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Selection and application of eutectic solvents for the valorization of jaboticaba processing by-product using high-pressure fluid technologies

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Profª. Dra. Sandra Regina Salvador Ferreira
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Florianópolis, 17 de março de 2022.

Dedico à minha *família* que me apoiou, aos *mestres*
que me incentivaram e a *todos que buscarem*
conhecimento por meio desta.

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"Não há no mundo exagero mais belo que a gratidão."

(Jean de la Bruyère)

"The future will either be green or not at all"

(Bob Brown)

RESUMO

Compostos bioativos obtidos de plantas têm sido intensivamente estudados devido aos seus benefícios à saúde e potencial de aplicação em formulações alimentícias e farmacêuticas. A casca da jabuticaba (família *Myrtaceae*), parte do subproduto do processamento do fruto, contém fibras, pectina, vitaminas e compostos fenólicos, especialmente antocianinas. A obtenção desses compostos pode ser efetuada por meio dos solventes eutéticos profundos (do inglês, DES), os quais apresentam baixa toxicidade e alta capacidade de solubilização. Nesse sentido, os objetivos deste estudo foram preparar, selecionar e aplicar DES visando a valorização do subproduto do processamento de jabuticaba por meio de processos "verdes". Primeiramente seis diferentes DES foram preparados e caracterizados. Baseado em análises *in sílico* e em dados experimentais, a solução de DES composta por cloreto de colina e propileno glicol, ChCl:Pro [1:1 (DES:água, m/m)] apresentou-se como o DES mais promissor para extração de antocianina, enquanto o DES formado por ácido cítrico, glucose e água, Ca:Glu:Wa [1:9 (DES:água, m/m)], foi o mais efetivo para extração de pectina. Na sequência, as soluções aquosas de DES foram associadas a tecnologias a alta pressão, buscando o emprego de métodos genuinamente verdes de extração. A fração rica em antocianina foi obtida por extração com líquido pressurizado (do inglês PLE) em condições otimizadas de 47% de DES, 90 °C e vazão de solvente de 5,3 mL/min. O efeito do solvente na termoestabilidade e bioatividade das antocianinas foi avaliado comparando dois DES (ChCl:Pro e ChCl:Ma - cloreto de colina e ácido málico) com solventes convencionais (água e água acidificada). O rendimento em antocianinas foi cerca de 50% maior com DES comparado com os solventes convencionais. Todos os extratos obtidos apresentaram potencial antidiabético e antiobesidade, entretanto, o extrato obtido com ChCl:Ma resultou em maior bioatividade e estabilidade da antocianina (E_a = 77.5 kJ mol^{-1}), indicando potencial de aplicação como corante natural com benefícios à saúde. A fração rica em pectina foi obtida por extração com água subcrítica (do inglês, SWE) modificada por DES (Ca:Glu:Wa) em condições otimizadas (122 °C, 8% de DES e vazão de 2 mL/min). O uso de DES como modificador da SWE foi comparado com ácido cítrico nas mesmas condições de temperatura e vazão de solvente. O método a baixa pressão, a extração com aquecimento e agitação (do inglês, HSE), foi empregado como controle. SWE foi mais eficiente para recuperar pectina, com rendimentos de 1,5 a 1,8 vezes maiores que HSE. Em geral, as pectinas obtidas do subproduto industrial de jaboticaba são de alto grau de metoxilação e apresentam capacidade de absorver óleo e água, podendo ser usadas como estabilizantes e emulsificantes. Comparado aos solventes e métodos controles, as pectinas obtidas por SWE-DES apresentaram maior concentração de GalA e a melhor atividade antioxidante. Portanto, a pectina obtida pelo método proposto apresenta alto potencial de aplicação devido a sua bioatividade e funcionalidades. O uso dos DES selecionados a alta pressão apresentaram alta efetividade na obtenção sequencial de frações ricas em antocianina e pectina. A partir de uma medida semiquantitativa, *Certificado Verde,* foi concluído que a extração sequencial utilizando soluções aquosas de DES pressurizado é uma abordagem genuinamente verde para a valorização de diferentes matrizes vegetais. Ademais, a seleção de DES combinando resultados *in silico* e experimentais para fracionar sistemas complexos é uma novidade com grande potencial de aplicação em matrizes vegetais complexas que ainda merece ser explorada.

Palavras-chave: Biorrefinaria, extração verde, DES, compostos bioativos, corante natural, propriedades funcionais.

RESUMO EXPANDIDO

Introdução

A relação entre dieta e saúde humana, associada a necessidade de suprir o mercado com produtos sustentáveis, tem aumentado o interesse por compostos obtidos das plantas visando aproveitar os recursos disponíveis. O Brasil apresenta uma ampla biodivesidade, no entanto, muitas espécies com potenciais usos são subutilizadas. Dentre estas, o fruto de Jabuticaba (*Myrciaria cauliflora)* vem sendo apontado como uma rica fonte de fitoquímicos, especialmente antocianina. O processo industrial desse fruto gera um subproduto que representa cerca de 40% do volume inicial, e apresenta composição semelhante ao fruto intacto. A recuperação sustentável dos compostos-alvos a partir de fontes vegetais pode ser realizada utilizando solventes GRAS (Geralmente Reconhecido como Seguro), como os álcoois. Recentemente, solventes eutéticos profundos (do inglês, DES) vem sendo utilizados como solventes alternativos ambientalmente amigáveis e com alta capacidade de extração. Além disso, tecnologias emergentes visam diminuir o gasto energético, o consumo de água e de solventes orgânicos ou petroquímicos, além de reduzir a emissão de carbono. Os métodos de extração a alta pressão como extração com líquido pressurizado (do inglês, PLE) e extração com água subcrítica (do inglês, SWE) são alternativas verdes que melhoram os rendimentos de extração, com menor tempo de processo e consumo de solventes. Poucos estudos avaliaram a a combinação de DES com tecnologias a alta pressão e, até o presente momento, nenhuma pesquisa avaliou a seleção de diferentes DES considerando dados *in silico* e experimentais visando o fracionamento de sistemas complexos a alta pressão.

Objetivos

A presente tese visa a preparação, seleção e aplicação de solventes eutéticos profundos (DES) associados a tecnologias de extração à alta pressão para uma extração verde sequencial de uma fração rica em antocianina e uma fração rica em pectina com potenciais de aplicação em formulações alimentícias e farmacêuticas a partir do subproduto agroindustrial da jabuticaba.

Metodologia

A composição centesimal do subproduto industrial da jabuticaba foi realizada por meio dos procedimentos oficiais descritos pela AOAC para umidade, cinzas, lipídeos, proteína e fibra bruta. A concentração de carboidratos não fibrosos foi determinada por diferença. Seis diferentes misturas eutéticas foram preparadas em escala laboratorial por meio da simples mistura dos componentes iniciais (em razão molar específica para formar ponto eutético), seguido de agitação e aquecimento à 80 °C até a formação de um líquido homogêneo. Os DES foram caracterizados por meio da determinação dos pHs, análise de DSC, viscosidade e custos. A seleção dos DES mais promissores para obtenção de uma fração rica em antocianina e outra rica em pectina foi realizada por meio de dados *in silico* realizados no software Cosmo-RS (short for Conductor-like Screening Model for Real Solvents) combinados com dados experimentais como viscosidade e capacidade de extração. As extrações combinando soluções aquosas de DES com a tecnologia a alta pressão foram otimizadas por meio de análise multiresposta associada a metodologia de superfície de resposta (do inglês, RSM). O extrato

rico em antocianina foi caracterizado por meio da concentração de antocianinas totais determinado pelo método do pH diferencial, porcentagem de antocianina recuperada em relação ao total presente na matriz, porcentagem de cor polimérica, atividade antioxidante, potencial antidiabético e antiobesidade, cor e perfil fenólico por HPLC. A pectina presente na segunda fração recuperada foi isolada e caracterizada em relação ao rendimento total por gravimetria, FTIR, grau de esterificação, concentração de ácido galacturônico (GalA), carboidratos totais, fenólicos totais, atividade antioxidante e funcionalidades como capacidade de absorver água e óleo e formar emulsões. A sustentabilidade da abordagem sugerida foi avaliada através do *Certificado Verde*, uma ferramenta semi-quantitativa que pode ser utilizada para mensurar quão "verde" um processo de extração pode ser.

Resultados e discussões

A partir das análises *in sílico* e dados experimentais, a solução de DES composta por cloreto de colina e propileno glicol, ChCl:Pro [1:1 (DES:água, m/m)] apresentou-se como a mais promissora para extração de antocianina, enquanto o DES formado por ácido cítrico, glucose e água, Ca:Glu:Wa [1:9 (DES:água, m/m)] foi mais efetivo para extração de pectina. Essa primeira etapa foi crucial para promover alta seletividade dos compostos-alvo. A combinação da solução aquosa de DES com PLE em condições otimizadas (47% de DES, 90 °C e vazão de 5,3 mL/min) foi eficiente na obtenção de um extrato rico em antocianina. O efeito do solvente na termoestabilidade e bioatividade das antocianinas foi avaliado comparando dois DES (ChCl:Pro e ChCl:Ma - cloreto de colina e ácido málico) com solventes convencionais (água e água acidificada). Ambos os DES apresentaram rendimentos de antocianina cerca de 50% maiores que solventes convencionais. Todos os extratos ricos em antocianina apresentaram potencial antidiabético e antiobesidade, embora o extrato obtido com ChCl:Ma resultou em maior bioatividade e estabilidade (E_a = 77.5 kJ mol^{-1}), mostrando-se promissor para uso como corante natural com benefícios à saúde. A fração rica em pectina foi recuperada por extração com água subcrítica (do inglês, SWE) modificada por DES (Ca:Glu:Wa) em condições otimizadas (122 °C, 8% de DES e vazão de 2 mL/min). O uso de DES como modificador da SWE foi comparado ao uso de ácido cítrico nas mesmas condições de temperatura e vazão de solvente. A extração a baixa pressão sob aquecimento e agitação (do inglês, HSE) foi realizada como método controle. SWE apresentou rendimentos 1,5 a 1,8 maiores que HSE. No entanto, o grau de metoxilação das frações a alta pressão foi menor. Em geral, pectinas obtidas a partir do subproduto industrial de jaboticaba apresentaram alto grau de metoxilação e capacidade de absorver óleo e água, podendo ser usadas como emulsificantes. A fração de pectina obtida por SWE-DES apresentou a maior concentração de GalA e atividade antioxidante, resultando em alto potencial de aplicação devido a sua bioatividade e funcionalidade. O valor do *Certificado Verde* obtido para a extração sequencial utilizando soluções aquosas de DES pressurizado foi de 90.35, representando uma abordagem genuinamente verde para a valorização do subproduto do processamento de jabuticaba. Devido ao efeito protetivo dos DES sobre os compostos bioativos e o baixo perigo associado aos DES utilizados, foi sugerida a aplicação dos extratos sem a necessidade de remoção do solvente.

Considerações finais

A extração de compostos de interesse utilizando soluções aquosas de DES selecionados, associados com tecnologia a alta pressão, apresentou altos rendimentos e elevada qualidade dos extratos recuperados. A abordagem sugerida pode ser considerada uma alternativa sustentável

para obter compostos com alto potencial de aplicação a partir de diferentes matrizes vegetais. Assim, para o adequado emprego desta metodologia alternativa é importante acompanhar o estudo de ampliação de escala, além de estabelecer o destino para o material sólido resultante após as extrações. Ensaios *in vitro, in vivo* e clínicos podem acompanhar futuros estudos para confirmar os benefícios à saúde das frações obtidas visando estabelecer suas aplicações como corante natural, emulsificante e estabilizante em formulações modelo.

Palavras-chave: Biorrefinaria, extração verde, DES, compostos bioativos, corante natural, propriedades funcionais.

ABSTRACT

Bioactive compounds obtained by plants have been intensively studied because of their biological properties that provide health benefits with potential applications in food and pharmaceutical formulations. The jaboticaba (family *Myrtaceae*) peel, a processing by-product, contains fibers, pectin, vitamin, and phenolic compounds, mainly anthocyanins. The recovery of these compounds can be performed by deep eutectic solvents (DES), which present high solubilization ability and low toxicity. In this sense, the main proposals of this thesis are to prepare, select and apply DES to value the jaboticaba peel employing green process. Initially, the DES were prepared and characterized. Besed on *in silico* and experimental analysis, a route for sequential extraction of an anthocyanin and a pectin-rich fractions was proposed using aqueous solution of DES. DES solution composed of chorine chloride and propylene glycol, ChCl:Pro [1:1 (DES:water, w/w)] was the most promising solvent for anthocyanin extraction, while the DES composed of citric acid, glucose, and water, Ca:Glu:Wa [1:9 (DES:water, w/w)], was the more effective for pectin recovery. Then, DES aqueous solutions were associated with high-pressure technologies, seeking to employ genuinely green extraction methods. The anthocyanin-rich extracts were obtained by pressurized liquid extraction (PLE), at optimized conditions of 47% of DES solution, 90 °C and flow ratio of 5.3 mL/min. The effect of solvent type on thermostability and biological properties of anthocyanin was evaluated comparing two DES (ChCl:Pro e ChCl:Ma – chorine chloride: malic acid) and conventional solvents (water and acidified water). The anthocyanin yields were about 50% higher with DES than conventional solvents. All anthocyanin fractions presented anti-diabetic and anti-obesity potential, however, the use of ChCl:Ma resulted in the highest bioactivity and anthocyanin stability (E_a = 77.5 kJ mol^{-1}), indicating their potential application as natural colorant with health benefits. The pectin rich fraction was recovered by subcritical water extraction (SWE) modified with DES (Ca:Glu:Wa) at optimized conditions (122 °C, 8% of NADES and 2 mL/min). The use of DES as SWE modifier was compared with the use of citric acid in the same conditions of temperature and flow rate. Besides, a low-pressure extraction method, heat-stirred extraction (HSE), was employed as a control method. SWE was more efficient to pectin recovery, with 1.5 to 1.8-fold higher yields than HSE. In general, the pectins obtained from jaboticaba pomace are high methoxylated and present oil and water holding capacity, presenting application potential as a stabilizer or emulsifier. Compared to the control solvents and methods, the pectin obtained by SWE-DES presenting higher GalA content and the best antioxidant capacity. Therefore, the pectin obtained by the proposal method showed high application potential due to their bioactivity and functionality. The use of selected aqueous solution of DES at high pressure presented high effectivity on sequential recovery of anthocyanin and pectin-rich fractions. Through a semi-quantitative tool, *Green Certificate,* it was concluded that the pressurized aqueous solution of DES is an effectively green approach for the valorization of different vegetal matrices. Besides, DES selection combining *in silico*/experimental results, to fractionate complex systems is still a novelty with high application potential in complexes vegetable matrices that must be explored.

Keywords: Biorefinery, green extraction, emergent solvents, DES, bioactive compounds, natural colorant, functional properties.

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CONCEPTUAL DIAGRAM

Why?
There is high demand for functional, natural, healthy, and economically viable products; The population growth associated with these food demands requires the development of sustainable food systems and better destinations for food industry residues; Brazil presents a large fruit diversity, which when processed generates low-value by-products with a similar bioactive composition to fresh fruit. Therefore, evaluating the technological and health benefits potential from an underexplored vegetable matrix or its by-products unites academic and industrial interests; The peel of jaboticaba processing by-product is an unexplored material rich in vitamins, fibers, pectin, and phenolic compounds, mainly anthocyanins; Deep Eutectic Solvents (DES) are emergent solvents with high recovery potential to bioactive compounds, and are very promisors as green solvents, suitable for green technologies; Data about the extraction using DES at high pressure are scarce in the literature.

