes, oligosaccharides with different chemical structures, polymerization degree and properties can be produced from pectins. For example, 4,5-unsaturated oligogalacturonides are formed from the pectin substrate by β-elimination using the pectate lyase, while oligogalacturonides are formed y hydrolysis of α-1,4-linkages of homogalacturonan by polygalacturonases (Roman Benn et al., 2023; Li et al., 2024). The extension of the degradation of the pectin chain can be affected by the enzyme concentration, time of enzymolysis, pretreatments, and other factors. Thus, POS with varied degrees of polymerization and different sugar moieties can be produced in the same process. This scenario results in a great challenge and high cost for the purification of POS individually, with the separation of these compounds into fractions the most common and economically viable approach.

In this sense, Sabater et al. (2021) described that several POS are produced from enzymatic hydrolysis of pectin from artichoke (*Cynara scolymus* L.), with notable variability in terms of

the chemical structure of these compounds depending on the fraction/type of hydrolyzed pectin. The results showed that di- and tri-POS were the most abundant oligosaccharides, being DiGalA (GalA-α(1,4)-GalA) and TriGalA (GalA- α(1,4)-GalA-α(1,4)-GalA) were the most abundant formed from HG ruptures. In addition, different di-POS such as Gal-β(1,4)-Rha, Rha-α(1,4)-GalA and GalA-α(1,2)-Rha as well as different tri-POS including Ara-α(1,4)-Rha-α(1,4)-GalA, GalA-α(1,2)-Rha-α(1,4)-GalA and Rha-α(1,4)-GalA-α(1,2)-Rha can be produced by hydrolysis of RG-I. Other POS with DP from 4 to 7 also were reported from HG, RG-I and XGA hydrolysis, and those with DP>8 don't were identified by limitation of the analytical technique employed, in this case the MALDI-TOF-MS.

In addition to the type of enzyme used to produce functional oligosaccharides, the system through which the hydrolysis process is conducted also plays an important role. Thus, functional oligosaccharides as POS can be produced by batch-wise enzymatic hydrolysis of pectic substrates using systems based on free or immobilized enzymes. Regarding immobilized enzymes, different techniques were recently reported for immobilization of pectic enzymes to POS production, including sol-gel encapsulation by polyvinyl alcohol, adsorption on functionalized porous silica, adsorption on mesoporous titanium oxide particles (MTOPs) and cross-linking with dialdehyde polysaccharide via the CLEA (Cross-linked enzyme aggregates) approach, (Long et al., 2022; Muller et al., 2022; Zheng et al., 2022; Abd Rahman et al., 2024). A recent study produced POS from citrus pectin and banana peels using four different commercial pectinases, investigating the impact when they were free or immobilized using the CLEA technique to form different cross-linked pectinase aggregates (CLPA) (Abd Rahman et al., 2024). The results showed that oligogalacturonic acid was most efficiently produced by enzymatic hydrolysis of citrus pectin using the free commercial pectinase Pectinex Ultra SP-L (PB, Novozyme) (50.68%), followed by pectinase from Aspergillus niger (PD, SolarBio) (45.78%), while that for enzymatic hydrolysis of banana peel pectin the highest total content of oligogalacturonic acids was achieved using PD (40.66%) followed by CLPA-D (22.24%). Although the approach is interesting, its industrial applicability would depend on further studies related to scale-up.

In general, most studies do not control or monitor the extent of hydrolysis using batch 8 processes, which most often employ crude multi-enzyme preparations with endo- and exo-activity, resulting in complex mixtures of POS and high content of free monosaccharides, mostly galacturonic acid, the end-product of the pectin hydrolysis (Babbar et al., 2016). To address this issue, the lasted trend in studies on enzymatic POS production suggests that the use of immobilized enzymes in systems other than batch wise enzymatic hydrolysis can be

considered promising. In this sense, immobilized enzymes are also applied in flow reactors such as packed-bed reactors (PBRs) and cross-flow continuous membrane reactors (MRs) (Long et al., 2022; Baldassarre et al., 2018).

The crude pectin extract obtained from onion skins was employed as substrate for POS production in a bioprocess carried using Viscozyme® L as biocatalyst in a cross flow enzyme membrane reactor (Baldassarre et al., 2018). In this study, it was demonstrated that an effective separation of POS can be accomplished using 10 kDa molecular weight cut-off membranes. The optimal conditions were residence time of 15 minutes, enzyme concentration of 41.4 U/mL, and substrate concentration of 50 g/L, what yielded the highest POS volumetric productivity at 22.0 g/L/h, with a corresponding high yield and POS/monosaccharide ratio of 4.5 g/g. Under these conditions, a consistent production of predominantly short POS (DP2, DP3, and DP4) was observed over time.

The enzymatic production of POS from mango peel waste employing PG immobilized through sol–gel encapsulation by polyvinyl alcohol and applied into a column to fabricate a PBR was recently described (Long et al., 2022). The optimal conditions were established using response surface methodology, consisting of an enzyme amount of 49U, flow rate of 2.0 mL/min, and substrate concentration of 0.1%, achieving a high Yield (94.56%) after continuously operated for 72 h and the predominant POS were DP4, DP5, and DP6 oligomers.

Previously, this immobilized enzyme was used for mango peel pectin hydrolysis in a batch process carried in different times (2, 5, 15, 30 and 60 min) at 50°C (Xue et al., 2021). The results showed that after 5, 15, and 30 min of hydrolysis were produced DP2 and DP4 oligomers, accounted for 57.4%, 59.4%, and 60.6% of the POS, respectively. Therefore, the composition of POS produced by the same immobilized enzyme is affected by the type of enzymatic production process chosen, making it necessary to develop reactors that ensure high yields and controlled hydrolysis of the pectic substrate.

The modulation of molecular weight of POS is a challenge to be achieved, and it is desirable the range between 1 and 3 kDa for heightened prebiotic activity. In this context, a recent study proposed the immobilization of pectinase on functionalized silica (amino, glyoxal, and amino-glyoxal groups) for POS production from citrus pectin, and the heterofunctional amino-glyoxyl support the improved the thermal resistance and pH stability compared to its soluble counterpart (Muller et al., 2022).

More recently, the chemical structures and functional properties of citrus pectin and oligosaccharides present in hydrolysates after enzymolysis by pectinase were investigated by Lu et al. (2024). Three main components named as SH, S-5, and S-35 were obtained from the

supernatant after enzymatic hydrolysis with yields of 16.4%, 4.2%, and 10.3%, respectively, and exhibited a low molecular weight (MW, 0.2–1.5×10^4 18 g/mol) and high content of RG-I (61.8–81.4%). For evaluation of prebiotic potential, were carried fermentation experiments and the results showed that compared with citrus pectin, the hydrolysates had higher total SCFA contents, probably because of their high RG-I content and low Mw. Moreover, the contents of all SCFAs were significantly higher in S-5 (the fraction with smallest Mw (0.2×10^4g/mol) when compared to citrus pectin and the Other hydrolysates, and these contents were considerably higher in S-5 than in inulin. Thus, this study corroborated with the hypothesis that enzymatic hydrolysates of citrus pectin, especially those with low Mw (e.g. S-5) can be exhibit prebiotic activity and enzymatic use of pectinase during the citrus juice processing lead more that technological goals (e.g. clarification) but can be considered an alternative approach to improve the functional appeal of citrus juice.

3.3.2 Chemical and related methods

The application of chemical methods for pectin hydrolysis/depolymerization was reported mainly for comprehensive study of the structure of this polysaccharide and some authors focused on the controlled production of oligomers (Renard et al., 1998). According to studies using the chemical hydrolysis of pectins for POS production, these approaches can be considered low cost, rapid, repeatable, and accurate (Singh et al., 2020).

The main questions about chemical methods for pectin depolymerization to produce POS and low molecular weight oligomers are related to the environmental safety and sustainability aspects of these processes. In recent years, efforts have been made to make processes more advantageous, reducing reaction time through association with other technologies, replacing metals in the reaction system, and optimizing processing parameters. The main findings of studies from this perspective will be discussed below.

Shi et al. (2017) employed the controlled acid hydrolysis to chemical characterization of RG-I domain (RG-I-3A) of ginseng pectin and the chemical hydrolysis procedure was carried out with 0.1 M trifluoroacetic acid (TFA) at 80°C in different times, including 0.5, 2, 4, 6, and 16 h. After chemical study using ^{13}C and ^{1}H-NMR analysis, the results suggested that RG-I domain was composed of →4)-α-GalpA-(1→2)-α-Rhap- (1→disaccharide repeating units as backbone, with β-1,4-galactan, α-1,5-arabinan, arabinogalactans-I and II (AG-I/II) side chains substituted via the O-4 of Rhap.

Another study reported the production of POS from apple pectin (AP), rhamnogalacturonan-I (RG-I) and homogalacturonan with different degrees of esterification, i.e. high-methoxy pectin

(HM) and low-methoxy homogalacturonan (HG) using an optimized chemical method using TFA (Singh et al., 2020). Initially, a screening using 10 mg of each pectin dissolved in 2 mL of 1.2, 1.5 and 2.0 M TFA was carried, and the chemical hydrolysis was performed on heating block at 110 °C for 3 or 4 h. Thereafter, bulk hydrolysis (1 g) of different pectins was performed under optimized conditions, including 1.5 M of TFA, incubation period of up to 4 h at 110°C. The results showed that the recovery of purified POS from 1 g of HG, AP, HM and RG-I was approximately 42%, 50%, 74% and 75% respectively. In addition, it was observed that POS with DP from 2 to 7 can be produced from different pectins. More specifically, thirteen different POS with DP between 2 and 7 were produced from AP and HM, while for the hydrolysis of HG twelve different POS with DP between 2 and 7 were produced. Finally, for the acid hydrolysis of RG-I only eight oligomers with DP between 2 and 6 were obtained. Therefore, it is once again possible to state that the type of pectin will directly affect the profile of POS produced, with this trend being notable in the acid hydrolysis method.

Previously, the preparation of POS through controlled degradation of citrus peel pectin by TFA and H_2O_2 at different concentrations and/or time of hydrolysis was reported by Zhang et al. (2018). The hydrolysis carried out with TFA 1.5M or 2.0M, at 85°C for 2.5h or with TFA 2.0M and modifying the last step of purification by lyophilization were obtained three different POS fractions, named POS_{T1}, POS_{T2} and POS_{sq}, respectively. The chemical approach was carried using 66.18mM of H_2O_2 for 3h at 90°C and 88.24mM of H_2O_2 for 4h at 90°C, resulting in two POS fractions, named as POS_{H1} and POS_{H2}. The main difference between these POS was due to the molecular weight and monosaccharide composition, with a decrease in molecular weight being attributed as the hydrolysis conditions became more drastic (for example, increasing the concentration of the hydrolysis agent and reaction time). In this case, different POS can be obtained from the same pectin by modifying the reaction conditions used in hydrolysis. Therefore, special attention must be paid to process parameters such as concentration of the hydrolysis agent and reaction time, so that an adequate design of the process is achieved.

Concerning the use of controlled oxidative chemical hydrolysis of pectin, this processes usually utilizes H_2O_2-dependent systems to generate reactive oxygen species that depolymerize the polysaccharide chain by attacking and breaking acid-labile glycosidic linkages producing different oligomers (Li et al., 2019a). In this context, Fenton reaction is one of the most innovative oxidation techniques used to depolymerize polysaccharides, including pectins, and is a process in which ferrous iron (Fe^{2+}) is combined with H_2O_2 at acidic conditions to in situ generation of •OH radicals (Amicucci et al., 2020; Pathania et al., 2020). These radicals can react with C-bonded H atom, attack the glycosidic linkage, resulting in the pectin

depolymerization into low molecular weight oligomers, and in some cases with improved biological properties (Li et al., 2019a; Zhi et al., 2017). Modifications in this reaction to produce non-metal or iron-free Fenton-like reactions or the association with ultrasound technology to obtain accelerated processes for pectin hydrolysis also have been reported (Li et al., 2019b)

Recently, POS were prepared from okra (*Abelmoschus esculentus* (L.) Moench) pectin by Fenton reaction using ferrous sulfate ($F_{eS}O_4 \cdot 7H_2O$) and H_2O_2 at a ratio of 1:1 (v/v) (Yeung et al., 2021). In this study the hydrolysis processes were carried out at room temperature for 2h using aqueous solutions with different concentrations of F_eSO_4, including 3 mM, 5 mM, and 7 mM, and the POS produced in these conditions were named as POS_3, POS_5, and POS_7, respectively. The results suggest that the Mw of POSs was decrease from 6.09 kDa to 1.79 kDa with increasing concentration of F_eSO_4 from 3 mM to 7 mM and significantly decreased GalA contents but significantly increased Rha contents, indicating that free radical generated by Fenton reaction preferentially attacked at the HG region of okra pectin. Regarding the antioxidant activity assessed by DPPH and ABTS assays, the results showed that the antioxidant activities of POS were significantly greater than those of okra pectin, which can be explained by the reduction of Mw. This same trend was observed for prebiotic activity, where POS exhibited higher prebiotic effects than pectin in bacterial populations *Lactobacillus rhamnosus* ATCC 7469 and *Bifidobacterium longum* ATCC 15707, promoting the generation of SCFAs and these effects were possibly attributed to their relatively lower Mw.

Previously, Li et al., 2019a described the application of a Fenton system for depolymerization of pectic polysaccharide into oligomers with molecular weights below 5 kDa using citrus canning processing water as substrate and replacing Fe^{2+} by Cu^{2+}. The results showed that when the reaction system is carried out with excess of H_2O_2, the increase of Cu^{2+} accelerate the generation of hydroxyl radicals per unit time, elevating the yield of pectic oligomers or as referred by these authors low molecular weight pectic polysaccharides (LPM). Thus, the cupric ion (Cu^{2+}) satisfies all basic redox criteria required to activate H_2O_2 for application in depolymerization of pectic substrates to produce POS.

POS derived from okra pectin also were produced through an ultrasound assisted metal-free Fenton (H_2O_2-ascorbic acid system) reaction carried out at 80% of the ultrasound amplitude, 20.0 mM of ascorbic acid, and 40.0 mM of H_2O_2 for 0.5h (DOPP-1), 1.0h (DOPP-2), and 3.0h (DOPP-3) (Wu et al., 2022). Compared to okra pectin (2.84×10^5 Da) these fractions showed lower molecular weights with increasing processing time, being 1.90×10^5 Da for DOPP-1, 8.27×10^4 Da for DOPP-2, and 4.65×10^4 Da for DOPP-3. Regarding the antioxidant activity, DOPP-3 exhibited strongest potential among the three fractions and okra pectin, whereas the

lowest antioxidant activities were observed in DOPP-1. The order of antioxidant activities was DOPP-3 > DOPP-2 > OPP > DOPP-1, and the lower antioxidant potential of DOPP-1 was related to the combined effect of the relatively high molecular weight when compared to other POS and the high degree of esterification when compared to pectin. These results suggested that ultrasound assisted H_2O_2/ascorbic acid treatment could promote the improvement of antioxidant activity of

POS as well as in vitro hypoglycemic and immunomodulatory activities by formation of lower molecular weight molecules. In this context, Li et al. (2019b) reported the use of an ultrasound-accelerated non-meta Fenton-like process (H_2O_2/ascorbic acid) for depolymerization of pectin from citrus canning processing water. The results showed that when H_2O_2 was combined with ultrasound the molecular weight of oligomers could be reduced to below 20 kDa, suggesting that while H_2O_2/ascorbic acid system is an efficient system to generate POS and low molecular weight pectin fragments, ultrasound enhances the efficiency of free radical depolymerization. This approach leads to formation of LPM enriched in RG-I, characterized by a highly branched arabinan structure, and POS. Findings indicated that one these fractions (LMP3) demonstrated greater antitumor activity against MCF-7 human breast cancer cells compared to native pectins, a difference that may be attributed to their molecular size.

3.3.3 Emerging technologies

When compared to pectin extraction, few studies describe the application of emerging technologies for POS production. This is mainly because interest in these oligosaccharides has only grown consistently in the last five years, with the predominant use of enzymatic hydrolysis mediated to pectinolytic enzymes and chemical methods, especially acid hydrolysis. However, considering the advantages described in subitem 3.2 regarding emerging technologies, their use in obtaining POS from pectins is promising.