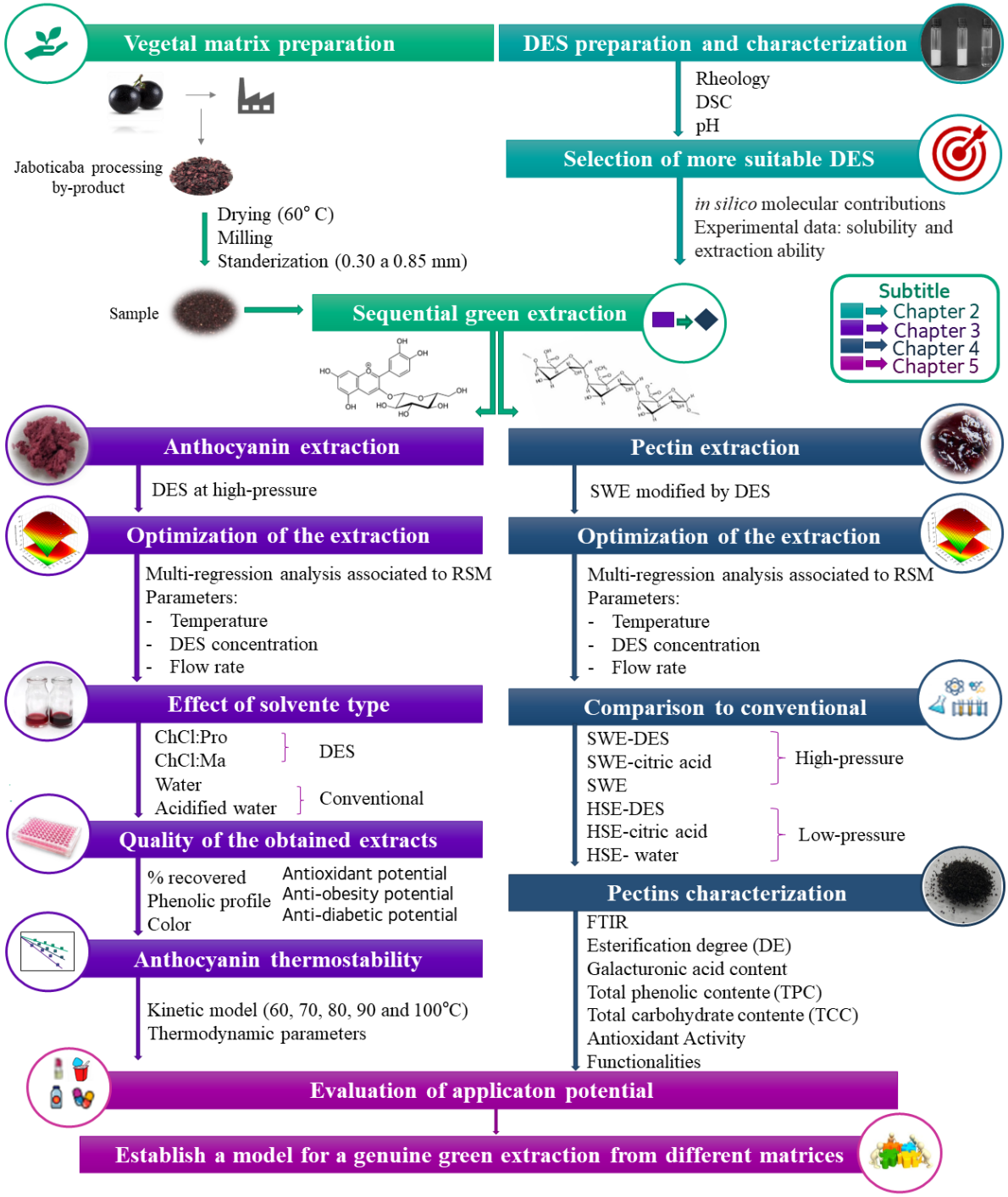
Who did?
Deep eutectic solvents (DES) have been employed as green solvents to extract bioactive compounds; Microwave-assisted extraction (MAE) and Ultrasound-assisted extraction (UAE) are the main processes using DES as extraction solvent; Anthocyanins were recovered by DES from carrots, mulberry, blue honeysuckle fruit, strawberry, raspberry, hibiscus, *Nitraria tangutorun*, *Lycium* fruit, among other natural matrixes; Anthocyanins are the most explored bioactive compound from jaboticaba peel, recovered mainly by MAE, UAE, and conventional extraction methods using ethanol and acidified ethanol-water solution as the solvents; Pectin and a galactose-rich heteropolysaccharide (GH) have also been recovered from jaboticaba by maceration method using acidified water with nitric acid (pH 1) as the solvent; The present study is the first to propose the recovery and fractionation of bioactive compounds from jaboticaba processing by-products using DES as solvents.

Hypothesis
Alternative solvents (DES) associated with emergent green technology can be a great alternative to obtain different extract fractions with high yield and quality; The DES can protect the anthocyanins from thermal degradation; Aqueous solutions of DES can exert effects on biological and functional characteristics of pectin; This approach is an eco-friendly alternative to valorize the peel of jaboticaba processing by-products.

Methodologies
Concepts of green extraction, biorefinery, and circular economy to valorize the jaboticaba processing by-product; *In silico* data combined with experimental results to define the most suitable DES; Multiple response analyses coupled to Response Surface Methodology (RSM) to define the extraction route and conditions for the sequential extraction methodology using high-pressure methods; Thermal and chemical analyses to provide the recovered extract characterization; *Green certificate* as a semi-quantitative tool to measure the sustainability of the sequential extraction approach.

Responses
Define the most suitable DES for extraction and fractionation of anthocyanin and pectin from the peel of jaboticaba processing by-product; Establish a wise way to select aqueous solutions of DES as low-cost green solvents, aiming to achieve selective recoveries of added-value compounds from different matrixes; Valorize the jaboticaba processing by-product in a sustainable system, recovering high-quality extracts with potential for use in food and pharmaceutical formulations. Eestablish a path for a genuine green extraction from different plant matrices.

THESIS METHODOLOGICAL SEQUENCE FLOWCHART



DES – Deep Eutectic Solvent, SWE – Subcritical Water Extraction, HSE – hot-stirred extraction, ChCl – Chlorine Choride, Pro – Propylene glycol, Ma – Malic Acid.

INTRODUCTION

The relation between diet and human health is increasingly evident, and this association has been changing the food choice of consumers (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020). Furthermore, sustainable food systems are still an industrial challenge, aiming to supply the constantly increasing food demand, allied with the decrease in food waste and its accumulation. Besides, the current COVID-19 pandemic evidences the need to ensure food security, and the supply of economically viable products that contribute to human health (GALANAKIS, 2020; GALANAKIS; TSATALAS; GALANAKIS, 2018). Therefore, natural food ingredients or additives have been evaluated as substitutes for synthetic ones due to their nutritional and technological effects, beyond health benefits (DOMÍNGUEZ DÍAZ; FERNÁNDEZ-RUIZ; CÁMARA, 2020; NG et al., 2020). Thus, the obtaining of natural compounds from underutilized resources or industrial by-products has been widely evaluated (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; KITRYTĖ et al., 2020; RODRIGUES et al., 2019; RUDKE et al., 2019; SORITA; LEIMANN; FERREIRA, 2020). This approach contributes to the seventeen Sustainable Development Goals (SDGs) adopted by United Nations Members States (2015), mainly the SDG2: zero hunger, SDG9: industry, innovation, and infrastructure, and SDG12: sustainable consumption, and production.

Brazil presents large biodiversity and many exotic species, mainly edible fruits, which can be useful as food, or in formulations of foods, supplements, medicines, and cosmetics (TEIXEIRA et al., 2019). Jaboticaba (*Myrciaria cauliflora)* is a Brazilian berry mainly cultivated in domestic gardens, small-scale agriculture, or in extractive system and consumed as fresh fruit. However, it is also industrially processed to produce juice, jam, syrup, liquor, and fermented beverages, among others (ALEZANDRO; GRANATO; GENOVESE, 2013;

GURAK et al., 2014; SANTOS; MEIRELES, 2009). These processes generate the jaboticaba by-product which represents 40% of total processed fruit among peel, seeds, and adhered pulp (GURAK et al., 2014). Both the jaboticaba fruit and its by-product show rich phytochemical composition including pectin, ascorbic acid, β-carotene, tocopherol, phenolic acids, gallotannis, and ellagitannins or hydrolyzable tannins and flavonoids as quercetin and anthocyanin (INADA et al., 2015, 2020; MORENO et al., 2016; RUFINO et al., 2010).

These valuable target compounds from plants are obtained by the extraction processes, mostly using aqueous-organic solvents such as hexane, benzene, methanol, chloroform, petroleum ether, and acetone (ALBERTI et al., 2014; DJANDE et al., 2018; RENARD, 2018). Recently, the use of GRAS (Generally Recognized as Safe) solvents, such as some alcohols (e.g., ethanol), has been highlighted as an alternative less harmful to the environment, operator, and consumer health (RENARD, 2018). In addition, it is increasingly evident the need to search for greener processes, aiming to decrease the energy, water, and solvent consumption, and carbon emission, and ensure the safety of the environment and final product (CHEMAT et al., 2019). In this proposal, new extraction technologies such as ultrasound-assisted extraction (UAE), microwave-assisted extraction, supercritical fluid extraction (MAE), pressurized liquid extraction (PLE), subcritical water extraction (SWE), supercritical fluid extraction (SFE), enzyme-assisted extraction, among others have been employed for the extraction processes mainly to increase the yield, and decrease solvent consumption and process time (RENARD, 2018). Furthermore, alternative solvents such as ionic liquid (IL), deep eutectic solvent (DES), and natural deep eutectic solvent (NADES) have been presented as eco-friendly alternatives that still are being enhanced and evaluated (BENVENUTTI; ZIELINSKI; FERREIRA, 2019; GULLÓN et al., 2020; MARTINS; BRAGA; DE ROSSO, 2017).

DESs are composed of a hydrogen bond acceptor (HBA) and one or more hydrogen bond donators (HBD) forming a eutectic mixture, i.e., a mixture with a melting temperature lower than the initial components due to the strong molecular interactions among them. These alternative solvents present low vapor pressure, being less harmful to the environment than organic solvents. Besides, DES presents thermal stability, adjustable viscosity, miscibility, high biodegradability, and high extraction ability (ABBOTT et al., 2003, 2004; BAKIRTZI; TRIANTAFYLLIDOU; MAKRIS, 2016). When natural components are used as HBA and HBD, usually plant primary metabolites (e.g. choline chloride, fructose, sucrose, glucose, malic acid, citric acid), they usually are denominated natural deep eutectic solvent (NADES) to highlight these less harmful eutectic solvents to the environment and nontoxic (CHOI et al., 2011). However, much caution is required with the use of the term *natural* since the HBA and HBD used are mostly industrially manufactured.

Only a few studies have reported the combination of DES solutions with high-pressure processes. Although, so far, no research was detected indicating the use of different DES solutions, prepared considering *in silico* and experimental combination, for the fractionation of complex systems employing high-pressure methods. In this sense, the main proposals of this thesis are the preparation, selection, and application of the DES solutions, combined with high-pressure fluid techniques, to provide a genuinely green sequential extraction of a rich-anthocyanin fraction and a rich-pectin fraction from jaboticaba processing by-product. Therefore, this study also aims to value and disseminate this rich underexplored material, as an alternative source of components with industrial application potential using emergent solvents and technologies.

OBJECTIVES

This thesis aims to select and apply Deep Eutectic Solvents (DES) to valorize the jaboticaba (*Myrciaria cauliflora*) processing by-product through an eco-friendly approach for the recovery of different chemical compounds with bioactive and technological potential. For this, the following specific goals were traced:

- *Prepare,* in lab scale, *and characterize* deep eutectic solvents (DES);
- *Select* the components (HBA and HBD) of DES to extract the target compounds (anthocyanin and pectin) using *in silico* and experimental methods;
- *Employ* the biorefinery concept for a sequential recovery of different fractions from the jaboticaba biomass;
- *Apply* high-pressure technologies, at different processing conditions and solvents, to recover bioactive compounds from jaboticaba biomass;
- *Optimize* the process conditions for the different procedures used;
- *Characterize* the bioactive extracts obtained;
- *Evaluate* the anthocyanin-rich extract stability obtained using aqueous solutions of DES combined with pressurized liquid extraction (PLE);
- *Study* the physicochemical and bioactive characteristics of the pectin fraction obtained using subcritical water extraction (SWE) modified by DES;
- *Evaluate* the sustainability of suggested sequential extraction by employing a green metric tool as a semi-quantitative approach;
- *Define,* based on previous results, the potential application of the recovered extracts.

CHAPTER 1

Literature Review

This first chapter aims to present an actual literature review about this thesis subject, presenting the growing interest in green extraction, mainly concerning emergent solvents for the obtention of bioactive compounds, highlighting Deep Eutectic Solvent (DES), the solvent used in this study. Additionally, the jaboticaba botanical characteristics, health benefits, industrial process, and main phytochemicals were summarized since the jaboticaba industrial by-product was the vegetal matrix valorized using DES in a green extraction approach. This content was based on two full review articles published at the journal **Trends in Food Science and Technology**: "Which is the best food emerging solvent: IL, DES or NADES?" (2019) (https://doi.org/10.1016/j.tifs.2019.06.003) and "Jaboticaba (*Myrtaceae cauliflora)* fruit and its by-products: alternative sources for new foods and functional components" (2021) (https://doi.org/10.1016/j.tifs.2021.03.044).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Review

Which is the best food emerging solvent: IL, DES or NADES?

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Jaboticaba (*Myrtaceae cauliflora)* fruit and its by-products: Alternative sources for new foods and functional components

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1.1 JABOTICABA

Jaboticaba (*Myrtaceae cauliflora)* is a native Brazilian fruit also find in Argentina, Paraguay, Bolivia, and Central American countries (GURAK et al., 2014). Its flowers grow directly from the tree stalk, an inherent characteristic of this species. The fruit ripens occur after 40-60 days of flowering (WU; LONG; KENNELLY, 2013). Ripe fruits appear as a spherical berry with a juicy pulp 2 to 4 cm in diameter, with a thin and fragile pericarp in a color that varies from red to black purple. This fruit is slightly acid (pH = 3.45-3.74, total acidity = 14.7-26.8 g citric acid/100g), sweetish, with a white gelatinous pulp, containing four seeds in the interior (ALEZANDRO et al., 2013; GURAK et al., 2014; TAO et al., 2014).

There are many jaboticaba species, but the most widespread and reported species are *Myrciaria cauliflora* and *Myrciaria jaboticaba* (SOBRAL, M.; PROENÇA. C.; SOUZA, M.; MAZINE, F.; LUCAS, 2020; WU; LONG; KENNELLY, 2013). Therefore, this research addresses studies with these two species. The *M. cauliflora* (Mart.) O. Berg, known as just *jaboticaba* or *jaboticaba assu*, has a tree of 3-4 m in height and leaves from 2 to 6 cm, with finely reticulated veins, while *M. jaboticaba*, called *jaboticaba sabará*, is typically a tree 3-9 m tall and leaves of 2-4 cm (WU; LONG; KENNELLY, 2013).

The jaboticaba is mainly cultivated in domestic gardens, in small-scale agriculture, or in extractive systems (Santos & Meireles, 2009). However, according to CEAGESP (Companhia de Entrepostos e Armazéns Gerais de São Paulo, Brazil), the formal jaboticaba activity in Brazil increases every year (CEAGESP, 2020). Jaboticaba can be found throughout the Brazilian territory, however, the State of São Paulo is the largest producer, with an average production of 2,646.8 tons per year. Jaboticaba trees produce fruits from August to September

or January to February and despite the short harvest period, the productivity is abundant (BAPTISTELLA; COELHO, 2019).

The jaboticaba is much appreciated for its sensory attributes (INADA et al., 2017). Despite its variable chemical composition, water, carbohydrates (mainly glucose and fructose), fibers, vitamins, phenolic compounds (mainly flavonoids), and minerals (e.g. potassium, nitrogen, and magnesium) are the main reported compounds (ALEZANDRO et al., 2013; INADA et al., 2015). The jaboticaba is mainly consumed as fresh fruit, although it is also industrially processed to produce juice, jam, syrup, liquor, and fermented beverages, among others (ALEZANDRO; GRANATO; GENOVESE, 2013; GURAK et al., 2014).

Several health benefits are associated with jaboticaba fruit consumption due to its phytochemical composition. Traditionally, this specie benefits gastrointestinal disorders (GASPAROTTO JUNIOR; DE SOUZA; LÍVERO, 2019), and presents high biological potential as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anti-obesity, cardioprotective, and antitumoral activities (ALEZANDRO; GRANATO; GENOVESE, 2013; CALLONI et al., 2015; GURAK et al., 2014; LAMAS et al., 2018; LEITE-LEGATTI et al., 2012; ZHAO et al., 2019). For these reasons, the jaboticaba fruit has been gaining interest from the scientific community and is highlighted as an emerging fruit useful to supply active fractions for food, cosmetic and pharmaceutical companies (WU; LONG; KENNELLY, 2013). A brief search on the Scopus database (www.scopus.com) including the keywords “jaboticaba” or “jabuticaba” resulted in 321 published documents. The main subjects detected within these published studies were related to extraction, preservation, and application of bioactive compounds with biological activity (ALBUQUERQUE et al., 2020a; PALUDO et al., 2019; RODRIGUES et al., 2015; SANTOS; MEIRELES, 2009).

1.1.1 Jaboticaba industrial process

The high nutritional value, pleasant taste, and fruit perishability encourage the industrial process of products derived from jaboticaba (INADA et al., 2017; WU; LONG; KENNELLY, 2013). Briefly, the industrial process of jabuticaba consists of selection, washing, sanitization, heating to extract the pigment from the peel, and, finally, the fruit is pulped in machines, which simultaneously separate the pulp from the by-product **(Figure 1)**. The pulp is packed and commercialized or destined for the formulation of juices, jams, liqueur, ice cream, and candies (ALEZANDRO et al., 2013; GURAK et al., 2014). Recent studies evaluated new technologies, e.g. high hydrostatic pressure and ohmic heating, to the development of innovative products. The employ of high hydrostatic pressure on jaboticaba juice instead of heat treatment resulted in higher phenolic compounds and antioxidant activity, decreased the microbial count, besides preserving the aromatic compounds, ensuring the sensorial acceptance (INADA et al., 2017). However, Mercali, Gurak, Schmitz, & Marczak (2015) reported that the ohmic heating treatment did not present a significant difference in anthocyanin degradation concerning conventional heat treatment in jaboticaba juice.

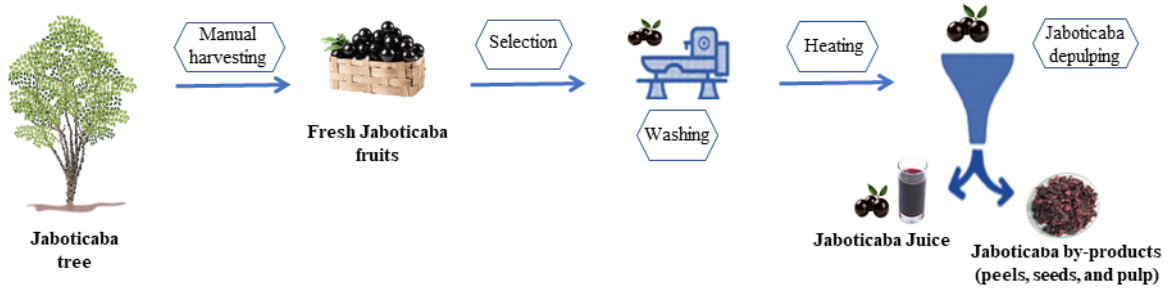

Figure 1 - General scheme of industrial processing of jaboticaba fruit.

As previously mentioned, the jaboticaba industrial process generates by-products (seeds, peel, and residual pulp) with a higher concentration of phenolic compounds, fibers, protein, and vitamins, compared to the whole fruit (ALBUQUERQUE et al., 2020b; GURAK

et al., 2014). Therefore, new alternatives involving the reuse of these materials have been identified and evaluated, aiming to reduce the environmental impact, and adding value to the by-products, such as direct incorporation into food (e.g. yogurt, cookies) and recovery of biomolecules (e.g. anthocyanin, ellagitannins) (ALVES et al., 2013; FERNANDES et al., 2020; FREITAS-SÁ et al., 2018; MARQUETTI et al., 2018), which are discussed throughout this review.

1.1.2 Phytochemicals from Jaboticaba

The jaboticaba fruit is a source of vitamins, dietetic fibers, and phenolic compounds (INADA et al., 2015). Ascorbic acid, β-carotene, and tocopherol are the main vitamins. According to Rufino et al. (2010), the ascorbic acid (vitamin C) content is about 277.06 mg/100 g of dry weight (dw). Inada et al. (2015) reported 873 μg/100g dw of β-carotene in jaboticaba, a moderate source of this vitamin, while the contents of α-tocopherol, β-tocopherol and γ-tocopherol detected were 0.72, 0.13 and 0.01 mg/100g dw, respectively. Therefore, the total tocopherol content (0.85 mg/100g dw) is lower than berries such as strawberry, blackberry, blueberry, and raspberry (from 4.2 to 21.5 mg/100g dw) (INADA et al., 2015).

Jaboticaba also has in its composition insoluble and soluble fibers (mainly pectin), which present technological properties such as a gelling, emulsifier, thickener, and encapsulating agent besides beneficial physiological effects in humans (GASPAROTTO JUNIOR; DE SOUZA; LÍVERO, 2019; MORENO et al., 2016; SOUZA; GURACK; MARCZAK, 2017). According to Lima et al. (2008) the whole jaboticaba fruit had 18 g/100g dw of fibers, the peel, 33 g/100g dw, and seeds, 28 g/100g dw. Moreno et al. (2016) reported a pectin yield of 4.5% w/w from jaboticaba peel, 2% w/w from pulp, and 6.0% w/w from the juice. These yields are lower than the main sources of commercial pectin, citrus peel (85.5%),

and apple pomace (14%) (CUI et al., 2021). However, the pectin from jaboticaba peel showed high galacturonic acid (GA) content, degree of esterification (DE), and methoxyl content (MeO) (GA: 69, 65 and 59%, DE: 67, 55, and 53% and MeO: 11, 7.5 and 8.4% for peel, pulp and juice respectively). These parameters, related to pectin quality and ability to form a gel, suggest that the pectin from jaboticaba peel is similar to commercial pectin of high esterification (HM), with 76% of GA, DE higher than 70%, and 12% of MeO. Therefore, jaboticaba peels and seeds can be explored as a source to recovery dietetic fiber and pectin.

The most studied phytochemicals from jaboticaba are polyphenols. According to the literature, the phenolic profile of jaboticaba is composed mainly of phenolic acid, gallotannis, and ellagitannins or hydrolyzable tannins, flavonoids (flavonols and anthocyanins), and depsides **(Figure 2)**. According to Inada et al. (2020) the unprocessed jaboticaba fruit presented eleven phenolic compounds. Among them, two are hydroxybenzoic acid derivatives (gallic, 3,4-dihydroxybenzoic or protocatechuic, and ellagic acids), two are hydroxycinnamic acid derivatives (*m*-coumaric and trans-cinnamic acids), four are flavonols [myricitrin (myricetin-3-*O*-rhamnoside), quercitrin (quercetin-3-*O*-rhamnoside), globularicitrin (quercetin-3-*O*-rutinoside), myricetin, and quercetin], and two are anthocyanins (cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside). Wu et al. (2012) quantified twenty-two compounds in jaboticaba fruit extract and juice. In addition to the aforementioned components were detected seven gallotannins (HHDP-galloyl-glucose, casuariin, casuarinin, pedunculagin, tellimagrandin I, tellimagrandin II, and casuarictin); two hydroxybenzoic acid derivatives (ellagic acid pentose and valoneic acid dilactone); flavonoids [isoquercitrin (quercetin-3-*O*-glucoside) and quercimeritrin (quercetin-7-*O*-glucoside)]; depsides [jaboticabin and 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid], besides a phenylpropanoid called

syringin and its glycoside derivative. Recently, Pereira et al. (2017) identified a new ellagitannin called cauliflorin, five other hydrolyzable tannins (alnusiin, strictinin, 1,2,3,4,6-penta-*O*-galloyl-glucose, castalagin, and vescalagin), and eight other phenolics mentioned above.

Inada et al. (2015) quantified the phenolic profile from different parts of the fruit by High-Performance Liquid Chromatography (HPLC) and reported that jaboticaba peel presents a higher amount of total phenolic content (2252 mg/100g dw), followed by residue (1658 mg/100g dwb), seeds (986 mg/100g dw), whole fruit (815 mg/100g dw) and pulp (20 mg/100g dw). The main phenolic compounds detected in jaboticaba were anthocyanins, with 42.82%, followed by gallotannins (16.94%), quercitin (13.06%), and gallic acid (5.84%) (WU et al., 2012). Anthocyanins are present in the fruit peel, from the ripening start (INADA et al., 2015; PEREIRA et al., 2017), while ellagitannins, such as castalagin and vescalagin, are mostly from seeds and pulp (PEREIRA et al., 2017). It is important to point out that the jaboticaba fruit composition ranges according to species, ripening stage, climate conditions, soil, among others (DE SOUZA et al., 2017; LIMA et al., 2008).

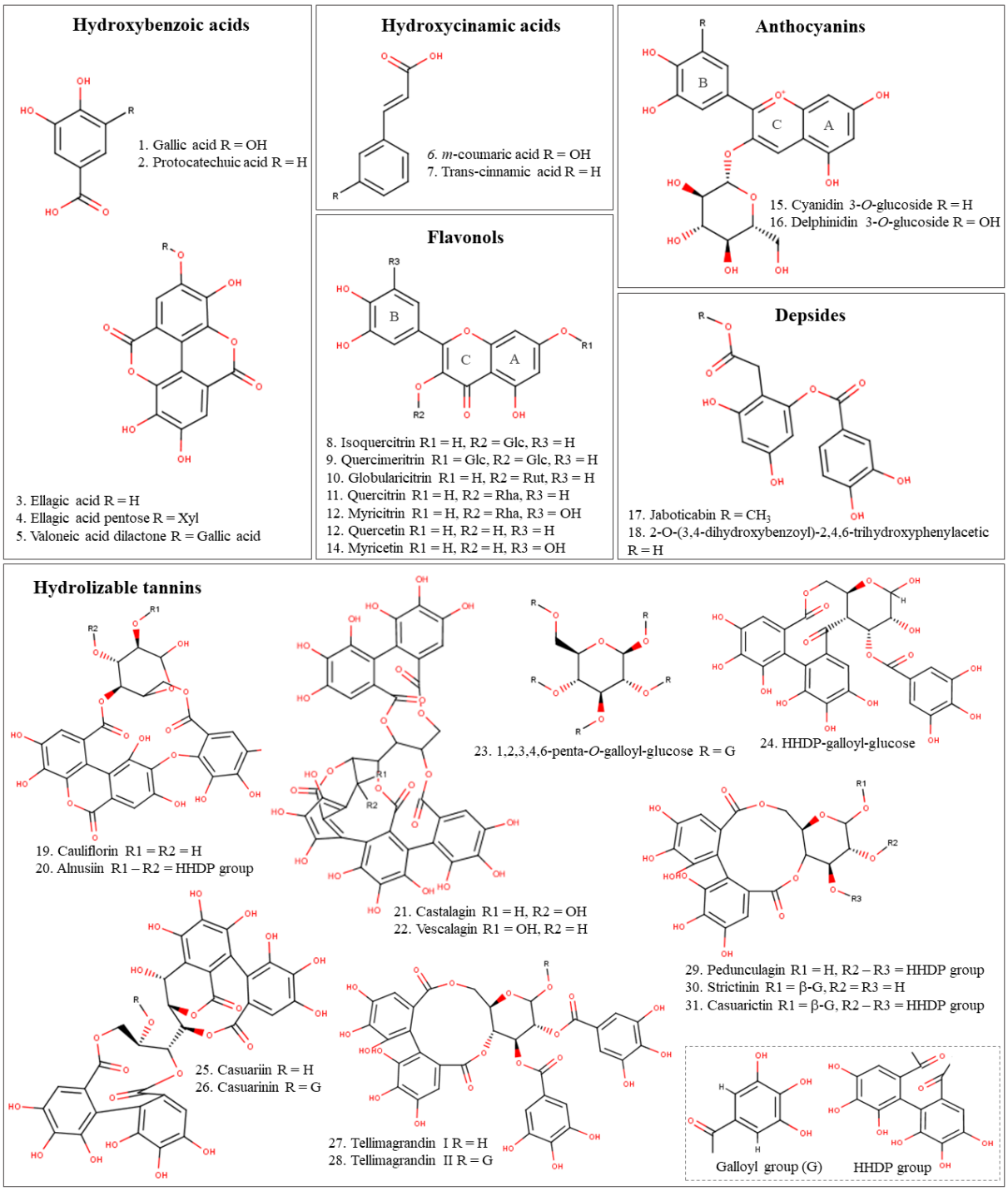

Figure 2 - Chemical structure of detected phenolic compounds in jaboticaba (*M. cauliflora*) separated into classes: phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (flavonols and anthocyanins), hydrolyzable tannins, and depsides.

1.1.4 Extraction of bioactive compounds from jaboticaba

In most cases, the first step for the application of bioactive compounds from the jaboticaba fruit or other vegetal matrix is the recovery of target molecules. **Table 1** summarizes the main methods, solvents, and process conditions to extract phytochemicals from different parts of the jaboticaba fruit. As can be seen, the jaboticaba peel is the most explored part of the fruit, probably, due to the high concentration of phytochemicals (INADA et al., 2015).

Among the different extraction methods applied for jaboticaba materials, the traditional procedures such as maceration, Soxhlet, and HAE (Homogenized Assisted Extraction), have been explored, as well as the environmental-friendly technologies such as UAE (Ultrasound-Assisted Extraction), PLE (Pressurized Liquid Extraction), SFE (Supercritical Fluid Extraction) and HPCDAE (High-Pressure Carbon Dioxide Assisted-Extraction) **(Table 1)**.