Previously, the feasibility of ultrasound technology to accelerate the chemical process based on the Fenton Reaction was discussed, demonstrating that when this approach is applied there is a notable improvement in the efficiency and yield of POS production, as well as making the process more sustainable in terms of environmental, with faster processes and in some cases without the need for the use of iron catalysts (subitem 3.3.2). This approach was described by Wu *et al*. (2022) and Li et al. (2019b) to production of POS through ultrasound assisted metal-free Fenton (H2O2-ascorbic acid) reaction by depolymerization of okra pectin and pectin from citrus canning processing water, respectively.

748 The effect of ultrasonication parameters in depolymerization of pectin from sweet potatoes
749 (*Ipomoea batatas* L.) was studied by Ogutu and Mu (2017), and this technology was able to
750 reduce the molecular weight by random scission of the pectin side chain. According to results,
751 increasing the ultrasound power, time and duty cycle led to increased sonolysis, resulting in
752 low molecular weight pectin fragments (probably POS), on the other hand, was proved that
753 pectin concentration had a negative correlation with sonolysis. More recently, similar effects
754 were described by Zhong et al. (2024) in a study that evaluated the impact of H2O2-assisted
755 ultrasonic bath on the degradation of commercial pectin (Mw of 466×103 Da). At a pectin
756 concentration of 0.3 % (w/v), H_2O_2 concentration of 2 % (v/v), and varying the ultrasonic power
757 of 30 to 120 W, these authors suggested that degradation rate of pectin is directly proportional
758 to the increase in ultrasound energy allowing low molecular pectic oligomers. The results
759 showed that degradation rates of pectin were 45.36, 50.35, 59.62, and 64.82 % and the Mw
760 were 8.22, 5.99, 5.39, and 3.39×103 Da when the ultrasonic power was 30, 60, 90, and 120 W,
761 respectively, at 30 min. Additionally, after H_2O_2-assisted ultrasonic bath degradation at H2O2
762 concentration of 0, 0.5, and 2 %, the Mw of pectin decreased to 304.5, 19.77, and 5.39×103 Da,
763 respectively. Thus, the results shown that pectin degradation rate was positively correlated with
764 the concentration of H2O2 and this can be related to enhance of reactive radicals formation
765 under H_2O_2-assisted ultrasound treatment and these radicals lead to more effective degradation
766 of pectin.
767 Another study compared the extraction of POS from sweet potato using hot-water extraction
768 (HWE), microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), and
769 optimized ultrasound- microwave-assisted extraction (UMAE) (Guo et al., 2019).
770 Comparatively, the optimized UMAE procedure (100 s extraction time, 300 W ultrasonic
771 power, and 200 W microwave power) exhibited higher extraction efficiency that HWE, MAE,
772 and UAE methods. This differential efficiency of UMAE was attributed to synergistic effects
773 between cavitation by ultrasound coupled with the heating and expansion due to microwaves,
774 which lead to severe rupture of the plant tissue used as substrate allowed accelerate the release
775 of POS, resulting in better yields.
776 The use of pressurized techniques also was reported for POS production. The production of
777 POS from depolymerization of apple pectin through dynamic high-pressure microfluidization
778 (DHPM) was described by Chen et al. (2013). Under optimal conditions (1.84% of pectin, 155
779 MPa, 63°C and 6 cycles passes) 605.7 mg/100 mL of POS was obtained, equivalent a yield
780 approximately of 33%. In addition, the POS extract obtained by DHPM increased the number
781 of *Bifidobacteria* and *Lactobacilli*, and produced a higher concentration of acetic, lactic, and

propionic acid than apple pectin, suggesting their prebiotic potential. POS derived from apple pectin were also produced using high hydrostatic pressure assisted enzyme (E-HHP) technology and this process was carried with 2% of pectin and 350 MPa for 20 min followed of enzymolysis for 60 min with 0.011 U/mL enzyme. In the optimal pressure-holding time (20 min) 0.956 mg/mL of POS were achieved and the mass spectrometry data suggested the presence of galacturonides with a DP from 2 to 6, mainly Di-GalA and Tri-GalA. Regarding the functional properties, POS exhibited inhibitory effects on cell degranulation, as well as can be inhibit the extracellular Ca^{2+} influx of RBL-2H3 cells by inhibiting SOCE channel function and IP3R function, suggesting their potential as promising targets for development of therapeutic agents for allergic diseases.

Finally, only one study reported the use of electrotechnology for POS synthesis. In this context, pulsed electric field (PEF)-assisted enzymatic treatment was used to produce POS from orange peel powder (OPP) and orange segment powder (OSP) (Thikham et al., 2023). The results showed that 19.16% of POS were achieved from OPP substrate using 10 kV/cm of field strength for 5 min combined with combined with 1.75% of Cellulase XL-531, pH 5.5, at 40 °C for 2 h, while 17.51% of POS were produced using 7.5 kV/cm for 5 min and the same enzymatic procedure described. In addition, these authors suggested that the POS consisted in oligogalacturonic acids with various degrees of polymerization. However, in this study fractionation or purification and complete chemical characterization of the oligomers produced were not carried out, which makes it difficult to compare the results of this study with others using other technologies. The main gaps in the published studies about the use of emerging technologies applied to degradation of pectin for POS production are due to the absence, in most cases, of robust chemical characterization and separation of the oligosaccharides produced, informing their DP, monosaccharide composition, and other structural features. If these studies provided which oligosaccharides were formed from the different treatments, it would be possible to effectively design processes for the generation of specific POS or standardized hydrolysates that would present the desired biological activities.

4. Fractionation and purification of POS

The extraction and hydrolysis processes lack selectivity and isolation capabilities, which requires the addition of supplementary techniques to remove unwanted compounds and even suspension particles. Several methods are available for the purification proposal, including, e.g., centrifugation, evaporation, precipitation, adsorption, and filtration (Castro-Muñoz and Fíla 2018). In the POS context, adsorption and filtration emerge as the favorite alternatives.

Simultaneously, these techniques are used as fractionation tools, enabling the production of POS products with different characteristics and offering prospects for diverse applications.

The most common technologies for purifying and fractionating POS are based on the adsorption process, which consists of mass transfer via the sorption of solutes onto a solid surface, referred to as an adsorbent (Hu and Xu 2020). Macroporous resins, ion exchange, and size exclusion represent the main adsorption processes in purifying POS. In this sense, Yan et al. (2023) used a resin column (AB-8 macroporous) to purify POS from orange peels, obtaining two fractions with a reduction in the polydispersity of molecular size distribution by 81% compared with crude extracts. For the proposal of fractionation, Li *et al*. (2019), Rajulapati et al. (2021), and Li et al. (2023) employed a size exclusion chromatography to obtain POS fractions with different molecular masses, via columns with diverse setups as Superdex 30, Polysep GFC-P6000, and Sephadex G-10. With a more specific proposal of separation, ion exchange columns are applicated. These columns are often combined with subsequent purification steps, such as size exclusion chromatography and resin columns. Liu et al. (2022) integrated the ion exchange and size exclusion columns (DEAE-cellulose and Sepharose CL-6B) to separate neutral and acidic POS from Osmunda japonica. At the same time, Gao et al. (2023) used a resin column (HPD-750 macroporous) combined with a size exclusion (Sephadex G-25) to isolation of seed melon POS.

Integration between adsorption and filtration technologies has been investigated to produce isolated POS fractions. For instances, Liu et al. (2021) developed a complex purification protocol for producing neutral oligosaccharides from crude POS extracts. They operated hyperfiltration tubes sequentially (according to the following order: 10 kDa > 3 kDa > 1 kDa) to fractionate POS as the first procedure. Then, the POS fraction of 1-3 kDa was employed in an ion exchange column (DEAE-sepharose CL-B6) followed by a nonporous graphite carbon column (Supelclean™ Envi-Carb™ 120/400) and, finally, was conducted Xbridge C18 (Xbridge Prep C18 OBD™) for a further purification. This approach resulted in a neutral POS with a molecular mass around of 1kDa and just composed of glucan, arabinoxyloglucan, and arabinogalactan. Despite the high selective capacity, the scale-up of protocols based on adsorption technologies can be very expensive and challenging for a long-term plan, such as absorbent acquisitions, regeneration process, and pollution risk (Gkika et al. 2022).

Furthermore, the inappropriate disposal of absorbents has the potential to evolve into an environmental problem, given their origin from mineral resources. However, the most complex issue about the use of the adsorption process on a large scale is the regeneration ability of the adsorbent, which method can be toxic and inefficient for removing the attached organic

compound and ions (Gkika et al. 2022). In this sense, filtration technologies via pressure-driven membranes have advantages for the proposal of POS purification due to, e.g., cost-effectiveness, operation simplicity, feasible scalability, and minimal risk of contaminants (Castro-Muñoz and Fíla 2018).

Membrane technologies consist of a mass transport approach via a semi-permeable barrier using a driving force to separate components from mixtures based on size, shape, and sometimes charge. These technologies use porous membranes to define the process selectivity, wherein pore size takes a key role in classifying filtrations. Membranes with 100 to 10,000 nm pore sizes are denominated as microfiltration (MF), retaining suspended particles and microorganisms. Ultrafiltration (UF), with pore sizes going from 2 to 100 nm, can retain diverse macromolecules, including smaller particles, colloids, and proteins. While nanofiltration (NF) uses porous membranes with smaller pores, varying from 2 to 0.5 nm, which can reject divalent ions, phytochemical compounds, peptides, disaccharides, and monosaccharides. Further, Some NF membranes can also have a dense structure without visible pores (Castro-Muñoz *et al.* 2020; Issaoui et al. 2022). Using membrane technology, it is possible to obtain different POS fractions with specific molecular weights, thus facilitating the purification of these substances from the hydrolyzed pectin solution (**Figure 3**).

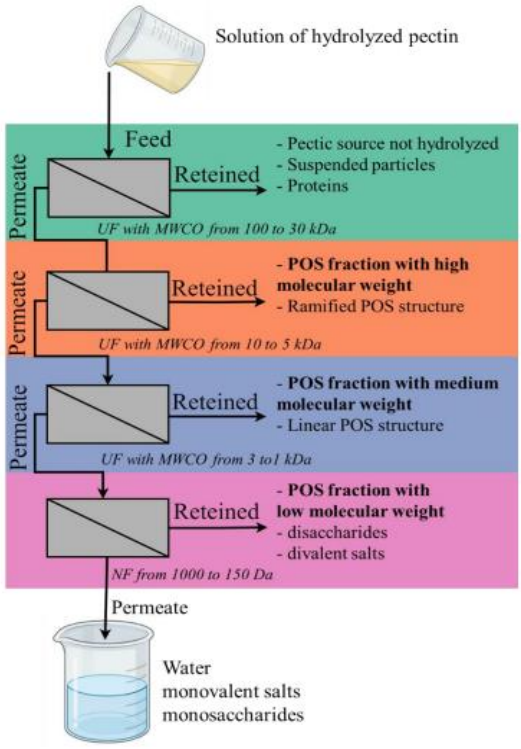

Figure 3. Schematic representation of the purification and fractionation of POS from hydrolyzed pectin using membrane technology.

In recent years, the application of membrane systems for POS purification has become usual. For instance, Ferreira-Lazarte et al. (2018) employed a device centrifugation with UF membranes (Ultracel®, Merck, Germany) with molecular weight cut-off (MWCO) of 3 kDa for the POS purification from sunflower and artichoke pectins. This method resulted in the POS fractions with molecular masses below 10 kDa. The device centrifugation coupled with UF membrane (MWCO: 1kDa) was also used by Sabater et al. (2021b; 2021a) for the POS fraction from artichoke pectin. In contrast, Baldassarre et al. (2018) developed a system of enzyme reactor coupled with a hollow fiber filtration unit (denominated as enzyme membrane reactor; EMR) via a crossflow pressure-driven system, which allowed the instantaneous POS separation from the onion skins not hydrolyzed. In a study extension, Elst et al. (2018) investigated the continuously operation of the EMR system for POS production from sugar beet pulp, drawing a comparison between conventional filtration and diafiltration (DF). It is worth noting that DF is a filtration configuration that enables molecular separation via solvent addition (dilute mode), acting as a carrier for small component. In a sequential work, Prandi et al. (2018) produced POS from sugar beet pulp using an EMR system. For fractionation purposes, they conducted POS product to an approach of sequential filtrations (according to the following order: 5-10 kDa > 1-5 kDa > 1000 to 700 Da > 700 to 400 Da > 400 Da) using flat polymeric sheet membranes.

Furthermore, other types of membrane system were also used for the POS purification. Gómez *et al.* (2019) investigated the POS purification from sugar beet pulp and lemon peel waste via a polymeric spiral membrane (MWCO: 1 kDa) in a concentration mode via DF operation. These authors achieved POS fractions with various molecular sizes (ranging from 5900 to 738 Da) and different degrees of oligosaccharide polymerization. While Zhu et al. (2019) obtained POS fraction from degraded products of hawthorn using flat sheet membranes with MWCO from 700 to 3000 Da. In a preliminary study, Foti et al. (2022) employed a sequential configuration of UF membranes (according to the following order: 100 kDa > 50 kDa > 30 kDa) to purify and fractionate POS from citrus peel waste. Considering all the information above, the membrane systems have demonstrated excellent performance in effectively separating POS from other components in a complex mixture and acting as a practical tool for fractionation. Due to these characteristics, several devices based on membrane filtration were developed, reflecting the need to address specific goals, volumes, and complexities inherent of each application. In this sense, Muhidinov et al. (2021) designed a pilot-scale crossflow filtration system to purify pectin from apple pomace and sunflower head residues, via a hollow fiber membrane operating at DF mode with a volume of 28 liters. This approach resulted in the removal of free impurities extracted along with pectin and in the concentration of neutral sugar.

Despite several advantages of applying membrane technologies, they have limitations, being the fouling the most relevant for POS purification. Membrane fouling is an inherent issue of filtration technologies, which consists of decreasing the mass transfer by accumulating unwanted compounds on the surface or within the pores. To mitigate this phenomenon, Muhidinov et al. (2021) employed a backwashing method using the feed flow during filtration, to decrease fouling formation and maintain high permeate flux. On the other hand, Elst et al. (2018) noted a decline in the mass trans after 32 hours of filtration, though they did not implement strategies to mitigate fouling during the operation of the EMR system. Indeed, the profound studies regarding fouling mitigation for POS purification have been little explored in the literature. Therefore, it is essential to increase the investigation of the fouling mechanism in the context of POS purification and fractionation in order to reduce the operational challenges and increase economic feasibility.

5. The biological effects of pectin and POS: focus in prebiotic-like effects and modulation of gut microbiota and related microorganisms

The main and widely accepted definition of oligosaccharides describes these compounds as carbohydrate oligomers composed of linked monosaccharide units via α- and/or β-glycosidic bonds, typically with a degree of polymerization (DP) ranging from 2 to 10. Moreover, some authors refer to prebiotic oligosaccharides as non-digestible oligosaccharides (NDOs) because these oligomers remain largely intact as they pass through the stomach and upper intestine, exerting their effects primarily in the colon (Roberfroid et al., 2010; Gibson et al., 2017; Hillman et al., 2017).

The colon (large intestine) harbors nearly 70% of all bacteria within the human body and this higher microbial diversity in the colon can be attributed primarily due to it being the primary location for bacterial fermentation of non-digestible food components, such as soluble fibers and prebiotic oligosaccharides (Donaldson et al., 2016; Hillman et al., 2017). Many of these bacteria are strict anaerobes and thus derive energy from fermentation of non-digestible substrates (Roberfroid et al., 2010). Additionally, the great microbial diversity in the colon is due to a much slower transit time as compared to stomach and small intestine and lower concentrations of endogenous antimicrobials (Donaldson et al., 2016).

The two major phyla found in the colon are Bacteroidetes and Firmicutes, with the predominant families being Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae, and Ruminococcaceae (Donaldson et al., 2016). Studies have demonstrated that diet-derived carbohydrates can influence the composition of the microbial community in the colon.

Moreover, maintaining an appropriate balance is essential for host health, while the development of dysbiosis is associated with various pathologies, including colon cancer, inflammatory bowel disease, non-alcoholic fatty liver disease, type II diabetes mellitus (T2DM), and others (Donaldson et al., 2016; Ou et al., 2022; Zhao et al., 2023).

Dysbiosis results from an imbalance in the microbial composition, which is often accompanied by a reduction in ecological diversity, the domination by few bacterial species, alterations in microbial functionality and metabolism, as well as shifts in their local distribution (DeGruttola et al., 2016; Roberti et al., 2022). On the other hand, the quantitative and qualitative structure of the gut microbiota, known as the eubiosis state, plays a crucial role in maintaining the overall homeostasis of the organism (Ou et al., 2022). In this context, oligosaccharides (e.g., FOS, GOS, XOS, POS) and certain polysaccharides, such as pectin, are crucial for maintaining intestinal homeostasis, as they can be fermented by microbiota bacteria, resulting in the production of short-chain fatty acids (SCFA) (Luo et al., 2021; Zhang et al., 2022). These products are related to health benefits including the inhibition of pathogenic bacteria, alleviation of constipation, lowered blood glucose levels, enhanced mineral absorption, reduced risk of colon cancer, and modulation of the immune system (Gullon et al., 2013). Many biological activities such as antioxidant, antimicrobial, anti-inflammatory, anti-allergic and antiproliferative have been reported to POS produced from different pectins by several methods, some of them are listed in **table 2.**

976 **Table 2.** Biological activities of POS obtained through enzymatic, chemical, and emerging technologies using different pectin substrates

Pectin source	Technological approach	Process conditions	POS characteristic	Biological effects of POS	Reference
Grapefruit (Citrus paradisi) waste	Enzyme technology	Viscozyme L with pectinase activity of 1198.5 U/mL, pH 4.6, substrate concentration of 1.0% w/v for 1 h and two different pectins: i) from Conventional method (CM) extracted with HCl; ii) from Pressurized carbon dioxide (pCO2).	DP range of 1–5	Antihypertensive effect POS from pCO2-pectin exhibited greater ACE inhibitory activity at the same amount as POS from CMpectin	Pattarapisitporn et al. (2024)
Mandarin (Citrus reticulata) peels	Enzyme technology	General conditions: 1 mL of reaction mixture containing 1% (w/v) of pectin,0.6 mM CaCl2 at 50 °C for 15 min. Reaction 1 (R1): 7 µg/mL of Recombinant clostridial pectate-lyase (CtPL1B) at pH 9.8. Reaction 2 (R2): 7 µg/mL of CtPL1B and 8 µg/mL of pectin-methylesterase CtPME at pH 8.5.	POS from R1: Monogalacturonate (DP1), unsaturated methylesterified di-galacturonate (DP2) and unsaturated methyl-esterified trigalacturonate (DP3); POS from R2: Monogalacturonate (DP1), unsaturated digalacturonate (DP2), and unsaturated trigalacturonate (DP3)	Biocompatibility with normal human kidney (HEK293) cells and cytotoxicity against colon cancer (HT29) cells ↓proliferation of HT29 cells in 24 h; ↓↓proliferation of HT29 cells in 48 h.	Rajulapati et al. (2021)
Haw (Crataegus pinnatifida Bge) fruit	Enzyme technology	0.2 U/mL commercial pectinase immobilized on an agar gel support; 2h of enzymolysis, pH 3.5 and 50 °C with 1% substrate concentration.	Chemical structure based in α-(1 → 4)-Dgalacturonic acid, DP5, Mw = 898; purity of 99%.	Anti-inflammatory effects ↓total liver fat content; ↓levels of TNF-α and IL-6; ↑level of IL-10; ↓gene and protein expression of NF-κB; ↓mRNA levels of RIP1,	Li et al. (2019c)

				NIK, IKKα, TNFR1, TRAF2 compared to high fat control (HFC).	
Commercial apple pectin	High hydrostatic pressure assisted enzyme treatment	Optimized conditions: 300 MPa, pressure-holding time of 20 min, enzymolysis time of 60 min with EPGM2 concentration of 0.017 U/mL.	0.960 mg/mL of POS were produced at optimized conditions. POS mixture: [GalA] = 60%; DE = 87.1%; DP from 2 to 6; 88% of GalA + Di-GalA + Tri-GalA	Anti-allergic effects ↓release of β-hexosaminidase and histamine; ↓ production of IL-4; ↓extracellular Ca2+ influx by inhibiting SOCE channel function and IP3R function.	Ma et al. (2022)
Swallow (Decalepis hamiltonii) root	Acid hydrolysis	100 mg of polysaccharide substrate, 10 mL of 2 M acetic acid in a boiling water bath.	Mw:831 Da, Rhamnogalacturonan I type, bearing arabinogalactan side chain with β-d-(1→4) galactose along with α-l-Araf (1→5)- α-l-Araf (1→3) structure on α-d-GalA-OAc-(1→2)- α-l-Rha-(1→4)- linear backbone.	Anticancer effects ↓galectin-3 mediated cancer promoting pathway; ↓mRNA levels of galectin-3; ↓galectin-3 and survivin levels.	Mallikarjuna, and Dharmesh (2018)
Pectic polysaccharides from citrus canning processing water	Ultrasound-accelerated non-metal Fenton-like chemistry	Optimal process conditions: 40°C, ascorbic acid concentration of 10 mM, hydrogen peroxide of 50 mM, 11.4 W/mL	Mw < 20kDa POS composed of residues of α-1,4-galactopyranosyl uronic acid and α-1,2- rhamnopyranose. β-1,3-linked-Galp units also suggests the presence of arabinogalactans.	Antitumor effects ↓ human breast cancer cells (MCF-7) growth; ↑Antitumor activity ↓Mw	Li et al. (2019b)

Orange peel wastes	Enzyme technology	The crude enzyme was produced by Aspergillus japonicus PJ01 through solid state fermentation using wheat bran as the substrate. Enzymatic hydrolysis of pectin: 4% of crude enzyme with pectinase activity of 48.3 U/mL; 45°C for 6h.	The results indicated that fungal crude enzyme cleaved the orange peel polysaccharides into different oligosaccharides, including glucooligosaccharides, OGalA, AraOS, and GalOS. Oligomers DP ≤ 5 were found in POS1-3, oligomers DP 6–8 was found in both POS2 and 3, and oligomers DP ≥ 8 were found only in POS3	Prebiotic effects POS with MW of 1–3 kDa; POS2 fraction showed higher prebiotic properties than POS1 and 3. Growth response of B. infantis and L. acidophilus after 24 h of cultivation: FOS > POS2 > POS1> POS3. Antimicrobial effects POS with MW of < 3 kDa; POS1-2 > POS 3. MIC against B. subtilis and S. aureus POS1-2 = 12.5 mg/mL; POS 3 = 25 mg/mL; MIC against E. coli POS1-2 = 25 mg/mL; POS3 = 50 mg/mL.	Li et al. (2016)
Apple pectin Citrus pectin Polygalacturonic acid	Alkaline hydrolysis	The hydrolysis was carried using H2O2 at 55°C, [H2O2] = 4% (v/v), and [NaOH] = 2 M for 8h.	Not provided.	POS react with HO radical to produce CO2 radical POS exhibited bacteriostatic effect. ↓ E. coli colonies 65% by addition of POS from apple pectin; 45% by addition of POS from citrus pectin;	Martinov et al. (2017)

Apple pectin; RG-I; High-methoxy homogalacturonan. pectin (HM); Low-methoxy homogalacturonan (HG).	Acid hydrolysis	10 mg of each type of pectin was dissolved in 2 mL of 1.2, 1.5 and 2.0 M TFA, and hydrolysis was then performed on heating block at 110 °C for 3 or 4 h.	POSs DP ranging from 2 to 7, and each starting material produced different structural forms	Immunomodulatory effects ↓ the inflammatory effect of LPS on macrophages Bacterio-modulatory effects ↑growth of several beneficial bacteria belonging to different classes. Bacterial members of the Firmicutes prefer low- molecular weight POS as compared to higher-molecular weight ones;	Singh et al. (2020)

977 POS: pectic oligosaccharides; GalA: galacturonic acid; Gal: galactose; Xyl: xylose; Ara: arabinose; DE: degree of esterification. EPG-M2: Endo-polygalacturonase;Tumor
978 Necrosis Factor-α: TNF-α; Interleukin-6:IL-6; Interleukin-10:IL-10; Receptor Interacting Protein Kinase 1:RIP1; NF-κB-inducing kinase: NIK; IκB kinase-α: IKKα; TNFα
979 receptors 1:TNFR1; Receptor-ssociated factor 2:TRAF2; DM: degree of methylation; DA: degree of acetylation

Studies have shown that both pectin and POS can reach and exert beneficial effects in the distal colon, an area associated with an increased risk of colon cancer and ulcerative colitis (Gullón et al., 2013). Additionally, there is evidence that the differential metabolism of pectic substances by the intestinal microbiota may be influenced by their structural characteristics. Considering the structural diversity of pectins, as discussed in section 2.1, it is expected that the oligosaccharides resulting from their depolymerization will also exhibit significant variability in their chemical structures. Thus, various types of POS can be generated, such as oligogalacturonides, arabinogalactan-oligosaccharides, rhamnogalacturonan-oligosaccharides, galacturonan-oligosaccharides, arabinoxylo-oligosaccharides, arabinoxyloglucan-oligosaccharides, arabino-oligosaccharides, and others (Babbar et al., 2014; Liu et al., 2021). However, there remains a need for further research to comprehensively understand their composition and prebiotic properties, given the limited available information about this promising class of molecules.

Structural features of pectins including degree of methyl-esterification, acetylation and rhamnogalacturonan I or rhamnogalacturonan II neutral side chains, affects their prebiotic and other biological activities, and the comprehensive studies about the structure-function relationships are considered interesting approach (Di et al., 2017; Beukema et al., 2020). Furthermore, some authors suggested that the pectin depolymerization it is carried by complex interaction of multiple bacteria consortia but recent understanding has established that a single strain can depolymerize the whole and complex structure of a pectin due to greater enzymatic machinery (Ndeh et al., 2017; Luis et al., 2018). It is possible to hypothesize that the structural factors may also be determinant for a different profile of microbiota modulation mediated by POS. The impact of structural arrangement, DE, and DP must be considered when evaluating the prebiotic effects of these low molecular weight oligomers (Li et al.,2016). Below we will discuss some studies focused on understanding how the differential degradation of pectins and POS occurs, as well as the repercussions in terms of modulatory effects and prebiotic activity.

Depending on their DM pectins undergo differential fermentation by the gut microbiota. According to the study performed by Tian et al (2016) using animal models, LM pectins are mostly fermented in the ileum, while HM pectins are predominantly fermented in theproximal colon. A recent study using an artificial colon model for in vitro batch fermentation demonstrated that pectin with different degrees of esterification influences the gut microbiota and metabolome in healthy adults (Huang et al., 2022). The results revealed that LM pectins exhibited more sustained effects on microbiota diversity, with increased abundance of families

Clostridiaceae and Lachnospiraceae, as well as genera Bacteroides and Lachnospira. On the other hand, HM pectin led to reduced levels of Enterococcus and Clostridium.

Recently, the prebiotic effects of two lemon pectins with different MW and DE on the gut microbiota of two donors were evaluated trought in vitro assay. These polysaccharides consisted in HMW-LDE (molecular weight of 308 kDa and 31% DE) and LMW-HDE (molecular weight of 122 kDa and 66% DE) (Firrman et al.,2022). The results suggested that LMW-HDE was able to modulate the community structure in a donor-dependent manner, with changes noted in Acidaminococcus, Paraprevotella, Psuedoramibacter, Alistipes, Oscillospira, and Fusobacterium, whereas HMW-LDE increased taxa within Lachnospiraceae in both donors. In addition, these pectins were able to increase the total SCFAs (1.49- and 1.46-fold, respectively) and increased acetic acid by 1.64-fold. These results corroborate the evidence that due to the complexity and structural variability of pectins, different modulation effects on the intestinal microbiota and consequent prebiotic activity will be observed.

Other study showed that HM pectins are degraded more slowly, and they are reported to persist in the intestine for up to 24h (Gómez et al., 2016). The pectin degradation depending on the microbiota-derived enzymes including methylesterases, acetylesterases, and lyases (Ndeh et al., 2017; Centanni et al., 2019; Elshahed et al., 2021). Thus, it can be hypothesized that the administration of POS could enhance the prebiotic effects of pectic substances, as these compounds would not necessitate this initial degradation step, potentially offering a quicker onset of prebiotic benefits.

As observed for pectin, in vitro and in vivo studies have demonstrated that the positive/stimulating effects of POS on the growth of probiotic microorganisms or on those derived from the gut microbiota depend on structural factors such as the type of monosaccharide constituent, their molecular weight distribution and degree of esterification (Singh et al., 2020; Wu et al., 2022).

Most in vitro studies focus on evaluating the viability of POS as a carbon source for the growth and stimulation of isolated probiotics, mainly Lactobacillus sp. and Bifidobacterium sp. strains, or gut microorganisms from feces and the quantitative and qualitative analysis of the SCFAs produced (Yeung et al., 2021; Thikham et al., 2023). Many authors have been used this approach as main tool to verify the prebiotic potential of pectic oligomers, but in fact, starting from the concept of prebiotic, there is a need for improvement to verify the clear health benefits. In vitro approaches lack robust correlation results between the ability of microorganisms to transform POS into SCFA and consequently generate a measurable beneficial effect for the host. However, it cannot be ruled out that this can be considered the first step as it demonstrates

that POS are capable of being used and modulating the intestinal microbiota, requiring in vivo approaches (animal models or clinical studies in humans) to consolidate them as prebiotics.

Regarding the effects of POS on probiotic microorganisms and gut microbiota, the main recent findings from this perspective will be discussed below. The modulatory effects of linear and branched POS (DP up to 7) on growth profile of gut commensal bacterial strains were investigated by Singh et al. (2020). These oligomers were produced from apple pectin (AP), rhamnogalacturonan-I (RG-I), and homogalacturonan (HM) through acid hydrolysis using trifluoroacetic acid. The results reveled that POS generated from HM and AG pectins were found to be more promising than the others for promoting the growth of Blautia producta, Bacteroides dorei, and Parabacteroides merdae, which can be explained by the presence of glycan-utilizing pathways and probably by the presence of CAZyme families and transporters observed in their genomes.

More recently, the influence of methyl-esterification and Δ4,5-unsaturation of galacturonic acid oligosaccharides (GalA-OS) produced from pectin using defined pectinases, on the fermentability by individual fecal inocula was assessed by in vitro batch fermentation (Zwolschen et al., 2024). The results showed that the metabolization of unsaturated GalA-OS (uGalA-OS) significantly increased butyrate formation compared to saturated GalA-OS (satGalA-OS), while satGalA-OS significantly increase propionate formation. Absence of methyl-esters within GalA-OS improved substrate metabolization during the first 18 h of fermentation compared to their esterified analogues and the data from the 16S rRNA sequencing suggested that GalA-OS stimulate unique microbiota compositions, depending on the methyl-ester substitution and saturation of POS.

It is reported that the main carbohydrate-degrading bacteria, including those able to fermentation of pectin, in the large intestine belonging the Bacteriodetes and that relatively few Gram-positive species of Firmicutes have been reported as able to ferment pectin or POS (Chung et al., 2016; Sheridan et al., 2016). Some species of this last phyla can be unable to utilize pectin but able to utilize specific POS as substrate. In this context, Eubacterium eligens DSM3376 was described as specialist pectin-degrading Firmicutes, able to utilize apple pectin due to pectate lyase of around 200 kDa that is expressed constitutively (Chung et al., 2017). Moreover, this specie was related to anti-inflammatory activity through promoting the production of IL-10 by epithelial cells in in vitro cell-based assays. In contrast, was described that some Faecalibacterium prausnitzii strains, although limited degrading pectin, shared with E. eligens the ability to utilize POS with DP4 and DP5, suggesting that these oligosaccharides can be used as a prebiotic fo promoting the growth of beneficial Firmicutes species.