Table 1 - Target compounds, extraction method, type of the solvent, conditions, and yields of the extraction processes from jaboticaba.

Target compounds	Extraction method	Solvent	Conditions	Product recovered	Remarks	Ref
Whole fruit						
Total phenolic compounds (TPC) and anthocyanins (ANC)	UAE	Methanol (51%)	TPC: 1.5:20 g/mL ratio, at 26 °C, 68.5% ultrasound amplitude, pH 7, for 0.5 seconds ANC:1.5:20 g/mL ratio, at 39 °C, 38% ultrasound amplitude, pH 7, for 0.47 seconds	TPC: 3279 µg/g and ANC: 520.5 µg/g	This is the first time that UAE was optimized for analytical purposes	Fernández-Barbero et al. (2019)
Peel						
Anthocyanins (delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside)	HAE	Ethanol and water	1:20 g/mL ratio, at 47.1 °C, for 21.8 min, and 9.1% ethanol (v/v)	14 mg D3G/g extract and 71 mg C3G/g extract	The conventional method (HAE) presented a higher anthocyanin yield than UAE.	Albuquerque et al. (2020a)
Galactose-rich heteropolysaccharide (GH)	HSE	Water acidified with nitric acid (pH1)	1:30 (w/v), 80 °C for 30 min	32.32 g/100g of raw material, containing 67.21% of galactose	The GC/MS and NMR showed galactose, glucose, arabinose, rhamnose, and traces of free and esterified uronic acids in the GH composition.	Miranda et al. (2020)
Total phenolic compounds (TPC), anthocyanins (ANC), organic acids, and tocopherols	HSE	Ethanol and water (80:20 v/v)	Non-anthocyanin compounds –5:65 mg/mL ratio, at 40 °C, for 1h. Anthocyanin compounds – ratio 1:3 mg/mL, at 40 °C for 1h with solvent acidified with 0.1% of citric acid	Total non-anthocyanin compounds: 3.59 mg/g dw, ANC: 24 mg/g dw, organic acids: 21.67 mg/100g dw, and tocopherols 1.71 mg/100g dw	Jaboticaba epicarp is rich in ellagitannins and anthocyanin, interesting compounds for the food and pharmaceutical industries	Albuquerque et al. (2020b)

Total phenolic compounds (TPC) and antioxidants	Microwave hydro diffusion and gravity (MHG) extraction	No solvent	In a 2.25 GHz multimode microwave oven, at atmospheric pressure, 400 W of power for 20 min, the extract was drained by gravity, condensed, and collected	TPC: 1.72 mg GAE/mL and antioxidant activity: 57741.67 μmol TE/mL	The extraction method was efficient and safe to obtain phenolic compounds from jaboticaba peel	Heck et al. (2020)
Phenolic content (TPC) and Anthocyanins (ANC)	UAE	Ethanol/water (50 v/v) acidified with formic, acetic or orthophosphoric acid	40 kHz and 150 W, 0.5:25 mg/mL ratio, at 30 °C for 60 min	TPC: 37 mg/g and ANT: 3.4 mg/g of raw material	The acetic acid in pH 3 was more efficient for TPC recovery and formic acid in pH 1, for anthocyanin	Barros et al. (2019)
Polyphenol (ellagitannin) and Anthocyanin (cyanidin-3-*O*-glucoside)	UAE	Water (acidified with HCl)	1:10 w/v ratio, 25 kHz for 20 min, pH 1.5	0.9 mg/g of ellagic acid and 7.9 mg/g of cyanidin dw	Time and pH are the main variables on UAE extraction of these phenolic compounds	Fernandes et al. (2020)
Phenolic compounds	UAE followed by d-SPE clean-up step	Ethanol (50%) acidified with citric acid (pH 3)	1:50 g/mL ratio, at 30 °C, for 30 min, 37 kHz and 320 W. d-SPE: 1mL of extract and sorbents (100mg DE + 100 mg GCB) were vortexed for 1 min and centrifugated (10 min at 4000 *g*)	Ellagic acid: 1643 mg/kg	d-SPE clean-up step was suitable for remove interferent compounds	Senes et al. (2020)
Pectin	HRE	Water and nitric acid solution (0.1M) (1:1)	5:200 g/mL ratio kept at boiling (97 °C) for 20 min. The pectin from the acid extract was precipitated with the addition of two volumes of 96% ethanol, filtered and dried	4.5%	This study reports the jaboticaba as a new raw material from pectin obtention	Moreno et al. (2016)

Total phenolic content (TPC) and total Anthocyanin (TA)	High pressure method	Ethanol (50%) acidified with HCl (pH 3.6)	152 MPa for 3h	TPC: 18.51 mg/mL TA: 2.10 mg/mL	The extract presented a high concentration and the study confirmed the viability of obtaining nanoemulsions	Di Maio et al. (2019)
Phenolic compounds (mainly epigallocatechin gallate)	Marination	Water and methanol (50%)	Water: 1:50 g/mL ratio, for 15 min at room temperature. MeOH: 1:50 g/mL ratio, for 15 min at 80 °C	Water: 517.83 MeOH: 724.73 mg/L	Aqueous and methanolic extracts of jaboticaba were able to inhibit enzymatic activity	Marques et al. (2019)
Phenolic compounds	HAE	EtOH 85% and 15% of 1.5 M HCl	1:15 (w/v) ratio, 12 h at 4 °C	314.3 mg/100g dw	The ethanolic and methanolic extracts of JSP had the highest contents of phenolic compounds	Oliveira et al. (2018)
Total phenolic compounds	MAE	Ethanol (50%, pH 3.6)	1:10 (w/v) ratio for 60 s	92.2 mg/g extract	The extract presented the cytoprotective effect	Pitz et al. (2016)
Total phenolic compounds	HAE	Ethanol (95%)	1:12 (w/v), at roon temperature for 1h	184.1 mg/g extract	The extract presents a promising potential to be used as a natural antioxidant	de Almeida et al. (2015)
Total phenolic compounds	HAE and sonication	I - MeOH: Water (70:30) II - MetOH: Water: Acetic acid (85:15:0.5)	I – 0.5:15.5 (w/v), vortexed and sonicated for 10 mn. II – 1:10 (w/v), vortexed 30s and sonicated for 15 min	48.61 mg/g extract	Jaboticaba is a good source of antioxidant compounds and the extract from jaboticaba peel presented an excellent application as a food additive	Lenquiste et al. (2015)
Phenolic and anthocyanin	UAE	Ethanol 46%	1:20 (w/v), at 30 °C	Gallic acid: 92.8 mg/g, Cyanidin-3-*O*-glucoside: 4.8 mg/g and Ellagic acid: 7.8 mg/g dw	The variables pH, ethanol concentration and time influence the UAE, which presented high yields than sophisticated extraction using $scCO_2$	Rodrigues et al. (2015)

Polyphenols	HAE	Methanol 50% and Acetone (70%)	500 mg of the sample with 40 mL of each solvent at room temperature for 60 min	1290 mg/100g dw	The major contribution of this research was the identification of delphinidin-3-glucoside and cyanidin-3-0-glucoside as the main anthocyanin in jaboticaba	de Castro et al. (2014)
Total Phenolic Compounds and Anthocyanins	PLE	Ethanol	ANT: 5MPa, 80 °C, for 9 min in the static system Phenols: 5 MPa, 120 °C for 15 min.	13%, 2.4 mg of cyanidin/g dw, and 7.8 mg of gallic acid/g dw	The results were compared with LPSE. PLE presented 2.15 and 1.66-fold more anthocyanin and polyphenols levels, respectively and the PLE cost is 40-fold lower	Santos; Veggi; Meireles (2012)
Total phenolic content and Anthocyanin	HPCDAE	Acidified water with HCl (pH 2.5)	HPCDAE: 117 bar, 80 °C, with 20% of the ratio of solid-liquid mixture/pressurized CO2	TPC: 12.89 mg/g dw and TA: 2.23 mg/g dw	HPCDAE presented higher yields than PLE	Santos e Meireles (2011)
Peel and seeds						
Total phenolic content (TPC) from seeds and Total Anthocyanin (TA) from peel	HSE	TPC - Ethanol: water (60:40 v/v) and TA: methanol:water:acetic acid (80:20:0.5 v/v/v)	0.5:15 g/mL ratio, 30 °C for 2h	TPC: 86.5 mg/g and TA: 1172 mg/100g dw	The optimized extraction conditions provided high efficiency	Paludo et al. (2019)
Seeds						
Phenolic compounds	HAE	Water: propanone (3:2 v/v)	0.5: 25 (w/v) ratie, at 25 °C for 30 min	8.65 g/100g dw	Time and temperature did not influence the phenolic yield.	Hacke et al. (2016)
Total phenolic content (TPC) and consdensed tannins content (CT)	HAE	Water:propanone (52%:48%)	1:20 (m/v) ratio, at 45 °C for 45 min	TPC: 57.75 mg/g GAE/g and CT: 5.28 mg CE/g	Vescalagin, castalagin, and ellagic acid were the major polyphenols detected	Fidelis et al. (2020)

Residue						
Antioxidants	SFE	$scCO_2$ using ethanol as modifier	20 MPa, at 50 °C wit 20% of the modifier	15%	The temperature exerted more influence in yield. The SFE showed as an economic viability technology.	Cavalcanti; Veggi; Meireles (2011)
Phenolic compouinds	Marination	Ethanol: Water (45:55 v/v)	3:10 (w/v) for 24 h	17.89%	The extract was dried by spray-dried which exhibited great potential for use in pharmaceutical and nutraceutical fields.	Borges et al. (2017)
Anthocyanin	HAE	Water	1:3 (w/v) for 6h in the dark	5.95 mg/g dw	The extract using only water as the solvent presented high anthocyanin content, especially, cyanidin-3-*O*-glucoside	Cavalcanti; Veggi; Meireles (2016)
	UAE	Aqueous solution of citric acid (1%)	10:50 (w/v), at 20 °C, for 15 min, 351 W/cm^2	510.35 mg/100g dw	It is possible to obtain a natural pigment with antioxidant properties from jaboticaba pomace	Souza; Gurack; Marczak (2017)
	HSE	NADES ChCl:Ca 1:1 (50%)0	1:30 (w/v), at 60 °C for 60 min	279.45 mg C3G/100g	The use of NADES:water solutions showed as a sustainable, selective, efficient, and low-cost alternative for the sequential obtention of anthocyanin and pectin from jaboticaba residue	Benvenutti et al. (2020)
Pectin	HSE	NADES Ca:Glu:Wa 1:1:3 (10%)	1:30 (w/v), at 80 °C for 2.5h	27.3%		
Total Phenolic Compounds and Total Anthocyanin	Shear	Water	1:2 (w/v) ratio, at 80 °C for 45 min.	TPC: 15.69 g/g. TA: 2.59 mg/g of extract	This approach is a means of harnessing the antioxidants from by-products	Rodrigues et al.(2018)

Note. HAE: Homogenization Assisted Extraction, HSE – Heating-Stirring Extraction, HHP: High Hydrostatic Pressure, HRE: heat reflux extraction, UAE: Ultrasound-Assisted Extraction, PLE: Pressurized Liquid Extraction, SFE: Supercritical Fluid Extraction, HPCDAE: High-Pressure Carbon Dioxide Assisted-Extraction, SPE: Solid Phase Extraction, TPC: Total phenolic content, TA: Total Anthocyanin, ANC: anthocyanin, D3G: delphinidin-3-*O*-glucoside, C3G: cyanidin-3-*O*-glucoside, dw: dry weigh.

Regarding the solvents used, most studies applied water, ethanol, methanol, and $scCO_2$ (**Table 1**). Oliveira et al. (2018) evaluated different solvents - 70% acetone, water, 85% ethanol with 15% of 1M HCl solution, and 50% methanol - to obtained phenolic compounds from jaboticaba peel. The acidified ethanol solution provided a higher phenolic recovery, followed by 50% methanol. Additionally, emergent solvents such as Natural Deep Eutectic Solvent (NADES) showed a good alternative for selective, efficient, low-cost, and environmental-friendly sequential obtention of anthocyanin and pectin fractions from jaboticaba residue with high potential for food and pharmaceutical applications (BENVENUTTI et al., 2020). Another eco-friendly approach is the Microwave by Hydrodiffusion and Gravity (MHG) extraction method, which allowed the efficient obtention of bioactive compounds (1.72 mg GAE/mL) from jaboticaba peel without solvent use. The extraction without solvent, besides eliminating the use of toxic solvents, reduces waste generation, and energy consumption (HECK et al., 2020).

Process conditions are highly related to the extraction yields and depend on the technique used and the target compounds. Therefore, some researchers optimize these conditions to increase yields, reduce costs, solvents, and energy consumptions, and still, facilitate the scaling up, which is highly needed to provide industrial applications (ALBUQUERQUE et al., 2020a; FERNÁNDEZ-BARBERO et al., 2019; PALUDO et al., 2019).

1.2. GREEN AND SUSTAINABLE EXTRACTION TECHNIQUES

The concept of green extraction derives from the green chemistry approach, which came up from awareness and responsibility about human health and the environment due to the

observed problems from human activities around the world (ARMENTA et al., 2019). From this idea, the green chemistry concept searches for the sustainability of processes and products, being firstly defined in 1999 by Anastas (1999). Since that, green analytical chemical (GAC) strategies stated be discussed, and in 2013, Gałuszka, Migaszewski and Namieśnik (2013) proposed the 12 principles of GAC with recommendations and guides destined to the scientific community. The 12 principles were based on the 5 main factors: sample, which must be minimum in number and size; reagent, that must be non-toxic, safe, from renewed fonts and use reduced; instrument energy-efficient and miniaturized; method preferably automated, *in-situ*, no sample treatment, no derivatization and with process and operations integrated; the waste must be zero or reduced and properly treated; and, finally, the operator must be safe.

Still on basis of green chemistry, more recently, Chemat et al. (2017) discussed "Green Food Processing", which englobe the discovery and design of technical processes with aim of reducing the consumption of water and energy, recycling the by-products through the biorefinery concepts and ensure the quality and safety of the final product. The same authors established a guideline to define the green extraction of natural products in six principles (CHEMAT et al., 2019). These principles are following the main factors of GAC principles (ARMENTA et al., 2019; ARMENTA; GARRIGUES; DE LA GUARDIA, 2015), but with specific adaptations. Regarding raw material, must be renewable and, and is recommended that be from a low exploited local food crop. In the extraction is discussed about the solvent used and not about the reagents. It is recommended that the solvent chosen is from natural origin, safe for the environment, operator, and consumer, with the possibility of recovery and reuse, suitable for industrial uses at an affordable cost. The use of innovative technologies, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized

solvent extraction (PSE), supercritical fluid extraction (SFE), pulsed electric field, among others, can provide higher extraction yields, reducing process time and solvent amount. A green extraction also should consider the co-products generation, instead of waste, following the biorefinery concept. Another need is to reduce or intensify the unit operations, improving the process control. Finally, the recovered extract must be safe, free from contaminants, and with quality and functionality.

Considering the aforementioned, there is a need to identify how green is the "green process". Therefore, since 2002 green metric tools have been proposed to evaluate the greenness of an analytical methodology. Among them, atom economy, environmental factor (E-factor), environmental quotient (EQ), effective mass yield, mass intensity, the process profile, life cycle analysis have been used and are better explained in the works developed by Galuska et al. (2012) and Van Aken; Strekowski; Patiny, (2006). From this idea, the *EcoScale* design aim to unique the green metric tools combining these goals. This scale is from 0 to 100, considering 6 main parameters that influence the process (yield, price of reaction components, safety, technical setup, temperature, and workup and purification). In each parameter, were attributed penalty points with relative weight, and the value of EcoScale is obtained from Equation (1) (VAN AKEN; STREKOWSKI; PATINY, 2006).

$$EcoScale = 100 - sum\ of\ idividual\ penalties \quad (1)$$

The evaluation of greenness is performed from the following ranking: >75 excellent, >50 acceptable, and <50 inadequate (VAN AKEN; STREKOWSKI; PATINY, 2006). More recently, were added to the penalty-points value, the volume of reagents consumed, and the waste generated using mathematical expressions to obtain the called "Green Certified" (ARMENTA; GARRIGUES; DE LA GUARDIA, 2015; ESPINO et al., 2018). These green

metrics were formulated for chemical reactions, but in the future, can be adapted to the extraction of natural products.

1.3. IL, DES AND NADES: EMERGENT SOLVENTS FOR BIOACTIVES OBTENCION

The search for an environmental-friendly, efficient and selective extraction process led to the development of new solvents. The ionic liquids (IL) can be defined as salts formed with an organic cation and an organic or inorganic anion, showing melting points lower than their former constituents – close to 100 °C (PASSOS; FREIRE; COUTINHO, 2014). This alternative solvent presents inherent thermodynamic properties such as low vapor pressure, thermal stability, adjustable viscosity, miscibility, solubility, and extraction capacity for many organic and inorganic compounds (BUBALO et al., 2014). However, there are difficulties to introduce them into the industry because they are not regulated by FDA, (Food and Drug Administration, USA), Codex Alimentarius (FAO/WHO), or European legislation, and their effects are not elucidated (MARTINS; BRAGA; DE ROSSO, 2017). Besides, currently has been considered inadequate to classify the IL as a green solvent according to twelve criteria of green chemistry due to its relatively high toxicity and price (ŞAHIN, 2019).

Deep eutectic solvents (DES) have similar thermodynamic properties as IL, but they are more easily prepared; generally, they are less harmful to the environment and present less toxicity than IL. Eutectic solvents are formed by a hydrogen bond acceptor (HBA), such as quaternary ammonium, with electric charge protected through a complex formed by hydrogen bond donators (HBD), such as urea, carboxylic acids, or ammine. The strong molecular interactions among these components form a mixture with a melting temperature lower than the

initial components (ABBOTT et al., 2003, 2004). DESs are defined as eutectic mixtures with a molar composition that result in an eutectic temperature lower than the resulting of the ideal eutectic point, as presented in **Figure 1** (MARTINS; PINHO; COUTINHO, 2018). **Figure 1** represents a phase diagram at constant pressure, for a binary system (components 1 and 2).

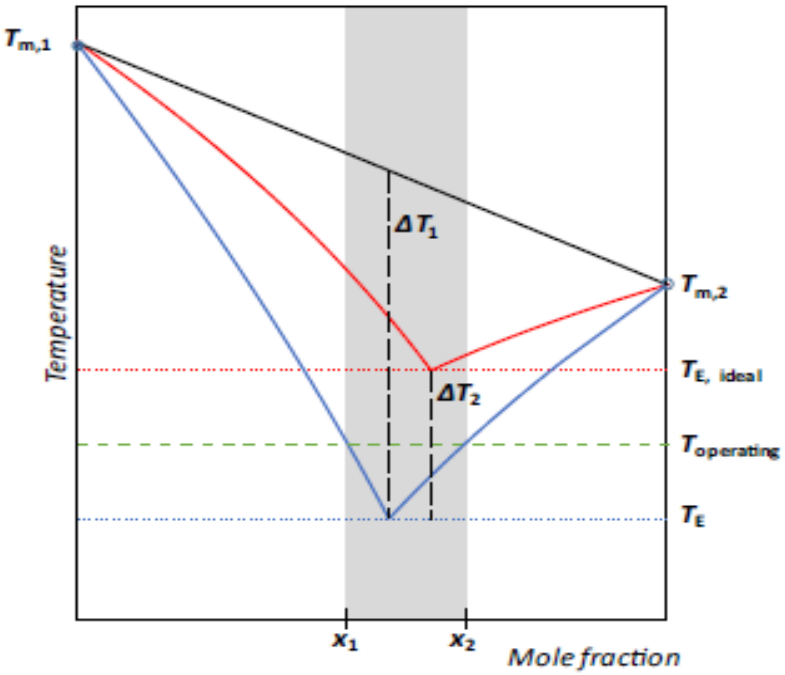

Figure 3 - Schematic representation of the comparison of the solid-liquid equilibria of a simple ideal eutectic mixture (red line) and a deep eutectic mixture (blue line).
Note: $T_{m,1}$ and $T_{m,2}$ – melting temperature of HBA and HBD, $T_{E,ideal}$ – ideal eutectic mixture, T_E – deep eutectic mixture, $T_{operating}$ – operating temperature.
Source: MARTINS; PINHO; COUTINHO (2018).

DES-based by natural compounds as starting molecules, usually plant primary metabolites (e.g. choline chloride, citric acid, malic acid, maleic acid, acetic acid, glucose, fructose, sucrose, trehalose, and water), have been termed as natural deep eutectic solvents (NADES) by Choi et al. (2011). Overall, NADES presents a lower environmental impact and toxicity than DES. However, some authors still use the DES denomination, even for mixtures formed by these “natural” compounds (CAO et al., 2018; NAM et al., 2015). This general denomination (DES) is explained by the fact that despite being formed by GRAS (Generally

recognized as safe) substances, and the eutectic mixture presents higher biodegradability and lower cytotoxicity (AHMADI et al., 2018; ASLAN TÜRKER; DOĞAN, 2021; RADOŠEVIĆ et al., 2016) than conventional solvents, the commercial HBA and HBD components (precursors), are mostly industrially manufactured. Therefore, much caution is required with the use of the term *natural*. Compared to other solvents, DES/NADES have the advantage to simulate the natural salvation way for water-insoluble primary and secondary metabolites in plants. Therefore, the DES/NADES work as an alternative liquid phase in nature (CHOI et al., 2011).

The replacement of organic and petrochemical solvents used in the recovery of bioactive compounds by emerging green solvents (IL, DES, or NADES) is of growing interest. This can be observed from the increasing number of research using these solvents in the SCOPUS Database Platform (www.scopus.com). The research carried out with the words "ionic liquid" AND "extract*" result in 12579 documents, and with the words "deep eutectic solvent" AND "extract*", 2142 documents, being the first one in 2003. The first publication using the NADES term was reported in 2011 (CHOI et al., 2011) and the database reported 553 documents with the words "natural deep eutectic solvent" AND "extract*". Therefore, the newness concerned with these solvents is evident, most particularly for DES and NADES, mainly due to the potential green approach associated with its use.

Although this thesis is the first to use these solvents to recover bioactive compounds from jaboticaba, DES/NADES already were employed for the extraction of anthocyanin and pectin, the main target compounds of this work (PANIĆ et al., 2019; SHAFIE; YUSOF; GAN, 2019).

1.4. DEEP EUTECTIC SOLVENTS

1.4.1 Preparation of DES

The preparation of DES is relatively simple **(Figure 3)**, the components are mixed and stirred at a specific temperature until the total formation of a homogeneous liquid. Then, it is cooled to ambient temperature, without the need for purification. In general, the DES synthesis can take from 30 min to 6 hours (BENVENUTTI et al., 2020; DAI et al., 2013a; HAYYAN et al., 2014), an advantage towards the IL.

Dai et al. (2013a) reported two methods for the synthesis of DES. In the first method, the compounds are diluted in water, subsequently stirred, heated (50 °C), and evaporated under vacuum. The resulting liquid is dried in a dissector with silica gel until constant weight. To obtain DES with a known water amount, a second procedure can be performed. It consists of a mixture of components including water, which is heated (50 °C) and stirred to obtain a limpid liquid. Although used less frequently, DES can also be obtained by the freeze-drying method, where an aqueous solution of components must be frozen and freeze-dried to obtain a clear and viscous liquid (GUTIÉRREZ et al., 2009).

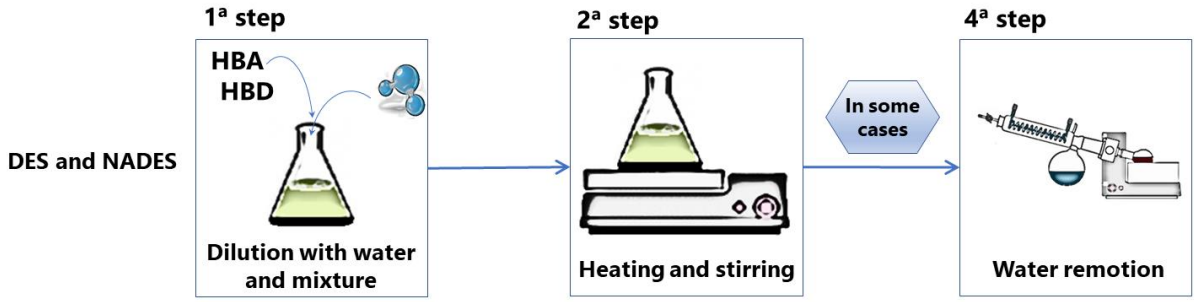

Figure 4 - Steps of preparation of deep eutectic solvents or natural deep eutectic solvents (DES and NADES).

The potential components used for the preparation of these emerging solvents (IL, DES, and NADES) are selected according to characteristics such as their ability to form a

mixture with a melting point lower than the individual constituents. The time and energy required for the synthesis are also considered for the selection of components to provide low process costs. Other important criteria for compound selection are related to environmental aspects, with higher standards attributed to greener solvents, allied with their ability to solubilize target compounds and associated with the solvent properties (CRESPO et al., 2017; DAI et al., 2013a).

As mentioned, DES/NADES can present water in their composition. The water molecule precipitates the supramolecular structure of these solvents, facilitating their formation. Furthermore, the water decreases the solvent viscosity, increasing the dissolution rate of the target compounds, and also decreasing the costs of the solvent mixture. However, the maximum water content in the DES solution must be carefully evaluated because excessive water concentrations may weaken the hydrogen bonds between HBA and HBD, reducing the solvation ability of this solvent (BAJKACZ; ADAMEK, 2017; LIU et al., 2018).

1.4.2. Properties of DES

The interactions between the starting components of DES confer their inherent properties. These associations form a network, favoring the solvation of different classes of compounds through interactions with the target compounds (LIU et al., 2018). Martins, Pinho & Coutinho (2018) defined DES as non-ideal mixtures due to the large differences between the molecular characteristics of their constituents. The main chemical and physical properties of these emerging solvents that must be well known to aid the definition of their applications are melting point, density, viscosity, conductivity, polarity, and solubility.

As previously reported IL, DES and NADES present melting temperatures (T_m) below their constituents. DES generally present T_m below 100 °C. The first reported DES, for instance,

presented T_m about 12 °C, being formed by ChCl (T_m = 302 °C) and urea (T_m = 133 °C) (ABBOTT et al., 2003). The low T_m values maintain the DES in the liquid state at ambient temperature. Therefore, processes using these solvents can be performed at mild temperatures, favoring the extraction of thermolabile compounds and decreasing energy costs compared to high-temperature operations.

The density and viscosity of the solvents are relevant properties affecting directly the transport phenomenon involved in the extraction process (MARTINS et al., 2018). DES and NADES can show highly variable viscosities and densities depending on the composition and temperature. DES can present viscosities from 5 to 6175 cP (ABBOTT et al., 2004; SAVI et al., 2019) and densities from 1.06 to 1.31 g/cm^3 (GUO et al., 2013; SAVI et al., 2019). Therefore, the viscosity of these eutectic mixtures can be as low as alike organic solvents or as high as pasty or gelatinous solutions, while density values are high compared to conventional solvents (RIBEIRO et al., 2015). These properties are directly affected by molecular size, structure, and intermolecular forces from the DES and NADES constituents (GUO et al., 2013; ZHANG et al., 2012). For instance, solvents containing lactic acid, with one carboxyl group, present lower viscosity than solvents formed by citric acid, with three carboxyl groups because of the increase in intermolecular forces (SAVI et al., 2019). Additionally, according to Dai et al. (2013a), solvents formed by polyalcohol have lower viscosity compared to the ones formed by acid or sugar, in that order, because smaller molecules, such as from polyalcohol, provide weaker molecular interactions. Therefore, the selection of the DES/NADES constituents is essential since high viscosity and density imply a low diffusion rate and mass transfer, decreasing its solvation ability (DAI et al., 2016).

The solvent polarity is another important property related to the solvation capacity. In general, DES and NADES are hydrophilic due to high electronegativity and the ability to form hydrogen bonds via dipole-dipole interactions. Therefore, they are normally comparable with polar solvents (ABBOTT et al., 2017; LIU et al., 2018), although some mixtures, such as citric acid and menthol, show mutually polar and non-polar properties (RIBEIRO et al., 2015). According to Dai et al. (DAI et al., 2013a), DES synthesized by organic acids shows the highest polarity (44.81 kcal/mol), followed by the synthesized by amino acids and sugar, reaching near water polarity (48.21 kcal/mol). Whereas, DES formed by ChCl and 1,2-propanediol presented the lowest polarity (51.89 kcal/mol), similar to methanol. Then, with this wide range of constituents, these solvents can be used to solubilize a large variety of bioactive compounds.

1.4.2 Toxicity and Biodegradability of DES

Environmentally acceptable solvents should present low toxicity and high biodegradability. These characteristics are related to the type and proportion of the constituents of IL and DES (GOUVEIA et al., 2014; RIBEIRO et al., 2015). From the environmental aspects, in general, DES are considered as green solvents due to their higher biodegradability (> 68% in 28 days) and lower bacterial toxicity compared to traditional solvents and IL (ZHAO et al., 2015a). Choline chloride (ChCl), the main HBA used, shows relevant characteristics as biodegradable, non-toxic, and low cost (FRANCISCO; BRUINHORST; KROON, 2013; ZHAO et al., 2015a). This amine salt is naturally present in the cell lipid membrane (CHOI et al., 2011). For commercial purposes, the ChCl is also easily synthesized by trimethylamine, ethylene oxide, and HCl. Because this chemical reaction does not generate residues, its Environmental (E) factor (mass ratio of waste to desired product) is zero, therefore it is considered a clean and sustainable process (ABBOTT et al., 2011; SHELDON, 2007).

Hayyan, Looi, Hayyan, Wong & Hashin (2015) evaluated the cytotoxicity of DES composed by ChCl combined with different HBD, such as glycerin (Gl), ethylene glycol (EG), triethylene glycol (TEG), or urea (U), using 5 human cancer cell lines and one normal cell. The evaluated DES presented lower human cytotoxicity compared to IL and their constituents. Ribeiro et al., (2015) stand out that DES as ChCl: glucose and ChCl: glycerol showed low cytotoxicity, while that ChCl: oxalic acid had moderate cytotoxicity. Some DES, especially those containing organic acids, exhibits antimicrobial effects against gram-negative and gram-positive bacteria due to their low pH (ZHAO et al., 2015a).