Regarding the ability of Bacteroidetes phyla to pectin degradation in human gastrointestinal tract, some studies reported that species belonging the Bacteoides genus possess a unique genetic structure, polysaccharide utilization loci (PULs) and the specific PULs of pectin degradation in Bacteroides species can be considered a new field to study pectin metabolism in gut microbiota (Li et al., 2024). In this sense, an interesting study performed by Ndeh et al (2017) showed that the gut bacterium Bacteroides thetaiotaomicron was able to degrade RG-II, recognized as the most structurally complex glycan known, cleaving all but 1 of its 21 distinct glycosidic linkages. The results reveled that depolymerization of this polysaccharide are coordinated to overcome steric constraints, and the degradation involves previously undiscovered enzyme families and catalytic activities. The transcriptomic results showed that RG-II upregulates three PULs (RG-II PUL1–PUL3), being that RG-II PUL1 encodes several proteins that are the founding members of new glycoside hydrolase and esterase families. Similarly, a transcriptomic RNA-seq approach associated with directed mutagenesis was employed to investigate the pectinolytic function of B. xylanisolvens, considered an important human gut symbiont (Despres et al., 2016). When this microorganism was grown on citrus and apple pectins at mid- and late-log phases highlighted six PUL that were overexpressed on pectin relative to glucose and two overexpressed PULs (49 and 50) were assumed targeted HG and RGI, respectively, showing that this species deploys a complex enzymatic machinery that probably it is related to structural complexity of pectins.

On the other hand, the study of enzymatic diversity in Bacteriodes can contribute to the prospecting of new enzymes applicable in biotechnological processes for POS production as well as for the comprehensive analysis of the structure of RG-I pectins. Recently, the degradation of RG-I by two novel (Bo4416 and Bo3128) rhamnogalacturonan lyases 27 (RGL) from Bacteroides ovatus ATCC 8483 was evaluated, and the results suggested a substrate preference for RG-I compared to other pectin types (Wang et al., 2022). Moreover, these authors found that the smallest product of Bo3128- and Bo4416-mediated degradation of RG-I from Adenophora tetraphylla was unsaturated R disaccharide and that the remaining oligosaccharide products of Bo4416 comprised a series of unsaturated RG oligosaccharides lacking side chains, while a series of unsaturated oligosaccharide linked to galacto-oligosaccharides and arabino- oligosaccharides as side chains were produced by Bo3128. Thus, the in-depth study of the bacterial degradation of pectic substrates can help both in understanding how the digestion and fermentation of these molecules occurs, the relationship between the pectin and POS structure and the required enzymes for its utilization, as well as

providing support for the prospecting of new microorganisms or enzymes of biotechnological importance.

6 CONCLUSION

Although pectin extraction is industrially consolidated, there is a growing demand for more sustainable and environmentally friendly processes. In this sense, in the last ten years there has been an increasing number of studies on the extraction of pectins from conventional sources (e.g., citrus and apple pomace) using emerging technologies, mainly high-intensity ultrasound and microwaves. Furthermore, the exploitation of waste and by products from the fruit production chain and other industrial sectors as substrates for pectin extraction is considered a trend.

Pectins with different structural characteristics have been described depending on the plant source used as substrate, the extractive method applied and with specific technological and biological properties profile. Several studies have demonstrated that structural factors such as type, DP, DE, and molecular weight are crucial for some biological effects, including the ability to modulate the gut microbiota and related microorganisms.

Furthermore, pectin proves to be a low-cost substrate to produce functional oligosaccharides, POS, which have been considered as emerging prebiotics, but not only that, being recognized as molecules with antimicrobial, antioxidant, anticancer properties, among other properties. This wide variety of effects places these compounds in a prominent position for the possibility of developing new products and ingredients. From the analysis of recent advances, it is noted that there is a need to standardize studies for the depolymerization of pectin by different methods, in addition to the need to use appropriate fractionation, purification and characterization techniques that allow characterization of the chemical structure of the POS produced. Enzymatic processes using pectinolytic enzymes are consolidated, in second place come chemical methods, which are also considered fast and efficient, but improvements in these two approaches have been proposed, mainly with the association of emerging technologies.

Author Contributions

Joseane Cardoso Gomes de Alencar: Conceptualization, Methodology, Validation, Investigation, desig and perform the experiments. **Klycia Fidelis Cerqueira e Silva:** Conceptualization, Methodology, Validation, Investigation, desig and perform the experiments, Data curation, Writing-review & editing. **Miriam Dupas Hubinger:** Conceptualization, Data curation, Formal analysis, Writing-review & editing, Supervision, Funding acquisition, Primary responsibility for the final content. **Carmen Lúcia de Oliveira Petkowicz:** Conceptualization, Formal analysis, Writing-review & editing, Project administration, Funding acquisition, Primary responsibility for the final content. **Bruno Nicolau Paulino:** Conceptualization, Formal analysis, Writing-review & editing, Project administration, Funding acquisition, Primary responsibility for the final content.

Conflicts of interest

All the authors declare no conflict of interest with regard to the described research, the publication of the results, and financial issues.

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Capítulo III

Manuscrito: Dual-objective optimization of ultrasound assisted organic acid extraction of pectin from umbu (Spondias tuberosa L.), a promising Brazilian native fruit from Caatinga biome

Dual-objective optimization of ultrasound assisted organic acid extraction of pectin from umbu (*Spondias tuberosa* L.), a promising Brazilian native fruit from Caatinga biome

Joseane Cardoso Gomes de Alencar[a], Denise Nathiele Santos Souza Batista[a], Jacqueline Carvalho de Souza[a], Isabelle Palma Patricio Santos[a], Juliano Lemos Bicas[b], Maria Eugênia de Oliveira Mamede[a], Bruno Nicolau Paulino[a]*

[a]Bromatology Laboratory, Department of Bromatological Analysis, Faculty of Pharmacy, Federal University of Bahia, Rua Barão de Jeremoabo s/n, Ondina, Salvador, Bahia, Brazil.

[b]School of Food Engineering – University of Campinas, UNICAMP, Campinas, São Paulo, Brazil.

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***Corresponding author:**

*bruno.nicolau@ufba.br. Department of Food Science, School of Pharmacy, Federal University of Bahia, Rua Barão de Jeremoabo s/n, Ondina, Salvador, Bahia, Brazil

Abstract

Fruit waste and by-products have considerable potential for valorization as a source of value-added compound of industrial interest and unconventional substrates, including native fruits, are promising source of pectins. This study investigated the potential of umbu (*Spondias tuberosa* L.) peel as a raw material for pectin isolation using high intensity ultrasound technology and organic acid for development of an eco-friendly extraction method aiming high yields and adequate degree of esterification. After optimization through Central Composite Design (CCD) with three independent variables (2^3) and five levels high yield close to 22% of low esterified pectin (DE = 46%) was achieved under ultrasound amplitude of 60%, SLR of 1:33 and pH 1, 5. The extraction process was validated and the effects of different acids on the yield of pectins and DE were evaluated, demonstrating that the use of citric acid allows yields of around 22% of LMP pectin to be achieved, confirming the reproducibility of the process, while using oxalic acid , nitric acid and hydrochloric acid led to the production of around 13% of HMP pectin. The quality of pectins, assessed through instrumental color, showed significant differences when compared to commercial citrus pectin CCP, which demonstrated the influence of the type of raw material and extraction method on the quality of the product obtained. Therefore, our study describes for the first time the extraction of pectins from umbu peels using ultrasound technology, proving that this material is a promising source of LMP and HMP pectins, and that this approach can considered as efficient green method to obtain different food grade pectins with higher yield and quality.

Keywords: Pectin; ultrasound technology; umbu; *Spondias tuberosa* L.; optimization.

1. Introduction

Caatinga is a dynamic and heterogeneous biome exclusively Brazilian and restricted to the northeast and part of the state of Minas Gerais [1-3]. In recent decades, the growing interest in characterizing the commercial and bioactive potential of plant matrices from this biome, including medicinal plants and fruits [4,2]. Fruits from the Caatinga have economic and nutritional importance for the populations of the Brazilian semi-arid region, requiring studies to strengthen production chains, develop new products and use their by- products.

In this context, the umbu tree (*Spondias tuberosa* L.) is a plant native from semi-arid northeastern region of Brazil and its fruits, known as umbu, are highly valued and appreciated due to their characteristic taste and aroma [5]. Umbu is an important food for local communities, especially in times of drought, when the fruit is harvested in large quantities, sold in natura or processed, traditionally been used to produce juices, frozen pulps, nectars, jellies, jams, ice creams, beers, etc [6-9]. It should be noted that these products can be prepared using only the pulp or the pulp with the peel, making it possible to verify a high viscosity of these substrates, which can be attributed to the presence of pectic substances.

Pectin is complex heteropolysaccharide widely consumed as dietary fiber in human diet and play an important role in the maintenance of health [10-11]. This hydrocolloid is recognized as food additive in industry (E440) and can be used as stabilizer, emulsifier, and texturizer in diverse food products such as jellies, canned fruits, fruit juices, jams, and confectionery products as well as also been considered as fat replacer for production of low-calorie products [12-13].

Structurally, pectins comprising homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), arabinogalacturonan (AG), and xylogalacturonan (XGA) regions and HG is the most abundant pectic polysaccharide, composed of a linear chain of (1,4)-linked α-D-GalA units, which can be partly methyl-esterified at O-6 position and at lower extent also acetyl-esterified at O-2 or O-3 [14-15]. The commercial pectins are mainly derived from three agro-industrial residues, including citrus peels, apple pomace, and sugar beet pulp, and in the last years many studies have been proposed novel unconventional substrates (e.g., food processing by-products) as alternative sources of pectins [13, 16-17].

The conventional extraction of pectin is carried at low pH values mainly by use of mineral acids (e.g., hydrochloric acid, nitric acid) and high temperature, which leads to environmental pollution, a time-consuming process, low quality and yields due to degradation of pectin [16]. To overcome these problems, the replacement of mineral acids by organic acids (such as citric

acid and oxalic acid) as well as the use of new technologies such as ultrasound, microwaves, high pressure, pulsed electric field, among others, have been proposed [13]. In this sense, due to their low dissociation constant, organic acids have a lower hydrolyzing capacity than mineral acids, causing less depolymerization of the pectin structure [14, 17]. Among emerging technologies, high-intensity ultrasound stands out as the most used approach for pectin extraction, providing less time consumption, high yields, and preservation of the pectin structure since it does not require high temperatures.

The ultrasound technology comprises mechanical waves with frequencies between 20 kHz and 10 MHz, higher than audible frequency range of human hearing (20 Hz to 20 kHz) [18]. The main mechanism involved in the ultrasound assisted extraction (UAE) consists in acoustic cavitation, which involves collapsing cavitation bubbles and the sound waves that may resulting in fragmentation, localized erosion, pore formation, shear force, increased absorption, and swelling index in the plant matrix. The efficiency of ultrasound-assisted extraction is influenced by different factors including the ultrasound intensity and frequency, the process time and temperature, the solid-to-liquid ratio, the acid type and concentration, pH of the extracting solution, and the sonicator duty cycle (continuous or pulsed) [19-21]. This technology has been proposed in pectin extraction processes as well as for the oriented modification of pectins aiming to modify their rheological, chemical, and functional properties. In recent years, many studies have been developed both using ultrasound and combining this approach with other methods, mainly enzymatic and microwave methods [22-24]. In general, higher pectin yields are observed using UAE when compared to conventional acid extraction. Panwar et al. [25] developed an optimized ultrasound-assisted extraction process for obtaining pectins from Citrus limetta peels. They achieved a maximum pectin yield of 28.82% after 24 minutes of sonication at 40°C, 37% amplitude, and a pH of 1.9. This approach demonstrated significant advantages over conventional acid extraction, even under optimized conditions, where the pectin yield ranged from 3.97% to 22%. Recently, Singhal et al. [21] investigated the feasibility of using new technologies including UAE and MAE for pectin extraction from Citrus limon and comparing with conventional acid extraction. The highest pectin yield observed was close to 32% using UEA, followed by CE and MAE, which allowed 19.61% and 15.56%, respectively. These results clearly demonstrate the great potential of ultrasound technology, and this promising yield was achieved due to the optimization of ultrasound amplitude, sonication time, and solid-to-liquid ratio (SLR). Thus, the effects of the extraction method must always be

considered since they can significantly influence the structure and, consequently, the functionality of pectin.

In addition to the use of ultrasound technology and other emerging technologies for the extraction of pectins, it has been observed in recent years that most of these studies use strategies to optimize the extraction process. Statistical tools for experimental design and process optimization have also been employed to determine the best extraction parameters, with the Box-Behnken design (BBD), Factorial design (FD), and Central Composite design (CCD) being the most used for this purpose [26-28]. Some these experimental designs allow the application of the Response Surface Methodology (RSM), which results in the establishment of optimal conditions for each type of substrate from which pectins are intended to be extracted. The independent variables most used in these experimental designs include pH, solid-to-liquid ratio, temperature, time, and variables linked to the technology used such as nominal power (W), frequency (kHz), amplitude (%), energy density (J/cm^3) related to ultrasound methods.

Although many of the pectin extraction studies have used conventional substrates based on agro-industrial by-products and residues, such as citrus peel and apple pomace, the potential of unconventional substrates is notable [13,10].Thus, considering that several countries have specific fruit production chains, some of them based on native fruits, there is a clear need to investigate the pectin content of by-products from non-conventional vegetable matrices as an alternative for their use and stimulation of biorefinery. In this context, Brazil is one of the largest citrus producers in the world, ranking as the largest producer of oranges in the world according to Food and Agriculture Organization of the United Nations (FAO) database [29]. Furthermore, this country is also recognized for its great biodiversity, including several types and species of native fruits, which have been extensively investigated for their bioactive and functional potential [30-34]. However, fruits from Brazilian biomes and their by-products have been investigated regarding the content, variety of pectins as well as the technological and functional potential of these polysaccharides [35-37].

Thus, considering that umbu is a fruit native to the caatinga biome, which is economically important for several communities of small farmers and cooperatives, this study aimed to develop and optimize an environmentally friendly process for extracting pectins from peels of this fruit using ultrasound technology and organic acid. Firstly, a univariate study to define the best sonication time was carried out aiming for high yield and lower time and energy consumption. After, a Central Composite Design (CCD) was applied to evaluate the effects of ultrasound amplitude, SLR and pH on the yield and degree of esterification of the extracted

pectins at fixed sonication time. Finally, the UAE method was validated under optimal conditions for greater yield with evaluation of the influence of the type of acid (organic and mineral) on the quantitative and qualitative aspects of the extracted pectins.

2. Materials and Methods

2.1 Sample preparation

The umbu fruits were purchased at the São Joaquim market located in Salvador city, Bahia state, Brazil (S12.9730401; W38.5023040). The fruits were selected and separated according to their stage of ripeness and structural integrity (absence of lesions). The fruits were carefully cleaned, and the peel was separated from the pulp and seeds, then dried in a forced-air oven at 60°C until constant weight.

2.2 Production of umbu peel flour and alcohol insoluble residue

The dried peel was ground in an industrial blender and the particle size was standardized to 20-mesh and named as umbu peel flour (UPF). This material was subjected to extraction step for removal of alcohol-soluble compounds (organic acids, sugars, pigments, etc.) with 80% ethanol (1:3 w/v) for 10 min under ultrasonic treatment with an amplitude at 50% of nominal power in pulsed mode with pulse duration of 30 seg, cycle time of 1 min, and duty cycle of 50%. Thereafter, the mixture was filtrated, and the solid fraction was dried in an oven at 40°C resulting in the alcohol insoluble residue (AIR, 76% w/w of UPF). Then, AIR was characterized according to item 2.6 and used as substrate for pectin extraction using conventional and ultrasound assisted extraction.

2.3. Proximate composition, physicochemical and physical characterization of UPF and AIR samples

The nutritional composition of UPF and AIR were determined through AOAC Official Methods for fruit and fruit products. The moisture was determined gravimetrically by loss on drying method at 105°C (AOAC 934.06). The ash content was determined gravimetrically after complete incineration of the sample at 550°C in a muffle furnace (AOAC 940.26). The fat fraction was determined in an intermittent Soxhlet extractor using petroleum ether as solvent (AOAC 920.39C). The total nitrogen content was determined by the Kjeldahl method, using a multiplication factor of 6.25, typical for determining proteins (AOAC 920.152). Titratable acidity was measured by titration of the samples with standardized 0.1 N NaOH solution and the results were expressed as g/100 mL of citric acid (AOAC 942.15). The total carbohydrate

content was obtained by difference. The instrumental color was measured in a CR-400 Chroma Meter (Konica Minolta) using the CIELAB as described in item 2.7.5.