According to Paiva et al. (2014), in general, choline-based DES are noncytotoxic. Furthermore, DES are more biodegradable and result in less environmental impact than IL, for example. Huang et al. (2017) checked the toxicity of thirteen choline-based and glycerol-based DES against two gram-positive bacteria (*S. aureus* and *L. monocytogenes)* and two gram-negative ones (*E. coli* and *S. enteritidis)*. According to the authors, no microbial growth inhibition was detected. Additionally, the authors evaluated the DES biodegradability and the results lay between 70.5 and 94.8% from 7 to 28 days. These data indicate that all DES evaluated are considered green biodegradable solvents.

1.4.4 Extraction process using DES and NADES

1.4.4.1 Solvent constituents

The solubility of the target compound is related to the thermodynamic properties of the system. Therefore, the extraction yield and selectivity using DES depend mainly on the substances used in the preparation of the solvent, the type of vegetal matrix, and the molecular structure of the target compounds (BAKIRTZI; TRIANTAFYLLIDOU; MAKRIS, 2016; MARTINS et al., 2018).

According to **Table 2**, ChCl is the most used HBA for the extraction of bioactive compounds, while for HBD, the main components used are organic acids, sugars, and alcohol. Several classes of bio-compounds have been extracted by DES and NADES from different combinations of HBA and HBD. Rutin, for instance, can be extracted using DES containing glycerol and triethylene glycol (PENG et al., 2018; ZHAO et al., 2015a). Procyanidins and anthocyanins are extracted by DES formed mainly by acids because the solubility of these target components increases in acidified medium (CAO et al., 2018; DAI et al., 2016). The DES formed by ChCl:ethylene glycol extracted essential oil from the *Amomum* species with higher hydrocarbons content compared to conventional solvents (YU et al., 2018). Extraction and separation of protein from an aqueous two-phase system (ATPS) using DES were reported by Li et al. (2016). The studied DES were formed by betaine, as HBA, and by urea, methyl urea, glucose, sorbitol, ethylene glycol, or glycerol, as HBD, with the best protein extraction detected by the green-based solvent betaine-urea. Therefore, the solvent selection depends on the target substances.

Bakirtzi et al. (2016) evaluated different compositions of DES [lactic acid: choline chloride (3:1), lactic acid: sodium acetate (3:1), lactic acid: ammonium acetate (3:1), and lactic acid: glycine: water (3:1:3)] for the extraction of phenolic antioxidants from native Greek medicinal plants. All evaluated DES showed a higher yield of total phenolic content compared to conventional solvents (ethanol 60% and water). The highest efficiency was provided by lactic acid: glycine: water (3:1:3), with yields about 2 times higher than ethanol 60% and water. However, regarding the total flavonoid content, in general, DES results in a higher yield than water, but lower than ethanol 60%. Furthermore, Ivanović et al. (2018) verified that NADES formed by ChCl:lactic acid provided higher extraction of phenolic compounds from *L.*

citriodora in comparison with methanol 80%. Also, according to Alañón (2020), the strongest aspect of DES/NADES, compared to organic solvents, is the low amount required for extraction, resulting in less residue.

Furthermore, the proportion of HBA and HBD for DES/NADES preparations is also relevant and directly affects the physicochemical properties of the solvent. Cui et al. (2018) verified that the increase in HBD proportion reduces the viscosity and surface tension of the solvent, promoting diffusion and enhancing the mass transfer. The authors studied the proportion of ChCl:1,4-butanediol (1:1, 1:2, 1:3, 1:4 e 1:5) for flavonoids extraction from sea buckthorn leaves. The extraction yield increased with the HBD proportion up to 1:3 and then decreased. Higher HBD proportions (1:4 and 1:5) reduced yield due to a decrease in flavonoids-chloride interactions caused by an increase in HBD-chloride interactions.

The viscosity of DES, the main drawback for extraction use, can be reduced by adding water (LIU et al., 2018). Bajkacz & Adamek (2017) evaluated the content of water in NADES [from 10 to 75 % (w/w)] for the microextraction of flavonoids from vegetal matrixes. According to the authors, the extraction yield increased with the increase of water content of up to 30%. For the water concentration of 75%, the higher dilution weakened the hydrogen bonds, reducing the DES or NADES solvation capacity (DAI et al., 2013a; GUTIÉRREZ et al., 2009; LIU et al., 2018).

It is also worth mentioning the importance of the pH of the DES on the extraction of target compounds. The DES pH varies according to the solvent constituents. In general, acid-based deep eutectic solvent (ADES), low pH solvents, present excellent dissolution properties due to their ability to donate protons and accept electrons, and can be used to recover low polarity compounds such catechin, as well as polar compounds such as anthocyanin

(BOSILJKOV et al., 2017; LI et al., 2015; QIN et al., 2020). Else, DES constituted by polyalcohol such as 1,2-propylene glycol, with neutral pH, present a high ability for extraction of phenolic acid. DES constituted of sugar components, also with neutral pH, present high selectivity towards flavonoid recovery (IVANOVIĆ et al., 2018).

Table 2 - Summarization of the extraction conditions of bioactive compounds from different vegetable matrices using DES or NADES.

	Matrix	Compounds	DES	Water (%)	Methods	Optimum conditions	Ref.
Herbs/ Leaves	Sea buckthorn	Genistin, genistein and apigenin (flavonoids)	ChCl:1,4-butanediol (3:1)	20	HRE, MAE and UAE.	MAE. 1:24 (g/mL), at 64 °C, 17 min.	Cui et al. (2018)
	Moringa oleifera	Hydroxycinnamic acids and flavonoids	ChCl: citric acid	40	HAE	1:10 (g/mL), at 50 °C, for 1h	Djande et al. (2018)
	Native Greek medicinal plants	Phenolic compounds and flavonoids	Lactic acid: glycine: water (3:1:3)	20	UAE	1:100 (g/mL), at 80 °C for 90 min, 140 W and 37 kHz	Bakirtzi et al. (2016)
	Juglans regia L.	Phenolic compounds	ChCl: butiric acid (1:2)	20	HAE	1.25:5 (g/mL), at 60 °C for 50 min. and 600 rpm	Vieira et al. (2018)
	Camellia sinensis	Catechin	ChCl: phenil propionic acid (1:2) and ChCl:lactic acid (1:2)	20	MAE	1:35 (g/mL), at 66 °C for 8 min.	Li et al. (2015)
	Cannabis sativa	Phytocannabinoids	Menthol: acetic acid (1:1)	-	HAE	20:1 (mg/mL), at 30 °C for 10 min.	Krizek et al. (2018)
	Ginko biloba	Proanthocyanidin	ChCl: malonic acid (1:2)	55	HSE	1:10.57 (g/mL), at 65 °C for 53 min.	Cao et al. (2018)
Flowers	Safflower *(Carthamus tinctorius* L)	Flavonoids	ChCl:1,4-butanediol (1:5)	30	UAE, HRE, HAE	HRE. 1:10 (g/mL), at 60 °C, 40 min.	Bi et al. (2013)
		Hydroxysafflor Yellow A (HSYA), Cartormin, and Carthamin	Proline:malic acid (1:1), ChCl: sucrose (1:1), and lactic acid: glucose (5:1)	25	Marination	30:1 (mg/mL), at 40 °C for 1h	Daí et al. (2013b)
	Petal *Catharanthus roseus*	Cyanidin	Lactic acid: glucose (5:1) and CHCl:1,2-propanediol (1:1)	50	HAE and UAE	UAE. 50:1.5 (mg/ mL) at 40 °C for 30 min.	Daí et al. (2016)

	Sophora japonica	Flavonoids	L-proline and glycerol (2:5)	10	UAE, HAE, HRE and HAE + HRE	UAE. 50 (mg/ mL), 330-450W for 45 min.	Nam et al. (2015)
		Rutin	ChCl: triethylene glycol (1:4)	20	HSE	1:10 (g/mL), at 55 °C for 20 min.	Zhao et al. (2015a)
						1:100 (g/mL), at 65 °C for 20 min.	Peng et al. (2018)
	Lonicera japonica	Phenolic acids	ChCl:1,4-butanediol (6:1)	10	UAE, HRE and MAE	MAE. 1:9 (g/mL), 60 °C for 20 min, 700 W.	Peng et al. (2016)
Fruits	Dry fruits of specie *Amomum*	Essential oil	ChCl:ethylene glycol (1:4)	-	MAE	1:7 (g/mL). Hot flash phase: 600 W, at 110 °C, 5 min. Hydrodistillation: 300 W, 110 °C, 30 min.	Yu et al. (2018)
	Goji Berry	Petunidin and malvidin	ChCl: 1,2-propanediol (1:2)	20	UAE	1:20 (g/mL), at 52 °C for 15 min, 300 W and 25 kHz.	Sang et al, (2018)
	Mangosteen pericarp	Xanthones and phenolic compounds	Citric acid: alanine (1:1)	70-90	SWE	160 °C, pressure of 5-10 MPa	Machmudah et al. (2018)
Food wastes	Shrimp waste	Astaxanthin	ChCl:1,2-butanediol (1:5)	10	UAE	1:15 (g/mL), 20kHz for 30 min.	Zhang et al. (2014)
	Orange peel	Phenolic compounds	ChCl: ethylene glycol (1:4)	10	HSE	1:10 (g/mL), at 60 °C for 100 min.	Ozturk et al. (2018)
		Antioxidants and cytotoxics	ChCl: fructose (1,9:1) and ChCl:xylose (2:1)	30	HAE	0.1/10 (g/mL), at 65 °C for 50 min.	Radosevic et al.(2016)
	Grape skin	Phenolic compounds	ChCl: oxalic acid (1:1)	25	MAE and UAE	UAE. 0.1/1 (g/mL), 35 kHz, at 65 °C for 50 min.	Bubalo et al. (2016)
		Total anthocyanin	Citric acid: maltose (4:1)	24	UAE, heating, and stirring	UAE. 0.1/0.83 (g/mL) at ambient temperature for 9. min.	Jeong et al. (2015)
	Olive pomace	Phenolic compounds	ChCl: citric acid and ChCl: lactic acid	-	HAE, MAE, UAE and HHPAE	HAE, 2:25 (g/mL), 60 °C, 12,000 rpm for 30 min.	Chanioti; Tzia (2018)
	Wine lees	Anthocyanin-3-*O*-monoglucosides and Anthocyanin-3-(6-*O*-*p*-coumaroyl) monoglucosides	ChCl: malic acid	35,4	UAE	0.1:1 (g/mL), 30,6 min., at 35 °C, 341,5 W and 37 Hz	Bosiljkov et al. (2017)

Other	Vanilla pods	Vanillin	Lactic acid: 1,2-propanediol (1:1)	25	HAE	50 (mg/ mL) with stirring in vortex at 50 °C for 1h.	González et al. (2017)
	Olive oils	Phenolic compounds	Glucose: lactic acid: water (6:1:6)	~85	HAE	1g of oil in 1 mL of hexane + 5 mL of DES with stirring in a vortex for 10 min.	Paradiso et al. (2016)
	Soy products	Daidzin, genistin, daidzein, genistein, and biochanin (isoflavones)	ChCl: citric acid (1:1)	30	UAE.	200:600 (mg/ µL), 616W, at 60 °C for 60 min.	Bajkacz e Adamek (2017)

Note: HRE – heat refluxed extraction, HAE – homogenate assisted extraction, HSE – Heat-Stirring Extraction, MAE – microwave-assisted extraction, UAE – ultrasound-assisted extraction, HHPAE – high hydrostatic pressure assisted extraction, SWE – subcritical water extraction.

1.4.4.2 Methods

Different extraction methods using DES as solvents have been considered and compared in the literature. The most common processes using these solvents are maceration, heat-reflux extraction (HRE), homogenate-assisted extraction (HAE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). However high hydrostatic pressure assisted extraction (HHPAE), and subcritical water extraction (SWE) modified by DES also were employed. A summarization of these studies is presented in **Table 2**.

Chanioti & Tzia (2018) compared the extraction of different methods HAE, MAE, UAE, and HHPAE for the recovery of phenolic compounds from olive pomace using four different NADES. The ChCl:citric acid and ChCl:lactic acid were the best promising solvents for phenolic recovery, particularly compared to water and ethanol (70%) as solvents. In this study, a process optimized was proposed by combining methods. HAE provided the highest process efficiency for the solvents tested due to the high temperatures and stirring involved, which are the most relevant factors for the selection of the extraction method.

Nam et al. (2015) evaluated several extraction methods using DES to extract flavonoids from the Chinese plant *Flos saphorae.* In this study, UAE resulted in a higher yield compared to methods using stirring, heating, and the combination of stirring and heating. Peng et al. (2016) evaluated the extraction of phenolic acids from *Lonicera japonica* using DES by the methods MAE, HRE, and UAE. The results indicate that HRE and UAE used larger solvent quantities, higher temperature, and process time compared to MAE. Therefore, MAE performed with DES was an efficient and promising method for the extraction of natural bioactive compounds from *Lonicera japonica*.

Among the emergent extraction process, the use of DES combined with high-pressure methods is still poorly explored. High-pressure techniques using liquid solvents are known as pressurized liquid extraction (PLE) or subcritical solvent extraction (SSE), pressurized hot-solvent extraction (PHSE), or accelerated solvent extraction (ASE). When the water is used as the solvent, the process is commonly denominated subcritical water extraction (SWE), superheated water extraction (SHWE), or pressurized hot-water extraction (PHWE) (GALLEGO; BUENO; HERRERO, 2019; ZIELINSKI et al., 2021). In these methods, the solvent is submitted to high pressures and temperatures. The solvent remains in a subcritical state at temperatures above its boiling point. These high-pressure methods provide high extraction yields due to the increase in the solubility of target compounds, improving the solvent diffusion due to the decrease in viscosity, facilitating the matrix penetrability, and improving the mass transfer rate (ZIELINSKI et al., 2021). Furthermore, solvents with hydrogen bond interactions have their superficial tension and dielectric constant highly altered with temperature changes. These physicochemical properties are mainly related to solute-solvent interactions, therefore, the solvent power can be tuned with adjustments in temperature, pressure, and solvent composition. It is also important to highlight that those high-pressure methods present low manufacturing costs and are considered green when GRAS solvents are used if compared with traditional extraction procedures (HERRERO et al., 2013; ZIELINSKI et al., 2021).

Moreover, the combination of extraction methods has been intensely studied to increase the application of these emerging solvents, enhancing the selectivity characteristics of the processes. Feng, Song, Dong, Yang & Yao (2017) performed two sequential extractions using the IL formed by benzothiazolium cation and p-toluenesulfonate. The first extraction was

performed in a thermostatic oscillator and the second, was assisted by ultrasound. Therefore, by a sequential extraction procedure, it was possible to recover two fractions from the *Polygonum multiflorum*, one enriched in stilbene glycoside and the other rich in anthraquinone.

1.4.5 Solvent recovery and recycling

An alternative to reduce the extraction process costs is associated with solvent recovery and recycling after the solute separation. Nevertheless, because the emerging solvents, IL and DES, present low vapor pressure, its separation from the target compounds by the vaporization method, the usual separation procedure, is difficult. Even considering this difficulty, there is a growing interest to study methods to separate solute and solvent, with the view of solvent recycling (LIANG; FU; CHANG, 2019; PASSOS; FREIRE; COUTINHO, 2014).

Consequently, methods such as solid-liquid extraction (SLE) using resin or molecular sieves, liquid-liquid extraction (LLE) with a focus on anti-solvent ability, ultrafiltration, and electrodialysis, or back-extraction and adsorption have been used to separate the solute from the emerging solvents (LIANG; FU; CHANG, 2019; PASSOS; FREIRE; COUTINHO, 2014; RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017).

Nam et al. (2015) extracted flavonoids from *Japanese acacia* using DES (L-proline: glycerol - 2:5). After the extraction, the solvent was regenerated by LLE using water as anti-solvent, with 75% separation efficiency, and by solid-phase extraction (SPE) in a reverse-phase cartridge (C18) with 92% efficiency. Peng et al. (2016) separated mixtures of phenolic acids and DES by SPE using NKA-9 resin reaching an efficiency of about 79%. Li et al. (2015) used an adsorption column with NKA-9 macroporous resin to recover up to 86.1% of the solute catechin from different DES. Aqueous two-phase systems (ATPS) with IL and DES have also been reported for the extraction and purification of various biomolecules. Besides the high

extraction efficiency, the ATPS improved the selectivity (partition coefficient) to aid the process scale-up for separation and purification of biomolecules (FARIAS et al., 2018).

Liang, Fu, and Chang (2019) recovered up to 96% of DES from lignocellulose constituents by the membrane-based process but pointed to the importance of further research for an effective industrial application of these solvents. Regeneration and recycling of these emerging solvents are important tasks that must be considered during process optimization. Several aspects affect the reuse of these solvents such as their characteristics, solute attributes, separation method and efficiency, energy requirement, process, and solvent costs.

Currently, DES has been used as a type of bio-sorbent when combined with magnetic molecularly imprinted polymer (DES-MMIP), which is a green alternative to selective adsorption of target molecules with magnetic separations property. Using a binary DES, this approach already was used to separate bovine hemoglobin from calf blood using with high selectivity. However, the high viscosity of the DES harms the adsorption capacity of the system (XU et al., 2019). The use of a ternary DES resulted in a DES-MMIP with high adsorption ability and selectivity of baicalein from the extract of *S. baicalensis* (WANG et al., 2020). Similarly, Qian et al. (2021) developed a bio-solvent immobilizing the β-cyclodextrin-grafted magnetic Fe_3O_4 nanoparticles with polymeric deep eutectic solvents (M-β-CD@PDES) to separate ovalbumin from real complex samples.

1.4.6 Stability of active compounds in DES and NADES

Dai, Verpoorte & Choi (2014) reported the influence of NADES on the stability of bio-compound, which is a result of the molecular interactions, mainly hydrogen bond between the solvent and the bioactive compound. In this research, the stability of carthamin, a natural colorant from safflower (*Carthamus tinctorius*), was evaluated in the presence of different

NADES, including ChCl: glucose, ChCl: sucrose, ChCl: xylitol, proline: malic acid, and acid lactic: glucose. The authors reported that the half-life time ($t_{1/2}$) of the compound diluted in NADES was 5 and 8 times higher compared to water solution, for storage at 40 °C and 60 °C, respectively. Carthamin diluted in NADES containing sugar showed lower light and heat degradation and higher stability compared to conventional solvents such as water and ethanol. Probably, the high viscosity of NADES containing sugar and the molecular interactions between the solvent and carthamin are responsible for the protective effects to heat, light, and time degradations, increasing the bio-component stability.

Anthocyanins from petals of *Catharanthus roseus* also showed higher stability during the extraction and the storage period when NADES was used as the solvent (DAI et al., 2016). The authors evaluated the effects of solvent type, temperature, and storage time using cyanidin as standard. Results showed higher cyanidin stability in the presence of NADES formed by lactic acid and glucose at 60 °C, compared to acidified ethanol with formic acid. The temperature influence on the anthocyanin stability was investigated at 25, 4, and – 20 °C, comparing cyanidin solutions with NADES and with ethanol. The NADES protective effect was evident at 4°C, with stability reaching 7 days, compared to a quick degradation in ethanol. The stability of the cyaniding in NADES is marked higher than in ethanol, most probably due to the hydrogen bonding between bio-compound and NADES.

Therefore, the stability behavior of bio-molecules in extraction solvents should be considered to warrant the biological activity associated with the target components. This characteristic is related to the type of biomolecule, the kind of starting constituents of DES or NADES, and the conditions of extraction and storage.

1.5. STATE OF ART

Jaboticaba fruit shows a phytochemical composition with high nutritional value, health benefits, and technological functions. Some emergent technologies such as UAE, MAE, and high-pressure fluid technologies have been applied as extraction processes to recover target molecules from jaboticaba fruits or their processing by-product. However, the protection of the most labile compounds, main anthocyanin, is still a challenge for the academic community depending on the desired application. Despite the need for further investigation in this subject, the literature review appoints that this unexplored Brazilian fruit may provide economically viable products. Furthermore, the use of jaboticaba processing by-products can promote a more efficient utilization of natural resources. This approach can be associated with circular economy and biorefinery concepts to the recovery of different fractions with high technological and bioactive quality, besides widener the application possibilities of jaboticaba fruit and by-product.

Santos, Veggi, and Meireles (2012) carried out the optimization and economic evaluation of the extraction of anthocyanins and other phenolic compounds from jaboticaba peel by PLE with ethanol. The PLE results show the anthocyanin yield is 2.15-fold higher than the low-pressure method with manufacturing costs 40-fold lower. The anthocyanin recovery from jaboticaba peel also was performed by maceration, UAE, HRE (heat reflux extraction), and MAE (**Table 1**). According to Moreno et al., (2016), the jaboticaba peel is an alternative source of pectin, with a content of 4.5% (w/w) recovered by high reflux extraction using 0.1 M nitric acid at boiling temperature. Fidelis et al.(2020), optimized the process to recover bioactive compounds from jaboticaba seeds and reported antiproliferative, cytotoxic, antimicrobial, antihemolytic, and antihypertensive effects from the optimized extract.

The use of emerging solvents in extraction methods joins the actual academic and industrial interest in green processes and the development of sustainable food systems due to the high demand for economically viable and healthy products. Among the emergent solvents, the DESs stand out because are formed by eco-friendly and low-cost components, attractive for food and pharmaceutical applications. Besides, a wide variety of bioactive compounds such as alkaloids, phenolic compounds, essential oils, and proteins have been extracted by these solvents, confirming the DES solvation capacity and versatility. However, there are few studies in the literature combining DES with high-pressure extraction. Just 26 documents in the SCOPUS database (www.scopus.com) including the words “pressurized liquid extraction” OR “pressurized solvent” OR “subcritical water extraction” AND “deep eutectic solvent” were found. Among these documents, 12 are review articles, 10 are research articles, 3 are book chapters and 1 is a conference review.

DES has already been reported as an enhancer to subcritical water extraction (SWE). For instance, 30% DES (alanine: citric acid) water solution, by SWE method, was highly efficient to recover xanthone from mangosteen pericarp (MACHMUDAH et al., 2018). Similarly, SWE with 30% DES (choline chloride: urea) provided an efficient recovery of phenolic compounds from winemaking byproduct (LOARCE et al., 2020), while SWE with 30% DES (choline chloride: oxalic acid) extracted anthocyanin from grape pomace (LOARCE et al., 2021). Also, 30% DES (choline chloride:glycerol) was efficient to recover polysaccharides from brown seaweed (SARAVANA et al., 2018). PLE associated with DES-based liquid-liquid microextraction was employed to extract pesticides from the chicken liver (MONAJJEMZADEH; FARAJZADEH; MOGADDAM, 2021). However, so far, there were

no reports of using DES aqueous solution combined with PLE for the valorization of an industrial by-product.

Commercially, DES is used to obtain bioactive compounds for cosmetics processing by the Naturex® company (Naturex, Avignone, France). However, the application of DES in the food industries is under viability evaluation. This feasibility study includes the environmental impact, toxicity, stability, solvent regeneration, solute recovery, and, evidently, the operational costs.

Then, the novelty of the present study is the sequential obtaining of an anthocyanin-rich fraction and a pectin-rich fraction, to valorize the jaboticaba peel, by using an eco-friendly approach of pressurized DES solutions selected by *in silico* and experimental analyzes. Furthermore, the use of DES to recover bioactive compounds from jaboticaba had not yet been reported in the literature.

CHAPTER 2

Deep Eutectic Solvents (DES) for anthocyanin and pectin extraction from *Myrciaria cauliflora* fruit by-product: *in silico* and experimental approaches for solvent selection

This chapter includes the preparation of six different DES, their characterization by DSC, pH and rheological analysis, and the selection of more suitable DES components for sequential extraction of anthocyanin and pectin from jaboticaba by-product. The content of this chapter was published as a full research article "NADES as potential solvents for anthocyanin and pectin extraction from *Myrciaria cauliflora* fruit by-product: *in silico* and experimental approaches for solvent selection" in the **Journal of Molecular Liquids** (2020). (https://doi.org/10.1016/j.molliq.2020.113761).

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NADES as potential solvents for anthocyanin and pectin extraction from *Myrciaria cauliflora* fruit by-product: *In silico* and experimental approaches for solvent selection

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ABSTRACT

In this work, an *in-silico* and experimental approach were combined for the selection of starting compounds of deep eutectic solvent (DES) aiming at a selective and sequential extraction of anthocyanin and pectin from Myrciaria cauliflora by-product. Six DES were prepared and characterized by DSC, pH, and rheological analysis. COSMO-RS was used to study the molecular contributions that confer affinity between DES and target molecules. Besides, experimental solubility, extraction ability, and DES cost were evaluated. DSC confirmed the formation of eutectic mixtures, which presented low pH values (0.77 to 4.01) and a wide range of apparent viscosity (0.070 to 36.922 Pa.s). The σ-profile [p(σ)], obtained by COSMO-RS, provided information on the polarity distribution of molecules, and σ-potential [μ(σ)] curves were indicative of affinity between solute and solvent. According to *in-silico* evaluation, both DES and target compounds exhibit amphoteric character, indicating affinities between them. Acid-based DES showed the highest affinity in the H-bond acceptor region while DES content betaine and water presented the best affinities in the H-bond donor region. However, all DES presented a low affinity for non-polar compounds. Choline chloride (ChCl): Propylene glycol (Pro) was the most promising DES for anthocyanin extraction and Citric acid (Ca): Glucose (Glu): Water (Wa), for pectin. The proposed approach established a wise way to select NADES: water solutions as low-cost green solvents, aiming to achieve selective recoveries of value-added compounds.

Keywords: Brazilian fruit, COSMO-RS, green solvents, molecular contributions, Deep Eutectic Solvents.

2.1 INTRODUCTION

Natural food ingredients have been evaluated as substitutes for synthetic additives due to their nutritional and technological effects associated with health benefits (RODRIGUEZ-AMAYA, 2016). In Brazil, there is a large variety of tropical fruits which generate, in their processing, a considerable number of co-products such as peels, seeds, and portions of pulp. In general, by-products present high contents of valuables nutrients and bioactive compounds, similar in composition to the intact vegetal matrix (GURAK et al., 2014). *Myrciaria cauliflora* is a blue Brazilian berry that belongs to the *Myrtaceae family*, rich in anthocyanin. Generally, it is commercially destined for fresh consumption and industrial processing for juice, jam, and fermented distilled liquors (GURAK et al., 2014; QUATRIN et al., 2019). It has been also reported that this industrial Brazilian berry by-product, representing nearly 40% of whole fruit, presents 2.5-fold higher phenolic content and 2.2-fold higher dietary fiber content than whole fruit (GURAK et al., 2014).

Anthocyanins are vegetal red-blue pigments belonging to the flavonoid group (CASTAÑEDA-OVANDO et al., 2009). Besides acting as a natural colorant, several beneficial health effects are attributed to anthocyanins such as improving human intestinal microbiota, antioxidant activity, and also inhibition of lipid peroxidation, cyclooxygenase enzyme, and cell tumor proliferation (VAREED et al., 2006; ZHOU et al., 2019). Another food ingredient found in this berry is pectin, a heteropolysaccharide with functionalities associated with prebiotic and bioactive agents. Furthermore, pectin is well-known for its technological properties as an emulsifier, gelling agent, stabilizer, and thickener of foods and beverages (MOHNEN, 2008; NAQASH et al., 2017).

Bioactive compounds, such as the aforementioned, are obtained from the vegetal matrix through several extraction methods like maceration, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, and enzyme-assisted extraction, among others (MARAN et al., 2017; PEDRO; GRANATO; ROSSO, 2016; RENARD, 2018; TAO et al., 2014). Conventionally, these processes are performed using organic solvents which recently have been substituted by GRAS (Generally Recognized as Safe) such as alcohols with low boiling points. Despite this, this type of solvent is not fully efficient to extract the non-polar to the polar range of molecules, being needed the use of solvent mixtures, high temperature-pressure conditions, or successive extraction stages for achieving the target molecule (RENARD, 2018). Besides, these solvents are highly volatile, therefore, they present a risk of fire and explosion. This release into the atmosphere also leads to air pollution (CHEMAT et al., 2019).

The search for alternative solvents has led to the development of Deep Eutectic Solvents (DES), which are composed of a hydrogen bond acceptor (HBA) with electric charge protected through a complex formed by a hydrogen bond donator (HBD). This type of solvent is nonreactive with water; and in general, they are biodegradable and less toxic regards conventional solvents and ionic liquids (ABBOTT et al., 2004; HAYYAN et al., 2015; RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017). Furthermore, DESs have been successfully used for obtaining added-value compounds from agri-food biomasses (DAI et al., 2013a; RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017; SHAFIE; YUSOF; GAN, 2019)

Molecular interactions between the DES components form an articulated network that confers inherent characteristics for these solvents such as negligible vapor pressure, high thermal stability, adjustable viscosity, high miscibility, and rare solvation ability, among others (PASSOS; FREIRE; COUTINHO, 2014). These characteristics allow the DES application in the extraction process. As the solvation power of DES is highly dependent on its thermodynamic properties, a good strategy to improve the selectivity and purity of the extracts is to understand the parameters related to molecular interactions between DES and target compounds (CAO et al., 2019; RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017).

Therefore, the aims of this chapter were i) to prepare different DES and to evaluate their physicochemical properties, ii) to study the interactions between DES and target molecules by an *in silico* tool (COSMO-RS) and relate them to the experimental solubility and extraction capacity and iii) to select the more efficient DES for selective extraction of anthocyanins and pectin from blue Brazilian berry by-product in a green approach.

2.2 MATERIAL AND METHODS

2.2.1 Materials

An industrial *Myrciaria cauliflora* by-product sample of 10 kg was acquired from *Sítio do Bello* (Piraibuna, SP, Brazil). Anhydrous glucose, citric acid P.A, malic acid P.A., and lactic acid (90%) were purchased from Êxodo Científica (Sumaré, SP). 2-Hydroxyethyltrimethylammonium chloride (Choline Chloride – ChCl) >98% obtained

from Sigma-Aldrich (Steinheim, Germany), propylene glycol (>99.5) from Neon Commercial (Suzano, SP) and betaine from Interprise® USA Corporation (Florida, USA). Other solvents such as 99.5% ethanol were obtained from Êxodo Científica (Sumaré, SP) and 99.5% acetone and ethyl acetate 99.5%, from Sigma-Aldrich (Steinheim, Germany).