2.4 Preliminary study of pectin extraction using UAE and statistical optimization

2.4.1 Selection of sonication time for UAE and its effects in pectin yield

Extraction time is an important variable and widely used in experimental designs to optimize pectin extraction, but it can generate interaction effects with other process variables, making them statistically significant. Furthermore, it is known that long periods of ultrasonic processing can lead to the degradation of biopolymers such as pectins, in this study we proposed to evaluate the influence of time by fixing other three variables as ultrasound amplitude, pH and SLR. The extraction was performed at different times, including 0, 6, 10, 18 and 23 min, using 1:33 (w/v) of solid to liquid ratio (SLR) based in AIR (w) and citric acid solution (v) at pH 2.0 and employing 50% of ultrasound amplitude. The extraction was carried out using an Ultrasonic Processor (Sonics Vibra-Cell VC 505), frequency of 20 kHz and maximum power of 500 W, equipped with a 13 mm diameter probe, in pulsed mode with pulse duration of 30 seg and cycle time of 1 min, resulting in a duty cycle of 50%. The energy density (ED) was calculated based on the nominal power input (W), processing time (s) and sample volume (mL) (Eq. 1). After the sonication process, the extracts were used for recovery of pectin as described below. Additionally, the temperature was measured before and after the ultrasonic treatment to establish the relationship between the increase of sonication time, ED and increase in temperature (ΔT). All experiments were carried out in triplicate and the best time was selected considering the binomial greater extraction yield and lower energy expenditure.

$$Energy\ density\ \left(\frac{J}{mL}\right) = \frac{Nominal\ power\ (W) * processing\ time\ (s)}{Sample\ volume\ (mL)} \quad (1)$$

2.4.2. Dual-objective optimization pectin extraction process

A CCD with three independent variables (2^3) and five levels was employed to optimize the ultrasound assisted extraction of pectin from umbu peel aiming to understand the effects of different variables on the quality of pectin and finally establish the best conditions to achieve high yields of pectin with different degrees of esterification. Thus, the target dependent variables in this study were pectin extraction yield (Y_1%) and degree of esterification (Y_2,%). The levels of independent variables including ultrasound amplitude (x_1), pH (x_2), and solid-to-liquid ratio (x_3) are presented in **Table 1.** Thus, appropriate content of AIR was mixed with the

extracting solution, citric acid at final volume of 200 mL. The extraction was carried out using the ultrasonic processor described previously with the best processing time selected in item 2.3.1. Multiple regression analysis was applied to determine the regression coefficients for the linear, quadratic and interaction terms. Experimental data were fitted to a second-order polynomial mathematical equation to express the relationship between independent variables and responses (dependent variables). The generalized form of second order polynomial equation was given as follows:

$$Y_n = \beta_0 + \sum_{y=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i}^{k} \sum_{<j=2} \beta_{ij} x_i x_j + e_i \quad (2)$$

Were, Y_n is the dependent variable, including Y_{py} to pectin yield (%) and Y_{DE} to degree of esterification; x_i and x_j are the independent variables ($_i$ and $_j$ range from 1 to k); β0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters (k = 3 in this study).

Table 1. Independent variables and their respective levels and values in Central Composite Design

Independent variable		Unit	Levels				
			-1,68	-1	0	+1	+1,68
x_1	Ultrasound amplitude	%	30	42	60	78	90
x_2	SLR	w/v	1:76	1:50	1:33	1:25	1:21
x_3	pH	-	1.5	1.9	2.5	3.1	3.5

2.4.3 Statistical analysis of optimization study

The reparameterization of the quadratic model was performed using only the significant terms. The significance of each effect and the regression coefficient were determined considering a significance level of 5% ($p < 0.05$). In addition, One-way analysis of variance (ANOVA) using the significant coefficients was performed to verify the statistical accuracy of developed mathematical models and included the F-test and obtention of the coefficient of determination (R^2). All the statistical analysis was carried out using the Statitica®10 package software (Stat Soft Inc., Tulsa, USA).

2.4.4 Validation and evaluation the effects of different acids on pectin yield and DE

The validation of mathematical models from optimization study was carried out usingextraction conditions to achieve high yield and low DE to verify the accuracy of of the models.The predicted

and experimental of responses were statistically compared and the percentage error and residual were calculated according to Zaid et al. [38], at which less than 10% of erroris relatively desirable [39]. Furthermore, the effects of acid type (organic and mineral) on theextraction yield and DE of pectin were verified as well as their impact in color quality parameters.

2.5 Conventional extraction of pectin

The acidic hot water extraction (AIWE) was performed according to the method described by Gharibzahedia et al [40] with adaptations. Pectins were extracted from AIR with water acidified with citric acid until stability in a thermostatic water bath under stirring at 70°C for 30 minutes, SLR and pH from the optimal conditions for higher yield from 2.4.2.

2.6 Pectin recovery and yield determination

After the extraction process, the extracts obtained were collected and centrifuged at 4.200 rpm for 20 min at 10°C. The supernatant was then separated by filtration, precipitated with ethanol 96% (1:3 v/v) and left to stand overnight at 4°C. The coagulated pectin was collected and washed three times with ethanol 96% and the wet pectin was dried in a forced air circulation oven at 35°C until constant weight. Finally, the dried sample was ground into powder and the yield of extracted pectin was determined as follows **(Eq. 3):**

$$Yield\ (\%) = \frac{Weight\ of\ dry\ extracted\ pectin\ (g)}{Weight\ of\ AIR(g)} * 100 \quad \textbf{(3)}$$

2.7 Chemical and physical characterization of pectin

2.7.1 Galacturonic acid content

The galacturonic acid content was determined by the metahydroxydiphenyl (MHDP) method developed by Blumenkrantz and Asboe-Hansen [41] with adaptations. An analytical curve was prepared using a standard solution of galacturonic acid (GalA) in Concentrations from 5 to 100 $\mu g.mL^{-1}$. Pectin solution (0.4 mL) was poured into a tube and sulfuric acid/sodium tetraborate (2.4 mL) was added and cooled in a cold-water bath. Your tubes were stirred by a vortex mixer, heated in a water bath and cooled. The above-mentioned color reagent was then added and stirred for 5 min before reading the absorption at 520 nm using a UV/Vis spectrophotometer.

2.7.2 Degree of esterification (DE)

The degree of esterification (DE) was determined using the titration method as reported by Panwar et al [25]. The pectin samples (100 mg) were moistened in ethanol (2 mL), dissolvedin distilled water (20 mL), and stirred until completely dissolved. Five drops of phenolphthalein were then added, and the solutions were titrated with 0.1 N sodium hydroxideuntil a pale pink color appeared (V1). Then 0.1 N sodium hydroxide (10 mL) was added to the titrated samples and stirred for 15 min. Next, 0.1 N hydrochloric acid (10 mL) was added,and the samples were shaken vigorously until the pink color completely disappeared. Finally,the samples were titrated again with 0.1 N sodium hydroxide until a pale pink color appeared(V2). The DE was calculated using the following formula **(Eq. 4):**

$$DE(\%) = \frac{V2(mL)}{V1(mL)+V2(mL)} \quad \textbf{(4)}$$

2.7.3 Instrumental color

Instrumental color of pectin samples was measured using a CR-400 Chroma Meter colorimeter (Konica Minolta) using the CIE L. The color coordinates obtained were L* (brightness ranging from 0 (black) to 100 (white)), a* (+ a* indicating tendency for red and - a* tendency for green), b* (+ b* indicating tendency for yellow and -b* tendency for blue) system. In addition, the hue angle (h0) which represents the qualitative attribute of color was calculated using **Eq. (6),** while the chroma index (C*) was calculated using **Eq. (7).** The colorimeter was calibrated in the reflectance mode, using the illuminant D65 and an observation angle of 10° [42].

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad \textbf{(6)}$$

$$h^0 = \tan\text{-}1\ (b^*/a^*) \quad (7)$$

3. Results and discussion

3.1 Chemical characterization of UPF and AIR

The plant material used as substrate (UPF and AIR) for pectin extraction were similar in terms of nutritional composition, with high values of total carbohydrates, around 85%, while the moisture content, total mineral content, of lipids and proteins were approximately 10%, 3%, 1.1% and 0.5%, respectively **(Table 2)**. These values are close to those recently reported by Cangussu et al. [43] where these authors evaluated the proximal composition of flours produced from the peels of ripe and semi-ripe umbu fruits collected from the Caatinga biome. The differences can be attributed to geographic and seasonal factors that notably affect the composition of fruits and vegetables, as the umbu fruits in the aforementioned study were

collected in the portion of the Caatinga biome in the state of Minas Gerais, in the southeast region, while the fruits of umbu in the present study come from the portion of the Caatinga biome in the state of Bahia, located in the northeast region of Brazil. A significant difference for titratable acidity was observed, where AIR presented a lower value (0.29±0.01) compared to UPF (0.42±0.01), indicating the process of producing insoluble residue by prior extraction with ultrasound was able to remove alcohol-soluble organic acids.

Table 2. Chemical and nutritional composition of umbu flour and alcohol insoluble residue

	UPF	AIR
Proximate composition		
Moisture (%)	10,16±0,50[a]	10,37±0,40[a]
Ash (%)	3,30±0,10[a]	3,20±0,40[a]
Lipids (%)	1,16±0,10[a]	1,15±0,10[a]
Protein (%)	0,50±0,10[a]	0,53±0,10[a]
Carbohydrates (%)	84,93±0,50[a]	84,71±0,70[a]
Physicochemical parameter		
Total titratable acidity (% citric acid)	0,42±0,01[a]	0,29±0,01[b]
Color coordinates		
L*	61,31±0,04[a]	68,01±0,27[b]
a*	4,00±0,06[a]	4,05±0,12[a]
b*	13,30±0,08[a]	10,89±0,27[b]
C*	13,89±0,10[a]	11,67±0,29[b]
h^0	73,27±0,04[a]	68,93±0,08[b]

Mean ± standard deviation for triplicate. Results followed by different superscript letters in the column indicate a significant difference ($p < 0.05$) using the Tukey test.

Regarding color analysis, AIR presented a higher value L* coordinate, indicating that this substrate was lighter when compared to UPF, probably due to the removal of natural pigments in the insoluble residue production stage. Significant differences were observed between the h0 values of UPF and AIR, which ranged from 73.27 to 68.93, respectively, indicating a yellowish/beige color for these substrates, with AIR being slightly lighter compared to umbu peel flour. Regarding coordinate a*, no significant difference was observed, with small positive values, close to 4.00, for both substrates. This result is related to the degradation of chlorophyll and carotenoids present in umbu peel, leading to browning reactions during the drying steps employed produce UPF and AIR, resulting in particulate matter with color tending from dark greenish to reddish, characteristic of fruit powders and flours produced after drying processes[44] Regarding the b* coordinate, a significant difference was observed between UPF and AIR, with a higher value for UPF (13.30±0.08), indicating a more yellowish tone for this

material, which may probably be related to a higher carotenoid content, which were removed during the extraction step that results in the production of AIR (10.89±0.27).

3.2 Selection of sonication time for UAE of pectin from umbu peel

The preliminary and univariate study for selection the best processing time for extraction of pectin from umbu peels using UEA was carried out using 1:33 of SLR at pH 2.0 and 50% of ultrasound amplitude. The results showed that 4.82% pectin yield was achieved from the solubilization of AIR in citric acid solution (pH 2.0) without ultrasonic processing **(Fig. 1A),** indicating the presence of a considerable content of pectic substances in this substrate. Using pulsed ultrasonic processing for 6 min at 50% amplitude (50W), the ED was 82.33 J.cm-^{3} and allowed a pectin yield of 12.20%, approximately. The pectin yield was significantly increased ($p<0.05$) to 13.76% and 13.26%, when the UAE method was carried out for 10min (143.04 J.cm-^{3}) and 18 min (258.30 J.cm-^{3}), respectively. These findings are associated with the greater intensity of the cavitation effects generated by ultrasonic waves on plant material particles of AIR resulting from the increase in sonication time. Additionally, a directly proportional relationship was observed between the sonication time with ED and the temperature in the extracting solution, which together contribute to the improvement of mass transfer, leading to greater amounts of extracted pectin **(Fig. 1B).**

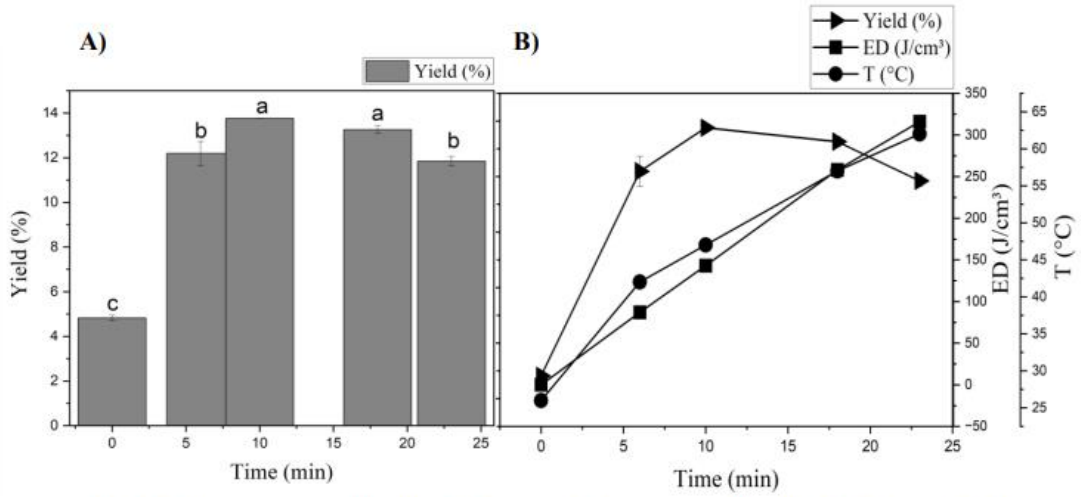

Fig.1 Influence of sonication time, temperature, and energy density on the pectin yield. A) Variation of pectin yield after different sonication times. B) Correlation between sonication time and increase of temperature and energy density and their impact on the pectin yield.

In contrast, a significant decrease in pectin yield was observed by increasing the extraction time to 23 min, where 11.85% of pectin was obtained. The reduction in pectin yield when extraction was carried out for 23 min can be explained by the high energy density (315.91 J.cm-^{3}) applied, which probably resulted in partial degradation of the polysaccharide chain into low molecular

384 weight oligomers. These degradation products may comprise mixtures of mono-, disaccharides
385 and mainly pectic oligosaccharides, which are more soluble in the acid extracting solution and
386 are not recovered in the alcohol precipitation step since they are removed in the subsequent
387 filtration and washing steps, reducing the amount of recovered pectin. In pectin extraction
388 processes, this effect must be avoided, but it is known that power ultrasound has been reported
389 as promising approach for oriented degradation and modification of polysaccharides, including
390 pectin, resulting in structural and functional changes [22, 45- 46].

391 In general, extraction processes of natural compounds of industrial interest (e.g., pectin) must
392 be robust, reproducible and it is desirable that they be low cost, with reduced time consumption,
393 less energy expenditure and consequently reduced environmental impact. These factors are
394 crucial for enabling the extraction processes for pilot scale and for scale-up purposes in
395 industrial level [47]. Although the application of other emerging technologies for extracting
396 value-added compounds, such as pectins, from agro-industrial by-products is onsidered
397 promising, it is known that some of them require pressurized apparatus such as those used in
398 high-pressure techniques or require specific components to generate heat or for strict
399 temperature control such as microwave-based methods [47-50]. In this context, high- intensity
400 ultrasound has been widely proposed for the extraction of pectins due to its clear advantages in
401 terms of improving extraction and reducing time consumption in the process [24].

402 Thus, to develop a simple, fast, and efficient extraction process of pectin from umbu peels, we
403 do not propose strict temperature control since ultrasound can generate an increase in
404 temperature during the sonication process. Therefore, UAE can generate heat depending on the
405 intensity and time applied, increasing the temperature of the medium, being desirable in some
406 processes as it leads to improved extraction without the need for thermostatic baths or complex
407 temperature control devices. Figure 1B shows the correlation between ultrasonic processing
408 time (min), energy density ($J.cm^{-3}$), and temperature and the impact of these variables on pectin
409 yield. It is worth noting that with sonication time of 23 min, a considerable increase in
410 temperature was observed, $\Delta T23' = 36°C$, when compared to the other sonication times
411 evaluated ($\Delta T0 = 0$, $\Delta T6' = 16°C$, $\Delta T10' = 21$ °C and $\Delta T18' = 31.5°C$). This phenomenon may
412 also have contributed, associated with high ultrasound intensity, to the lower yield as it is
413 reported that depolymerization rate of pectin increases with temperature [51]. On the other
414 hand, we must highlight that this pectin degradation was attenuated by conducting the
415 sonication process in pulsed mode, as if we had opted for continuous mode the extent of the
416 temperature increase, and degradation would probably be greater. In pulsed mode, the

sonication is regulated on a periodic basis and through a switch on and off mode, in our study 30 sec, that allows a moderate heat in the solution [20]. This approach is interesting because causing lesser destruction of bioactive compounds through such intermittent relaxation when compared to continuous mode. However, variables such as pH, type of acid employed for extraction and temperature will influence the extent of the effects in pulsed mode and must be considered [22,52-53].

The negative effect of prolonged extraction time yield was reported by Sengar et al [54] in study about extraction of pectin from tomato processing waste using UAE, where a maximum yield of 15.21% was obtained at ultrasound power of 600 W after 8.61 min, while 14.29% was achieved after16 min using same ultrasound power, suggesting that inadequate power input can be affect the efficient extraction of pectin. These authors also associate that the phenomenon of lower yield at higher ultrasound power can be explained by the fact that long 420 exposer time and increasing power input causes degradation of pectin into low molecular weight compounds. A similar finding was found in UEA of pectin from waste custard apple peel (Annona squamosal), where the yields were close to 9.1% with a sonication time of 20 min, while with a time of 30 min, under the same conditions, the pectin yield decreased. to around 8% [55]. Previously, Xu et al [56] demonstrated that with increasing power density from 0.20 to 0.40 W/mL, the pectin yield increased from 22.67% to 27.27% and this can be explained by the fact that the cavitation bubble collapse became more energetic with amplitude or power increased. Moreover, these authors observed that power density was higher than 0.40 W/mL led to a significant decrease of pectin yield, concluding that degradation effect on pectin increased with the increasing of ultrasound intensity.

In this study, the statistical analysis of results revealed no significant difference ($p<0.05$) between the pectin yield achieved in 10 min and 18 min of ultrasonic processing, demonstrating that a UAE process conducted by 10 min would be the most advantageous as it allows reduction in process time (8 min) and energy consumption (difference of approximately 115 J.cm-3). Therefore, this sonication time was selected and fixed for the optimization study of ultrasound-assisted extraction of pectin from umbu peel.

3.3 Model fitting and experimental data analysis

In this study, a CCD was successfully applied to optimize the UAE of pectin from umbu peel. The experimental design consisted of a total of 19 experimental runs and the experimental and predicted values of pectin yield and DE obtained at these different extraction conditions are

shown in Table 3. The pectin yield varied between 10.97% and 21.82%, while the DE ranged of 46.48% to 83.54% indicating that by varying the extraction conditions, important differences are observed in the quantity and characteristics of the pectins obtained. The pectin content in umbu peels obtained using UAE are close to the described by Cangussu et al [43] where were extracted 16.69% of pectin from mature umbu peel and 20.41% from semi-mature umbu peel using microwave assisted method and citric acid solution. However, these authors did not carry out the chemical characterization of obtained pectins. Therefore, our study presented here is the first to extract pectins from semimature umbu peel (called "de vez" maturation stage) using ultrasound technology and the first to describe the chemical characteristics of pectins from this fruit.

Table 3. Central Composite Design with experimental and predicted yield (%, Y_1) and DE (%, Y_2) of pectin extracted from umbu peel using ultrasound-assisted extraction (UAE).

Run	x_1	x_2	x_3	Y_1		Y_2	
				Experimental	Predicted	Experimental	Predicted
1	42	1:50	1.9	16,06	16,73	62,68	64,13
2	78	1:50	1.9	15,60	16,41	63,59	64,95
3	42	1:25	1.9	15,64	16,25	60,82	62,45
4	78	1:25	1.9	14,43	15,36	61,58	62,97
5	42	1:50	3.1	13,62	13,18	82,12	84,12
6	78	1:50	3.1	12,24	12,12	82,18	83,93
7	42	1:25	3.1	13,28	12,96	83,39	85,41
8	78	1:25	3.1	10,97	11,33	83,01	84,94
9	30	1:33	2.5	12,46	12,71	83,06	80,47
10	90	1:33	2.5	12,01	11,07	82,96	80,76
11	60	1:76	2.5	13,98	13,99	83,54	81,27
12	60	1:21	2.5	13,63	12,93	83,23	80,71
13	60	1:33	1.5	21,82	20,59	46,48	44,64
14	60	1:33	3.5	13,67	14,22	82,86	79,91
15	60	1:33	2.5	13,48	13,62	82,33	83,17
16	60	1:33	2.5	13,26	13,62	83,44	83,17
17	60	1:33	2.5	13,65	13,62	83,01	83,17
18	60	1:33	2.5	13,76	13,62	83,61	83,17
19	60	1:33	2.5	13,83	13,62	82,64	83,17

Applying multiple regression analysis on the experimental data were obtained the regression coefficients and statistical parameters presented in **Table 4**. These coefficients were employed to generate the quadratic polynomial models which could express the relationship between the evaluated independent variables, including ultrasound amplitude (x_1), pH (x_2), and SRL (x_3), and our responses of interest, in this case represented pectin yield (Y_1, %) **(Eq.8)** and DE (Y_2,%) **(Eq. 9).**

$$Y_1(\%) = 13.62 - 0.49x_1 - 0.32x_2 - 1.89x_3 - 0.61x_1^2 + 1.33x_3^2 \quad \textbf{(8)}$$

$$Y_2(\%) = 83.17 + 10.49x_3 - 0.90x_1^2 - 0.77\,x_2^2 - 7.39x_3^2 + 0.75x_2x_3 \quad \textbf{(9)}$$

Table 4. Regression coefficients estimates and statistical parameters for Central Composite Design models of pectin yield (%, Y_1) and DE (%, Y_2) for pectin extracted from umbu peel using UAE.

Source	Coeff.	Std.Err.	t(4)	*p*
(a) Y_1				
Model	13.61955	0.102573	132.7791	0.000000
x_1	-0.48743	0.062137	-7.8444	0.001426
x_2	-0.31696	0.062137	-5.1009	0.006978
x_3	-1.89404	0.062137	-30.4814	0.000007
$x_1\,x_2$	-0.14250	0.081187	-1.7552	0.154075
$x_1\,x_3$	-2.2787	0.084908	-0.18500	0.081187
$x_2\,x_3$	0.06500	0.081187	0.8006	0.468203
x_1^2	-0.61085	0.062153	-9.8282	0.000601
x_2^2	-0.05577	0.062153	-0.8973	0.420291
x_3^2	1.33723	0.062153	21.5153	0.000028
(b) Y_2				
Model	83.1701	0.238897	348.1427	0.000000
x_1	0.08654	0.144721	0.5980	0.582077
x_2	-0.16778	0.144721	-1.1593	0.310809
x_3	10.48657	0.144721	72.4608	0.000000
$x_1\,x_2$	-0.07375	0.189087	-0.3900	0.716391
$x_1\,x_3$	-0.24875	0.189087	-1.3155	0.258667
$x_2\,x_3$	0.74625	0.189087	3.9466	0.016866
x_1^2	-0.90210	0.144756	-6.2319	0.003377
x_2^2	-0.76952	0.144756	-5.3160	0.006022
x_3^2	-7.38627	0.144756	-51.0257	0.000001

The effect of independent variables in the studied responses can be predicted by the sign of the regression coefficient of the models, where positive sign suggest that the linked variable has positive impact on the response, whereas those with negative sign lead to an opposite effect [57]. The statistical significance and quality of the constructed quadratic models were evaluated through analysis of variance (ANOVA) shown in **Table 5**. The results showed that the F-value of the model for yield and DE were 8.42 and 20.90, respectively, while the p-value was less than 0.05 for both models constructed. The calculated F-value was higher than critical F value ($F_{9;9;0.05} = 3.18$) obtained from F-distribution table considering $\alpha = 0.05$, indicating that the models were adequate and could be selected to explain and predict the variation of pectin yield and DE. Although the lack of fit for both models appeared significant (less than 0.05), this can be attributed to the very low value of the pure error calculated from the replicates represented by the central points, where the values are very similar. Furthermore, high correlation coefficients were observed for the pectin yield and DE models, with R2 of 93.80% and 97.41%, while the Adj-R2 were 87.61% and 94.38%, respectively. Similar findings regarding the adjustment of predictive models for pectin yield and DE were described in a study to optimize

the conventional extraction of pectin from orange peels, where the lack of fit these responses also proved to be significant [58]. However, high F-values (27.57 for pectin yield model and 6.40 for DE model) were observed as well as correlation coefficients R2 of 96.13% and 85.20%, and Adj-R2 of 92.64% and 71.88%, respectively, which indicating that the obtained models are fit to experimental data and suitable to predict the relationship between the dependent variables and responses. From the ANOVA data the linear terms of all variables were significant, while only the quadratic terms of ultrasound amplitude and pH were significant for the yield predictive model. For the DE predictive model, only the linear term for pH was significant, while all quadratic terms and the interaction between SLR and pH were significant. Thus, the substitution of the coded values relating to the independent 488 variables in the predictive models obtained in our study leads to predicted values (Table 3) very close to those found experimentally. Naturally, variations related to extraction process and even in the pectin recovery stages may cause small variations, but these do not invalidate the robustness and quality of the models.

Table 5. Analysis of variance (ANOVA) for yield (%, Y_1) and DE (%, Y_2) of pectin extracted from umbu peel using ultrasound-assisted extraction (UAE).

Source	Sum of squares	*df*	Mean square	*F-value*	*p-value*
(a) Y_1					
Model	87.89325	9	9.76591	8.4188	< 0.0001
x_1	3.24475	1	3.24475	61.5352	0.001426
x_2	1.37199	1	1.37199	26.0191	0.006978
x_3	48.99237	1	48.99237	929.1175	< 0.0001
$x_1 x_2$	0.16245	1	0.16245	3.0808	0.154075
$x_1 x_3$	0.27380	1	0.27380	5.1925	0.084908
$x_2 x_3$	0.03380	1	0.03380	0.6410	0.468203
x_1^2	5.09337	1	5.09337	96.5933	0.000601
x_2^2	0.04245	1	0.04245	0.8051	0.420291
x_3^2	24.40916	1	24.40916	462.9084	< 0.0001
Lack of fit	5.59288	5	1.11858	21.2133	0.005570
Pure error	0.21092	4	0.05273		
Total	93.69705	18			
R^2				0.9380	
Adj-R^2				0.8761	
(b) Y_2					
Model	2,253.689	9	250.4098	20.896	< 0.0001
x_1	0.102	1	0.102	0.358	0.582077
x_2	0.384	1	0.384	1.344	0.310809
x_3	1,501.820	1	1,501.820	5250.569	< 0.0001
$x_1 x_2$	0.044	1	0.044	0.152	0.716391
$x_1 x_3$	0.495	1	0.495	1.731	0.258667
$x_2 x_3$	4.455	1	4.455	15.576	0.016866
x_1^2	11.108	1	11.108	38.836	0.003377
x_2^2	8.083	1	8.083	28.260	0.006022
x_3^2	744.714	1	744.714	2603.624	< 0.0001
Lack of fit	58.566	5	11.713	40.951	0.001576
Pure error	1.144	4	0.286		
Total	2,313.399	18			
R^2				0.9741	
Adj-R^2				0.9438	

$F_{9;9;0.05} = 3.18$

3.4 Effects of extraction process variables on the response variables

Considering the fitted models described in the previous section, 3D response surfaces graphs and contour plots were constructed for study of the interactive effects of the independent variables on the pectin yield **(Fig.2)** and DE **(Fig.3).** In each figure the interactive effects between two independent variables and response could be observed which the remaining independent variable was fixed at the central point level, where in A) pH = 2.5, B) SLR= 3 % w/v and C) ultrasound amplitude = 60%. Thus, the main results of the interactive effects of the independent variables on the yield of pectin and DE will be discussed, understanding how each variable affects the responses to establish the best process conditions.

3.4.1 Effect of process variables on pectin yield

Generally, extraction processes are designed to achieve high yields of the compound of interest. In this context, most studies that propose improving pectin extraction aim to achieve high yields. From the analysis of the response surfaces and contour curves in **Fig.2**, it is possible to observe using moderate ultrasound amplitude, close to 60%, and SLR around 1:50 to 1:30 in acidic conditions with pH less than 2.0, higher yields can be achieved. **(Fig. 2A).** The characteristics of the plant material used as a substrate for pectin extraction as well as its proportion in the extraction medium affect the yield. Considering the analysis of the response surfaces and contour curves A and C **(Fig.2),** it is possible to verify that the increase in the solid-liquid proportion negatively affects the yield, with greater amounts of pectin being obtained with SLR up to 3 g/100mL or 1:33. The negative effect related to the increase in solute in extractive processes can be explained by the saturation of the extracting solution with a consequent decrease in the mass transfer rate and this saturation can compromise the efficiency of the cavitation process (e.g rupture of cavitation bubbles), leading to a decrease in yield. Similarly, Shivamathi et al. [55] reported the influence of SLR on yield in a study that aimed the optimization of pectin extraction from waste curtard apple peel. The yields obtained with 1:10 (6.54%) for example, was lower than the most assays employing 1:15 g/mL (6.52-7.94%), 1:20 (6.69-7.73%), 1:25 (7.12-8.22%) and 1:30 g/mL (7.58%) of SLR, which is probably related to the increase in saturation of the acid extracting solution that occurs when there is a large increase in the amount of solid and a decrease in liquid.

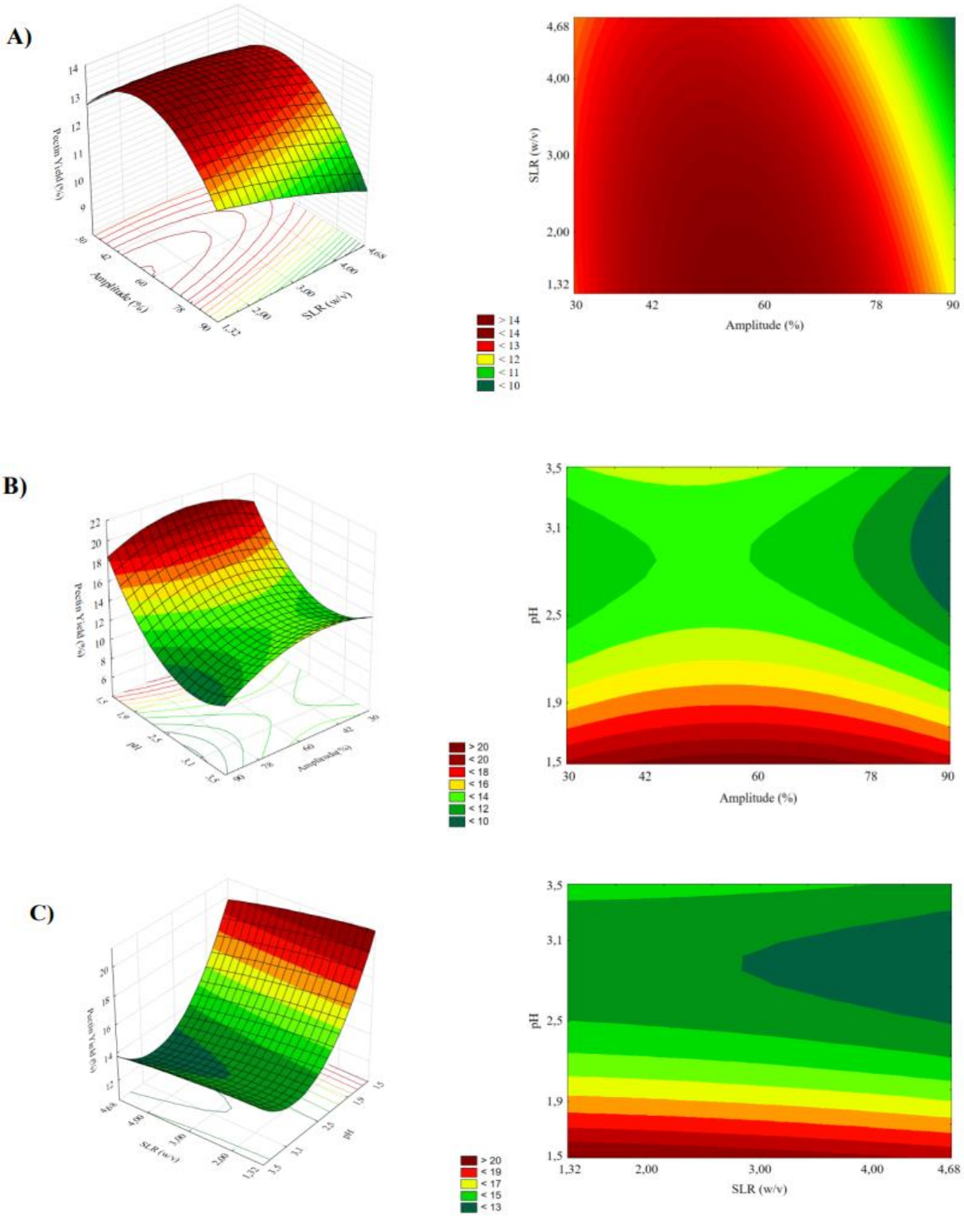

Fig.2 Response surface and contour plots of the interactive effects of two independent variables on the pectin yield. A) ultrasound amplitude and SLR. B) Ultrasound amplitude and pH. C) SLR and pH. The UAE processes were carried out room temperature (26°C) with sonication time of 10 minutes.

Furthermore, it should be noted that increasing the volume of extracting solution (liquid) allows a greater amount of pectin to be contained in the extraction solvent, thus obtaining a greater yield of pectin. This is due to difference of concentration that is established between the interior of the plant cell and the exterior solvent, thus increasing the mass transference of pectin from the solid matrix to the liquid solvent, resulting in a greater amount of pectin [59, 27]. Throughout extraction, the concentration of pectin in the solvent increases and the extracting liquid becomes viscous, leading to a decrease in the pectin mass transfer rate until it reaches the saturation point [48, 27]. This phenomenon depends on the total amount of pectin available on the substrate to be extracted, the particle size (which affects the surface area) and other process variables. Therefore, SLR is an important independent variable to be considered in pectin extraction optimization studies.

Due to its mechanical wave nature, characteristics of the ultrasonic wave such as frequency, wavelength and amplitude can influence the acoustic cavitation and therefore extraction [60]. In higher amplitudes, the ultrasound wave contains a high number of cycles of compression and rarefaction, leading to a more intense collapse of bubbles, allowing the targeted compound to be released into the solvent rapidly [61]. This parameter is directly related to power parameters that can be expressed as ultrasonic intensity (UI, $W.cm^{-2}$), power density (PD, W.mL-1) and energy density (ED, J/cm-^{3}), where the increase of amplitude leads to increase of these parameters, resulting in improvement of sonochemical effects and many cases lead to an increase of the extraction efficiency [60, 25]. The analysis of response surfaces and contour curves A and B **(Fig. 2),** it is possible to verify that the best ultrasonic amplitude range is between 50 to 60%, leading higher yields of pectin. These higher yields are achieved under conditions of low pH values and intermediate substrate amounts, between 1:50 to 1:33 of SRL. From the mathematical model for pectin yield (Eq. 8), we infer that there is a negative effect of increasing amplitude, since high amplitude values are related to an increase in energy density in the solution as well as temperature, which can contribute to depolymerization and reduced pectin extraction yield. Therefore, the ultrasound amplitude used in pectin extraction processes must be evaluated and optimized to have an adequate range for processing. Our results show that the use of 60% amplitude, equivalent to a power density of 0.26 W.mL-1 and ED of 149.08 J/cm-^{3}, the yields were greater than 13.26%, reaching a maximum yield of 21.82%. In contrast, using 90% amplitude, equivalent to 0.45 W.mL-1and 262.63 J/cm-^{3}, a yield of only 12.01% was observed, probably due to the degradation of the pectin structure into low molecular weight compounds. When 30% amplitude (0.11 W.mL-1 and 60.17 J/cm-^{3}) was used a yield of 12.46%

641 was achieved, like the yield using 90% amplitude. The lowest yield was observed at 78%
642 amplitude (0.385 W.mL-1 and 221.25 J/cm-^{3}), 1:25 of SLR and pH of 3.1. Probably, this finding
643 can be related to a greater quantity of AIR used, in addition to the pH of 3.1, a value greater
644 than the best yield range (pH < 2.0). The negative effect related to the increase in solute in
645 extractive processes can be explained by the saturation of the extracting solution with a
646 consequent decrease in the mass transfer rate and this saturation can compromise the efficiency
647 of the cavitation process (e.g rupture of cavitation bubbles), leading to a decrease in yield.

648 Some studies show that depending on the plant matrix used, maximizing amplitude can
649 contribute to higher yields. For example, in recent optimization study of the UAE of pectin
650 from Assam lemon (Citrus limon Burm f.), the ultrasound amplitude was found to have a higher
651 effect on the extraction, where the increase in the amplitude from 20 to 100 % resulted in
652 increase of pectin yield from 2.9 to 32.17% [21]. Similarly to our results, an optimization study
653 of UAE of pectin from Citrus limetta peels performed by Panwar et al [25] demonstrated
654 increase in amplitude till 40%, the yield of pectin increased linearly, but further increase in
655 amplitude beyond 40% reduced the pectin yield, demonstrating that the ultrasound amplitude
656 is a variable that must be controlled appropriately depending on the plant matrix used to extract
657 pectins.

658 One the most important variable evaluated in optimization studies of conventional and
659 unconventional extraction of pectin is pH or acid concentration [62-63]. Several studies
660 describe that pectin yield is greater at low pH values or in more acidic conditions. Traditionally,
661 pectin is extracted using strong mineral acids at pH ranging 1 to 3, and acidic conditions are
662 essential to hydrolysis of the complex cross-linked networks of the cell wall in plant material
663 and the insoluble pectin constituents, promoting the release of soluble pectin [62-64]. Our
664 results show that when pH 1.5 was used in UAE the pectin yield was 21.82% and with the
665 increase in pH to higher values a reduction in yield was observed. At pH 1.9 and 2.5, pectin
666 yields varied from 14.43 to 16.06%, and from 12.01 to 13.98%, respectively. At a pH of 3.1 the
667 yield varied from 10.97% to 13.12% and at a pH of 3.5 the yield was 13.67%. According to the
668 mathematical model obtained for yield, a strong negative effect of pH is observed, which
669 indicates and explains the results obtained where, in more acidic conditions, greater yields are
670 obtained. This result corroborates the study reported by Colodel et al. [65], which demonstrated
671 that pH was the most influential variable in the study to optimize the conventional extraction
672 of pectins from grape pomace.

673 The effect of pH in pectin extraction from orange peel was also observed in recent study
674 performed by Iñiguez-Moreno et al [66], where the results from Box-Behnken Design showed
675 that at pH 2.0 the pectin yield ranging 22.77 to 31.20%, while at pH 4.0 the pectin yield varying
676 from 3.00 to 3.99%. The effects of pH may vary depending on the duty cycle applied in the
677 UAE, where pulsed mode and low pH values, in some cases, have been shown to be more useful
678 for achieving higher yields when compared to continuous mode. In this context, the UAE of
679 pectin from pomelo fruit using citric acid at different pH values showed that in continuous mode
680 at pH 1.5 the yield ranged from 22.55 to 33.9%, while in pulsed mode the yield varied from
681 33.46 to 46.4% [20]. With the increase in pH to values above 1.5 to 3.5, a decrease in pectin
682 yields was observed, demonstrating that in ultrasound-assisted processes the duty cycle must
683 be considered to improve the extraction process. The UAE of pectin from navel orange peels in
684 continuous and pulsed mode was reported by Patience et al [53]. The results showed that in
685 continuous mode, pH 2 and power density of 0.24 W.mL-1 a yield close to 11% can be
686 achieved, with only a 1.3% difference between this yield to achieved at pulse ultrasound mode
687 at the same conditions. These authors concluded that pulsed mode can be considered more
688 efficient due to less energy consuming (80 kJ) when compared to continuous mode (190 kJ).

689 Therefore, in our study we proved that high levels of pectin with low and high DE can be
690 extracted from umbu peels, and a higher yield (greater than 21%) of low DE pectin (close to
691 44%) can be achieved using 60% ultrasound amplitude in pulsed mode, SLR of 1:33 and pH
692 1.5., while pectins with higher DE (greater than 80%) can be extracted with a yield of around
693 13% under the same conditions by only varying the pH of 1.5 to 2.5.

694 **3.4.2. Effect of process variables on degree of esterification of pectin**

695 Among the main chemical characteristics of pectins, the degree of esterification (DE), also
696 expressed relatively as the degree of methoxyl (DM), can be considered one of the main
697 chemical guidelines for the applications of this polysaccharide [64]. Other chemical
698 characteristics, including molecular weight (MW), monosaccharide composition (MC), RG-
699 I/HG ratio and degree of branching, are also reported to be important for the functional
700 properties of pectins, such as modulation of the gut microbiota [67].

701 The DE has been used mainly as indicator of the gelling and emulsifying properties of pectin.
702 Moreover, this parameter has been used to classify pectins into two groups, high methoxyl
703 pectins (HMP) and low methoxyl pectins (LMP). HMP pectins have a DE value greater than
704 50% and require solutes such as sucrose and pH lower to 3.5 for effective gelation, which occurs

705 through hydrogen bonds and hydrophobic interactions. LMP pectin, on the other hand, has a
706 DE of less than 50% and for gelation it depends on divalent cations, such as calcium ion, which
707 interact with the free carboxyl groups of galacturonic acid residues, forming gels over a broad
708 pH range. Recent studies have been reported the interesting applications of pectins with low
709 DE as for increase the water holding capacity and viscoelasticity of gluten proteins [68].
710 Considering the analysis of the response surfaces and contour curves of **Fig .3**, it is possible to
711 verify that all variables have some effect on DE, being pH the most important variable to be
712 considered, with linear, quadratic and interaction terms significantly. Thus, the positive effect
713 of pH it is evident, suggesting that increase of pH close to 3.0 leads to extraction of HMP, while
714 acidic conditions allowing the extraction of LMP. It can be seen in **Fig. 3 B** that regardless of
715 the amplitude and SLR used, the DE tends to increase with increasing pH. Thus, based on Fig.
716 3A it was possible to verify that higher DE are achieved using SLR of 1:33 and amplitude of
717 60%. These observation supporting the experimental data showed in **Table 3**, where it is
718 observed that at pH 1.5 was extracted higher amounts of LMP with DE of 46.48%, while at pH
719 of 1.9 the DE ranged from 60.82 to 63.59% and at pH of 2.5 the DE percentages ranged from
720 82.33 to 83.61%. DE percentages increases at pH of 3.1 to values varying from 82.12 to 83.39%
721 and at pH 3.5 the DE observed was close to 80%.

722 Similar effects were observed in optimization study of UAE of pectins in continuous and pulsed
723 mode from pomelo peels at different pH values, where in continuous UAE the DE ranged from
724 40.73 to 52.09% were observed when the extractions were carried out at pH of 1.5, while DE
725 ranged from 63.26 to 72.25% and 76.92 to 84.90% at pH of 2.5 and 3.5, respectively [20]. When
726 the UAE was performed in pulsed mode, the DE values at pH of 1.5 were slightly higher than
727 those obtained in continuous mode, ranging from 47.6 to 55%, while at pH of 2.5 the DE
728 varying from 67.8 to 75.2% and DE of 82.43 to 88.6% at pH of 3.5. This small increase in DE
729 by varying the duty cycle is possibly associated with a greater cavitation effect that can be
730 generated in continuous mode when compared to pulsed mode, thus in continuous mode there
731 is greater de-esterification of the pectin chain due to interactive effects of ultrasonic waves with
732 a more acidic environment. Acid de-esterification by hydrolysis is a pH-dependent reaction and
733 it occurs mainly under acidic conditions using strong inorganic acids and the increase of pH
734 lead to β-elimination as main mechanism of de-esterification of pectin [69]. Another study
735 reported that at pH of 2.0 the DE of extracted pectins from orange peel ranged from 28.93 to
736 34.76%, at pH 3.0 the pectins showed DE from 62.84 to 84.46% and at pH 4.0 the DE varied

from 72.46 to 90.86%, depending on temperature and time of depending on temperature and time of extraction [66].

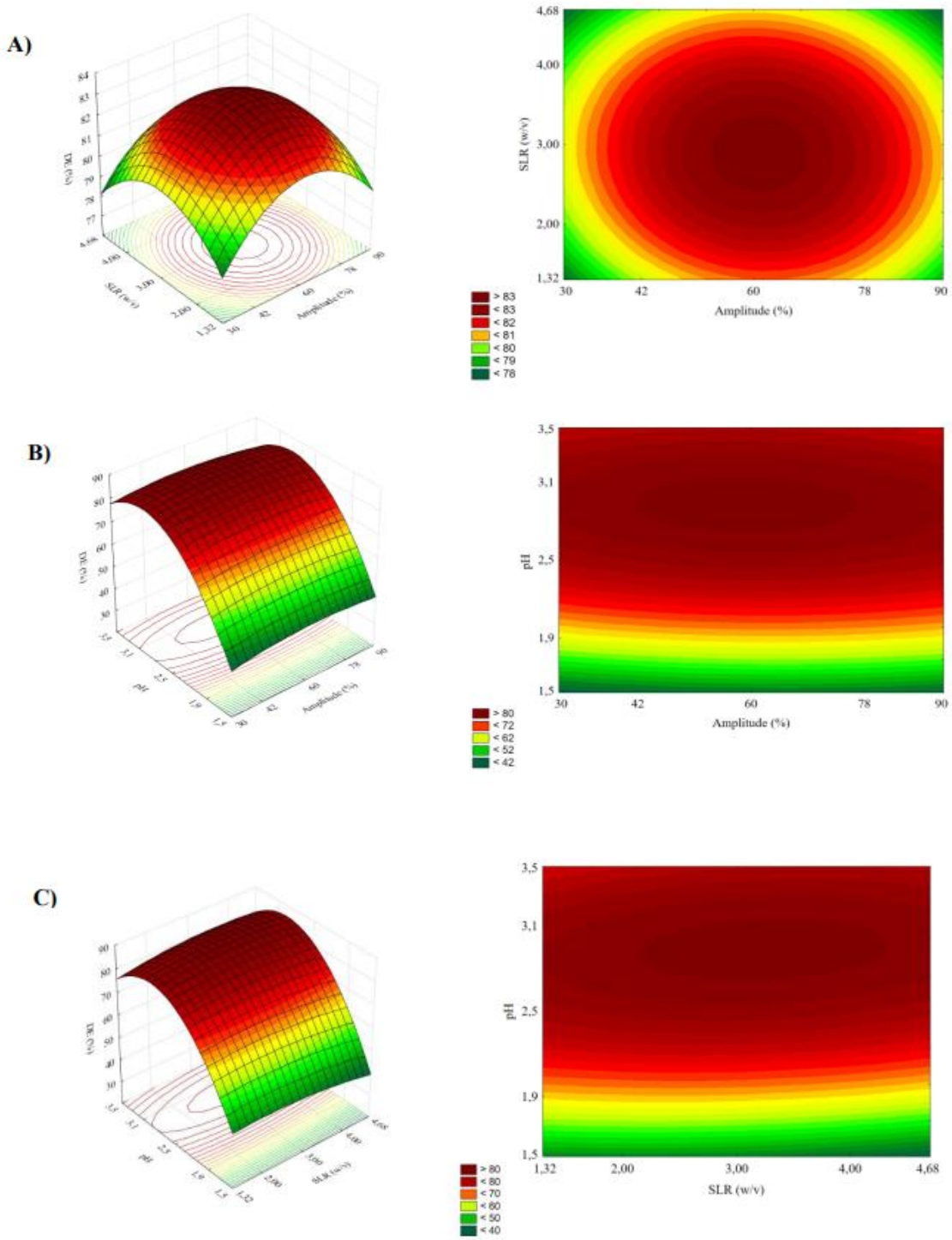

Fig.3 Response surface and contour plots of the interactive effects of two independent variables on DE. A) ultrasound amplitude and SLR. B) Ultrasound amplitude and pH. C) SLR and pH. The UAE processes were carried out room temperature (26°C) with sonication time of 10 minutes.

In comparative study performed by Tran et al. [70] pH was the most influencing variable on DE of pectin from jackfruit rags. For both conventional and UAE methods, DE of pectin decreased when pH was decreased, and lowest DE values of 11.71% in conventional method and 8.48% in UAE method were found at pH of 1.5. This can be explained due to increase de-estericationof the polygalacturonic chain under severe pH conditions [71].

Therefore, our results suggest that the use of experimental design would facilitate the establishment of conditions for the extraction of different types of pectin, making it possible to obtain HMP and LMP from umbu peels using UAE in pulsed mode by varying the experimental conditions.

3.5 Comparison between conventional heating extraction and ultrasound-assisted extraction

The conventional methods to extraction of commercial pectins are based in the use of hot diluted acids. In general, long times of extraction, high temperatures and low pH leads to high pectin yields but can be influence to the physicochemical properties. Thus, the exact extraction conditions are adjusted for each raw material to obtain pectins with suitable properties [14,72].

Many studies use reflux or stirring methods, generally employing temperatures greater than 60°C under acidic conditions using strong mineral acids. Furthermore, conventional methods are conducted for prolonged periods (several hours) which lead to high costs related to energy consumption [14]. In general, these processes are recognized as being expensive and there is concern about their alignment with the principles of “Green Chemistry”, as they employ strong mineral acids that result in waste that needs to be adequately treated after the extraction process [73]. In this context, many studies strengthen the trend towards using new technologies such as ultrasound, microwave, high pressure techniques, ohmic heating, and others, for pectin extraction [74]. Among the proposed technologies, ultrasound appears as the main emerging technology used in the extraction of pectins, with several advantages reported for this method alone or in combination with others, which has resulted in several studies in the last ten years using this approach. In this study, using the conventional method of hot acid extraction a yield of 12.16% was achieved and the extracted pectin presented a DE of 49.63%, while the UAE method proposed in this study allowed a high yield of LMP close to 22%.

Previously, significant differences on pectin yield from dragon fruit peel were reported using conventional and UAE at different times and temperature [75]. Using conventional extraction for 30 min at 45°C, 60°C and 75°C, the pectin yields were 4.98%, 8.42% and 10.44%,

respectively. On other hand, UAE for 30 min at same temperatures achieved Yield of 9.38%, 15.30% and 16.30%, respectively, demonstrating that ultrasound technology can be leads to higher yields using same raw materials. In addition, different extraction methods influenced the DE of pectins, where the conventional extraction for 30 min at 45°C, 60°C and 75°C leads to DE of pectins of 49.87%, 41.37% and 46.06%, respectively; while using UAE method the DE values were 37.64%, 36.80% and 35.44%, respectively. Recently, the efficiency of an optimized UAE method for extracting pectin from Assam lemon (Citrus limon Burm. f.) was described with yield close to 32%, while conventional extraction approach leads to pectin yield of 19.61% [21].

Therefore, based in our results UAE can be considered a favorable approach for obtaining pectins from umbu peels when compared to conventional method as it allows higher yields of different pectins (HMP and LMP) in a reduced processing time (10 min of sonication), which results in a process that is economically viable in terms of energy consumption. time and energy. Furthermore, the use of an organic acid increases the appeal and suitability of the process in environmental terms and in obtaining a product for the food industry.

3.6. Validation and evaluation of the impact of different acids in pectin characteristics

Considering the results obtained finding in the **Table 2** and the discussion above-mentioned through analysis of surfaces and contour plots, the optimum conditions were considered as ultrasound amplitude of 60%, 1:33 of SLR and pH of 1.5 for obtaining a maximum pectin yield. These conditions were set because reducing pH to values lower than 1.5 would result in a highly acidic environment that could, when associated with ultrasound, lead to pectin depolymerization. Furthermore, the reduction in pH would depend on an increase in the concentration of citric acid and the consequent increase in process costs for this substrate. The experiments were carried out in triplicate and to assess whether the results obtained so far were dependent on citric acid, different acids were evaluated, including oxalic acid, nitric acid, and hydrochloric acid, to investigate the impact on the yield and DE as well as the color parameters, here used as quality parameter, of pectins from umbu peel. Considering the results obtained in Table 6 and comparing with the predicted values in **Table 2**, percentage errors less than 10% were obtained for both the yield and the DE under the conditions established as optimal and using citric acid, indicating that the process is reproducible and can be considered validated [27]. The results shown that citric acid allowing higher yields of LMP, while HMP (DE > 50%) were obtained using both organic acid (POA) as mineral acids (PNA and PHA), demonstrating that the type of acid it is determinant on the yield and DE of pectins **(Table 6).**

Table 6. Influence of different acids on the yield and chemical and physical characteristics of pectin extracted by UAE from umbu peels under validation conditions.

Pectin	Yield (%)	DE (%)	GalA (%)	Color		
				L*	a*	b*
PCA	22.52±0,15^{a}	46.07±0,19^{e}	56.26±0,65^{d}	63.43±0,03^{e}	7.42±0,03^{b}	7.6±0,02^{c}
POA	11.93±0,47^{b}	75.82±0,23^{d}	70.8±0,72^{b}	72.31±0,02^{b}	7.14±0,01^{c}	12.86±0,08^{a}
PNA	11.29±0,66^{b}	82.94±0,17^{c}	61.92±0,28^{c}	69.21±0,10^{d}	8.16±0,02^{a}	11.23±0,02^{c}
PHA	10.20±0,17^{b}	84.00±0,2^{b}	70.57±0,53^{b}	71.91±0,01^{c}	6.41±0,01^{d}	12.07±0,02^{b}
CCP	-	89.08±0,17^{a}	75.86±0,47^{a}	91.34±0,03^{a}	0.97±0,02^{e}	10.49±0,01^{d}

Results followed by different superscript letters in the column indicate a significant difference ($p < 0.05$) using the Tukey test. PAC: pectin extracted with citric acid; POA: pectin extracted with oxalic acid; PNA: pectin extracted with nitric acid; PHA: pectin extracted with hydrochloric acid; CP: Commercial citrus pectin.

No significant difference was observed ($p < 0.05$) between the pectin yields obtained with the other acids evaluated, yields varying between 10.20 and 11.93%, while in relation to DE significant differences were observed, with PHA exhibiting the highest DE (84%), followed by PNA (82.94%), POA (75.82%) and PCA (46.07%). These results demonstrate that LMP can be obtained in high yields using the optimized UAE method proposed in our study and that by varying the type of acid HMP can also be obtained under the same conditions with a yield reduction of around 50% **(Fig. 4).** The extraction of LMP can be considered an interesting approach, as this pectin type has been pointed as suitable additive in the food industry. In this context, recent study described that LMP increased the water holding capacity (WHC) and viscoelasticity of gluten protein, as well as promoted the conversion of sulfhydryl groups (-SH) to disulfide bonds (-S-S-), inducing gluten aggregation and strengthened gluten network [68]. The addition of LMP led to improvement in technological and sensorial characteristics of low-fat set yoghurt, being able to reduce whey loss, improve firmness, rheology, quality, and overall liking of this dairy product [76].

The different effects of the type of acid, mineral and organic acids, on the pectin yield and DE were previously described for various raw materials, including jackfruit peel, pomelo fruit peel, grape pomace, and others [20,77-78]. The type of acid and pH of the acid used in the extraction process play a critical role in achieving higher pectin yields [79]. In this study, at pH 1.5, important differences were observed in the yield of pectins obtained from umbu peel using UAE. Using citric acid, the weakest acid of those evaluated, a higher yield and lower DE and GalA content were obtained, while the other acids led to yields between 10-11%, approximately, but with high DE and high GalA content. Probably, the stronger acids associated with the effects of ultrasound at 60% amplitude at pH 1.5 contributed to the hydrolysis of pectin

and the consequent reduction in yield. These findings diverge from what is generally expected in relation to acid strength and pectin yield, where many authors report that the greater the acid strength, the greater the pectin yield. However, at extreme acid strength, such as pH 1, severe acid hydrolysis and degradation of pectin is observed [79].

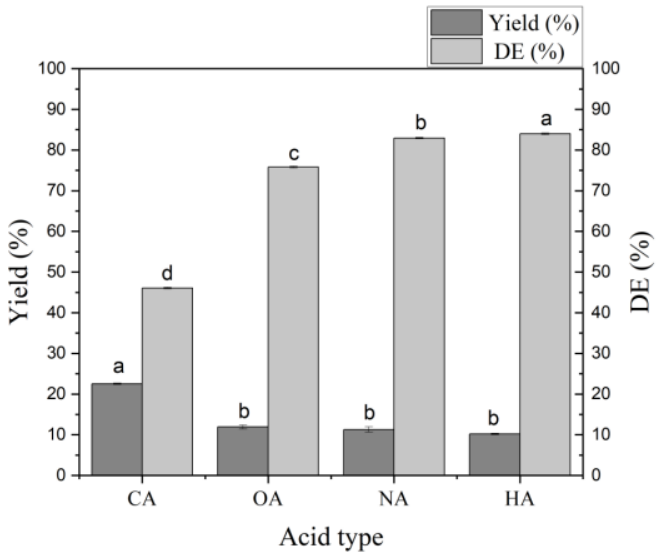

Fig.4 Influence of different acids on the pectin yield and DE under optimized UAE conditions: ultrasound amplitude of 60%, 1:33 of SLR and pH 1.5.

Regarding the galacturonic acid content, it was observed that pectins with a high GalA content, around 70.5%, can be extracted using oxalic acid and hydrochloric acid, without a statistically significant difference ($p < 0.05$). However, this content can be considered statistically diferente but close to the GalA content of commercial citrus pectin, which has a content close to 76%. Pectins obtained with nitric acid and citric acid have GalA contents close to 62 and 56%, respectively. These levels are statistically different from each other, from the commercial one and from the other extracted pectins. The extraction of pectin with high GalA content (74.4 - 77.9%) from lemon peels through conventional method and using oxalic acid was recently reported [80]. The GalA content in pectins extracted by conventional hot method and UAE may vary depending on the acid type used, according to study performed with onion (Allium cepa L.) waste as raw material [81]. Similar trend to that found for umbu pectins was observed for citric acid and hydrochloric acid, in which in the study with onion waste lower GalA contents were found for pectins extracted with citric acid using the conventional method (37.82%) and UAE (27.03%), while pectins extracted with these methods and using hydrochloric acid showed higher levels, around 55.12% and 50.79%, respectively. In contrast, the effects of organic acids on GalA content of pectin from different pomelo varieties showed that pectin extracted using citric acid contained higher GalA contents (76.5-85.1%) than those extracted by lactic acid

(60.4-63.8%) and acetic acid (65.1-68.2%) [82]. According to these authors, the observed variations can be attributed to fact that probably pectin extracted by acetic acid or lactic acid contained high amounts of neutral sugars such as fucose, rhamnose, arabinose, galactose, glucose, xylose, and mannose, resulting in low galacturonic contents.

Color is an important quality parameter for pectins as it affects the appearance of the gel produced and the characteristics of the product to which it will be added, influencing product acceptance [54, 83-84]. The color of pectin can be affected by the type of extraction process, raw material used as substrate, presence of associated pigments, as well as the pretreatment and purification steps [54]. For the color analysis of pectins, significant differences were observed for all coordinates evaluated. According to the data in table 6, significant differences ($p< 0.05$) were verified for the three parameters (L*, a* and b*) of the pectins obtained with different acids, indicating that the type of acid used for extraction affects the final color of the extracted pectin. Commercial citrus pectin (CCP) had the highest lightness value (91.34), while the lowest lightness values were observed to pectins obtained with oxalic acid (72.31), hydrochloric acid (71.91), acid nitric acid (69.21) and citric acid (64.43).

Regarding the a* coordinate, positive values indicative of redness palettes was observed for all pectins, with the highest values being found for PNA (8.16%) and PCA (7.42), followed by POA (7.14%), PHA (6.41%) and CCP (0.97). In relation to coordinate b*, the highest value was observed for POA (12.86), followed by the pectins PHA (12.07), PNA (11.23), CCP (10.49) and PCA (7.6), where higher positive values this coordinate indicates the yellowish color of the pectins. Therefore, establishing commercial pectin as a reference regarding the color quality parameter and that it is desirable that pectins for industrial use are not brownish or darkened, pectins extracted with different acids can be grouped in the following order of color quality: CCP (reference)>POA>PHA>PNA>PCA. Although the acid pectin obtained with citric acid is listed as the most brownish pectin among those obtained with different acids,its L*, a* and b* values result in a color considered more interesting than that described for pectins extracted by UAE and MAE a from tomato processing waste where L*, a* and b* values of 54.92, 13.49 and 24.22, and 50.96, 11.07 and 24.9 were observed, respectively, indicating that they are darker brownish pectin with a reddish hue [54]. This reddish color is strictly related to the raw material used for extraction, tomato processing waste, which presents high levels of lycopene that were not properly removed in the pretreatment stages and that remained until the pectin recovery stages, remaining associated with them and affecting its final coloring.

4. Conclusion

In this study, ultrasound assisted extraction of pectin from umbu peels using organic citric acid was investigated under different processing parameters and Central Composite Design associated with response surface methodology were used for extraction optimization. Two second-order polynomial models were developed using multiple regression analysis for prediction the pectin yield and degree of esterification. Preliminarily, the effect of sonication time on the pectin yield was evaluated, demonstrating that the highest yield, around 13.7%, was achieved after 10 min, and no statistically significant difference was observed ($p > 0.05$) when compared with the yield obtained after 18 min. After optimization study, the highest yield, approximately 22%, of low esterification pectin (DE = 46%) was achieved under ideal conditions, established under ultrasound amplitude of 60%, SLR of 1:33 and pH 1, 5. Process validation was carried out under established optimal conditions and the effects of different acids on the yield of pectins and DE were evaluated, demonstrating that the use of citric acid allows yields of around 22% of LMP pectin to be achieved, while using oxalic acid , nitric acid and hydrochloric acid led to the production of around 13% of HMP pectin. Furthermore, LMP pectins have a lower galacturonic acid content (56%) when compared to HMP pectins extracted with other acids, where the content varied from 61.9% to 70.8%. In terms of quality, assessed through instrumental color, pectins extracted with different acids under optimized UAE conditions showed significant differences when compared to commercial citrus pectin CCP, which demonstrated the influence of the type of raw material and extraction method on the quality of the product obtained. Therefore, our study describes for the first time the xtraction of pectins from umbu peels using ultrasound technology, proving that this material is a promising source of LMP and HMP pectins, which can be obtained in yields of 13 to 22% epending on the type of acid using. Thus, this study demonstrates the potential of high intensity ultrasound to obtain compounds of industrial interest from native Brazilian fruits, particularly from the Caatinga biome, thus contributing to the valorization of biodiversity, preservation of the environment and innovation in the food sector.

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Contributions

Joseane Cardoso Gomes de Alencar: investigation, experimental performance, formal analysis, data analysis, manuscript preparation; Denise Nathiele Santos Souza Batista, Jacqueline Carvalho de Souza, Isabelle Palma Patricio Santos: experimental performance, formal analysis; Juliano Lemos Bicas: investigation, data analysis, manuscript preparation; Maria Eugênia de Oliveira Mamede: data analysis, Bruno Nicolau Paulino: investigation, data analysis, supervision.

Ethics declarations

Ethical approval

The authors claim that none of the material in the paper has been published or is under consideration for publication elsewhere.

Competing interests

The authors declare no competing interests.

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