2.2.2 Preparation of Deep Eutectic Solvents (DES)

Six DES types were prepared using the HBA and HBD were weighed in analytical balance (Shimadzu, AUY220, SP, Brazil) in the molar proportion that form deep eutectic points as shown in **Table 3**, according to Dai, Spronsen, Witkamp, Verpoorte, & Choi (2013a) and González & Wilson (2017). Then, the mixtures were heated at 80 °C, at continuous stirring, by a type Dubnoff bath (Ethik technology, 304 TPA model, SP, Brazil), until homogeneous and transparent liquid solvents were observed.

2.2.3 DES characterization

2.2.3.1 pH measurements

The pH values of DES were measured in triplicate according to AOAC (2016), by direct-reading in a bench pH meter (KASVI, model K39-2014B, PR, Brazil) calibrated with buffer solution pH 4.01, 7.01, and 10.01.

2.2.3.2 Differential scanning calorimetry (DSC) analysis

All DES synthesized (section 2.2), as well as their starting constituents were evaluated by DSC (Jade-DSC Model; Perkin Elmer, SP, Brazil) on the temperature range from 233 to 673 K, at 10 K/min, after equilibration for 3 min at 233 K. The analyses were

performed under nitrogen atmosphere (20 mL/min), with 3.55-14.30 mg of sample in aluminum pans covered with lids (AROSO et al., 2017).

2.2.3.3 Rheological measurements

Rheological parameters of the DES were obtained in a concentric-cylinder rheometer (Brookfield, model LVDV-II + Pro, Middleborough, MA, USA) using a spindle SC4-34 (SAVI et al., 2019). The data were obtained by Rheocalc software (v. 3.3) and the analysis was performed in triplicates, at 293, 323, and 353 K, controlled by a thermostatic bath with an accuracy of ± 0.25K (TECNAL, model TE-2005, SP, Brazil).

Flow curves were obtained ranging the shear rate (from 0.11 to 198 s^{-1}) and shear stress (from 71 to 1578 Pa) according to the rheological behavior of the solvent. The parameters *K* and *n* were estimated by non-linear regression solution based on Ostwald-de-Waele model (OW), Equation (2), using the OriginPro 2016 software (OriginLab, Northampton, MA, USA). This model was reported by Savi et al. (SAVI et al., 2019) as adequate to describe the rheological behavior of different DES.

$$\tau = K\ (\dot{\gamma})^n \tag{2}$$

where τ is the shear stress (Pa), K is the consistency coefficient ($Pa.s^n$), $\dot{\gamma}$ is the shear rate (s^{-1}) and *n* is the flow behavior index ($n > 1$ dilatant fluid, $n < 1$ pseudoplastic fluid and n = 1 Newtonian fluid).

The temperature effect on apparent viscosity was evaluated by the Arrhenius model fitting, Equation (3) (SATHIVEL; HUANG; PRINYAWIWATKUL, 2008).

$$\eta = \eta_0\, e^{-\left(\frac{E_a}{RT}\right)} \tag{3}$$

where η is the apparent viscosity (Pa.s), η_0 is the pre-exponential factor, E_a is the activation energy (J.mol^{-1}) and R is the universal gas constant (8.314 J.K^{-1}mol^{-1}). The fitted models were evaluated based on the coefficient of determination (R^2) and the root means square error (RMSE).

2.2.3.4 Costs of the evaluated solvents

By way of comparison, the costs of the DES were calculated considering the value of their starting components in the used proportion. These values were compared with conventional solvents as water and ethanol as well as their dilutions in water.

2.2.4 COSMO-RS predictions

To understand the dissolving mechanism of NADES and its potential as bio-solvents, COSMO-RS (short for Conductor-like Screening Model for Real Solvents) was employed. COSMO-RS calculations include a two-step procedure. In the first one, the molecular structures of each component of the six synthetized DES (including each HBA and HBD used), as well as the target compounds (cyanidin 3-*O*-glucoside as a representative molecule for anthocyanin, and β-D-galacturonic acid for pectin), were optimized to find the minimum energy state. For that, the software Marvinsketch (ChemAxon Ltd, version 19.24) was used to generate the structure of the molecules and transform them from 2D to 3D. Afterward, considering its geometry and electron density, the molecules were adjusted in their lowest energy level conformer based on the Dreading force field (Appendix B). For the second step in COSMO-RS predictions, using the 3D distribution of the polarization charges σ of each molecule, the surface composition functions (σ-profiles) noted p(σ) were calculated and plotted using COSMOtherm

software (Version 2019.301). Subsequently, based on the obtained σ-profiles, the σ-potentials (chemical potential from the DES and target compounds) noted μ(σ) were calculated from the thermodynamics of the molecular interactions. This approach allows the interpretation of affinity between DES solvents and the target compounds, represented by p(σ) and μ(σ) curves (SICAIRE et al., 2018).

2.2.5 Experimental solubility of anthocyanin and pectin in DES

2.2.5.1 Sample preparation and extraction

The industrial blue Brazilian berry by-product sample was manually separated into two fractions: seeds (JS) and peel containing remaining pulp (JP). After separation, only JP was used for the present research work. The JP sample was dried (moisture ≅ 15.7 ± 0.3 %) in an oven with air circulation at 333 K (Lucadema, Model 82/27, SP, Brazil), ground in a knife mill (Marconi, Model MA340, SP, Brazil), and sieved to standardize particle size between 20 and 48 mesh. The target compounds were extracted from BP by the maceration method. For anthocyanin extraction, the process was performed at 323 K, a solid-to-solvent ratio of 1:30 (g/mL), and a time of 60 min using acidified ethanol solution (50% (v/v) with 0.1 M of citric acid) as solvent (PEDRO; GRANATO; ROSSO, 2016; TAO et al., 2014). After the extraction period, the extract was centrifuged at 412 *x* g for 15 min (Quimis, Model Q222T, SP, Brazil). The supernatant was further purified (as explained in the next section) and the solid-matrix residue was used for the extraction of pectin. This residue was dried by convection air oven at 333 K and the pectin was extracted using a citric acid solution (0.1 M) at 353 K, the solid-to-solvent ratio of 1:30 (g/mL) for 2.5h (SHAFIE; YUSOF; GAN, 2019). In

sequence, the solid matrix was removed from the aqueous extract by centrifugation (412 *x* g for 15 min).

2.2.5.2 Partial purification of enriched-anthocyanin and pectin extracts.

To have a better approximation of the solubility of target compounds in the synthetized DES, the obtained extracts were partially purified. The anthocyanin-rich extract was semi-purified following the methodology described by Pedro et al. (2016) with minor adaptations. Briefly, the extract obtained by maceration was concentrated by rotary evaporation under vacuum (Fisatom, model 801, SP, Brazil), followed by freeze-drying (Liotop, model LD101, SP, Brazil). The lyophilized extract was resuspended in an acidified aqueous solution (7% (v/v) acetic acid) in a ratio of 1.5:5 (g/mL). To remove the nonpolar compounds, 10 mL of resuspended extract was washed twice using 15 mL of ethyl acetate. Sequentially, the extract was added in a glass column (1.0 cm x 30 cm) filled with 10 g of Amberlite XAD-7HD resin (Sigma-Aldrich, Steinheim, Germany). For the removal of sugars and aliphatic acids, the elution was firstly made with 150 mL of ultrapure water. Then, the semi-purified anthocyanin extract was desorbed by elution with 50 mL of acidified hydroethanolic solution [50% (v/v) ethanol with 7% acetic acid (v/v)]. Lastly, the recovered anthocyanin solution was concentrated by rotary evaporation under vacuum and freeze-dried.

In the case of the pectin, it was precipitated from the supernatant portion (obtained in section 2.2.5.1) employing two volumes of 99% ethanol at 277 K for 2h. After this period, the precipitated portion was separated from the aqueous phase using a muslin fabric. The pectin gel fraction obtained was washed twice with 15 mL of 70% ethanol solution and dried in a forced-air drier at 333 K (LIEW et al., 2018).

2.2.5.3 Solubility assays

The solubility assays of anthocyanin and pectin in the six synthesized DES were evaluated in triplicate according to the methodology described by Mokhtarpourh et al (2019). Since it was not possible to perform experimental solubility for the most viscous NADES, all of them were diluted in water at the same proportion [1:1 w/w for anthocyanin and 1:9 (w/w) for pectin] used in the extraction step. Excess amounts of the freeze-dried purified target compounds were added to 5 mL of each DES aqueous solution and maintained under stirred conditions (60 rpm) for 2 hours at a controlled temperature (298 K ± 1). The anthocyanin solutions formed were filtered using a 0.22 μm nylon syringe filter (UNICHRO®), followed by volume standardization. Finally, the Monomeric Anthocyanin Pigment (MAP - mg/mL) was quantified by pH differential method described by Giusti & Wrolstad (GIUSTI; WROLSTAD, 2001). 20 μL of sample and 280 μL of 0.025 M potassium chloride buffer (pH 1.0) or 0.4 M sodium acetate buffer (pH 4.5) were mixed and the absorbances were measured at 420 and 700 nm in a microplate reader (Multileader Infinite M200 TECAN, ZH, Switzerland). The concentrations of MAP were expressed in mg of cyanidin-3-*O*-glucoside per 100 g of dry sample (mg/100g), since it is the most abundant anthocyanin in this berry by-product (QUATRIN et al., 2019).

On the other hand, the pectin fraction solubilized in each DES was precipitated according to described in section 5.3.5.2, dried at 333 K, and quantified. Finally, the solubility of anthocyanin (quantified in MAP) and pectin (gravimetrically quantified) were calculated in terms of molar fraction according to Equation (4):

$$x_1 = \frac{\frac{x_i}{M_i}}{\frac{x_i}{M_i} + \frac{x_j}{M_j}} \quad (4)$$

where M_i and x_i are the molar mass and mass fractions of cyanidin 3-*O*-glucoside and D-galacturonic acid (representative molecules for anthocyanin or pectin, respectively) and M_j and x_j different DES evaluated in the saturated solution.

2.2.6 Potential of NADES for anthocyanin and pectin extraction

The potential for anthocyanin and pectin extraction of the six studied DESs were evaluated by the maceration method. All extractions assays were performed in triplicate and the yields were compared to reference solvents such as ethanol solution [50% (v/v)], acidified ethanol solution [50% (v/v) with 0.1 M of citric acid – pH 2.66 ± 0.01], pure water and citric acid solution (0.1 M – pH 2.16 ± 0.02).

2.2.7 Anthocyanin extraction

Similar to the solubility assays methodology, each studied DES was diluted in water (1:1 w/w) and the anthocyanin extractions were performed using the conditions described in section 2.3.5.1. The BP sample was separated from the extract by centrifugation at 412 *x g* for 15 min (Quimis, Model Q222T, SP, Brazil). The supernatant was stored at 255 K until analysis and the solid portion was dried in a forced-air drying oven at 333 K for subsequent pectin extraction.

The content of Monomeric Anthocyanin Pigment (MAP) from the extracts obtained by the six studied DES and by the conventional solvents was measured by pH differential method as described in section 2.3.5.3.

2.2.8 Pectin extraction

The pectin extraction was performed according to conditions described in section 2.3.5.1 using DES: water solutions (1:9 w/w). The extracts were centrifuged at 412 *x* g for 15 min, and the pectin was separated from the aqueous extract as described in section 2.3.5.2. The yield was expressed in the percentage of dry pectin obtained from the dry sample.

2.2.9 Statistical analysis

All data were shown as average results followed by the standard deviation. Firstly, the homogeneity of variance was verified by Levene's test ($p \geq 0.05$). Significant differences between the samples were evaluated by one-way ANOVA ($p \leq 0.05$), followed by Ficher's LSD test. The statistical significance of the models used was also determined by ANOVA and the quality and adequacy of the adjustments were assessed by the determination coefficient (R^2), adjusted R^2, and the root-mean-square error (RMSE).

2.3 RESULTS AND DISCUSSION

2.3.1 DES preparation

The DES were prepared by mixture and stirring of HBA and HBD in an appropriate molar ratio to form a deep eutectic mixture already mentioned in the literature (DAI et al., 2013a; GONZÁLEZ; WILSON, 2017). The preparation time varied from 21 minutes to 7 hours, depending on the starting components (**Table 3**). To produce NADES composed of citric acid and glucose, water had to be added to the solution, and the final

molar ratio was 1:1:3 (Ca:Glu:Wa) (AROSO et al., 2017; CASTRO et al., 2018). The visual appearances of six DES synthesized were presented in Appendix C. All studied DES showed as clear viscous liquids.

2.3.2 DES characterization

2.3.2.1 pH values

According to **Table 3**, all evaluated DES presented low pH values (0.77 to 4.01), with significant differences ($p < 0.05$) among them. As can be observed, citric and malic acids as HBD result in lower pH values. According to Qin et al. (2020) the pH values of DES are mainly related to the HBD chemical structure. In the present data, DES containing the same HBAs presented variation in pH values, confirming the influence of HBD type on this property of DES, providing an increase in the order of ChCl:Ca < ChCl:Ma < ChCl:Pro and Ca:Glu:Wa < Ca:Pro. According to the literature, acid-based DES solvents provide a high extraction yield of bioactive compounds. This phenomenon is due to the high dissolution ability of NADES at low pH values, which present a high capacity to donate protons and accept electrons, solubilizing both polar and nonpolar compounds (BOSILJKOV et al., 2017).

Table 3 - Composition, molar ratio, time and pH values of the six deep eutectic solvents (DES) evaluated, as well as the experimental solubility of anthocyanin and pectin.

Starting Components of DES				Characteristics		Experimental Solubility	
HBA	**HBD**	**Molar ratio**	**Code**	**Time of prepare**	**pH**	**Anthocyanin (mmol/mol)**	**Pectin (mmol/mol)**
Choline Chloride (ChCl)	Propyleneglycol	1:2	ChCl: Pro	21 min	$4.01^{a} \pm 0.05$	$2.48^{a} \pm 0.04$	$1.07^{d} \pm 0.01$
	Citric acid	1:1	ChCl:Ca	6h	$0.77^{e} \pm 0.0$	$1.75^{b} \pm 0.09$	$0.91^{e} \pm 0.01$
	Malic acid	1:1	ChCl:Ma	6h	$0.99^{d} \pm 0.01$	$1.40^{c} \pm 0.11$	$1.29^{a} \pm 0.01$
Citric acid	Glucose and water	1:1:3	Ca:Glu:Wa	50 min	$1.01^{d} \pm 0.03$	$0.74^{e} \pm 0.03$	$1.07^{d} \pm 0.01$
	Propylene glycol	1:1	Ca:Pro	5h	$1.86^{c} \pm 0.04$	$1.01^{d} \pm 0.03$	$1.18^{b} \pm 0.01$
Betaine	Citric acid	3:1	Be:Ca	7h	$2.90^{b} \pm 0.10$	$0.79^{e} \pm 0.02$	$1.13^{c} \pm 0.01$

^{abc} - different letters indicate significant differences ($p \leq 0.05$).

2.3.2.2 Characterization by Differential scanner analysis (DSC)

Figure 4 shows the DSC thermograms for the starting components (HBA and HBD) and the six studied DES. The presence of low endothermic peaks in DES thermograms during heating could be explained by their high viscosity (AROSO et al., 2017). However, in general, the DES thermograms did not present the characteristic endothermic peaks of the individual constituents. This behavior confirms the DES formation and its stability in a liquid form for a wide temperature range (233 at 673 K), which is probably due to the strong hydrogen bonds between HBA and HBD (AROSO et al., 2017; CASTRO et al., 2018; DAI et al., 2013a).

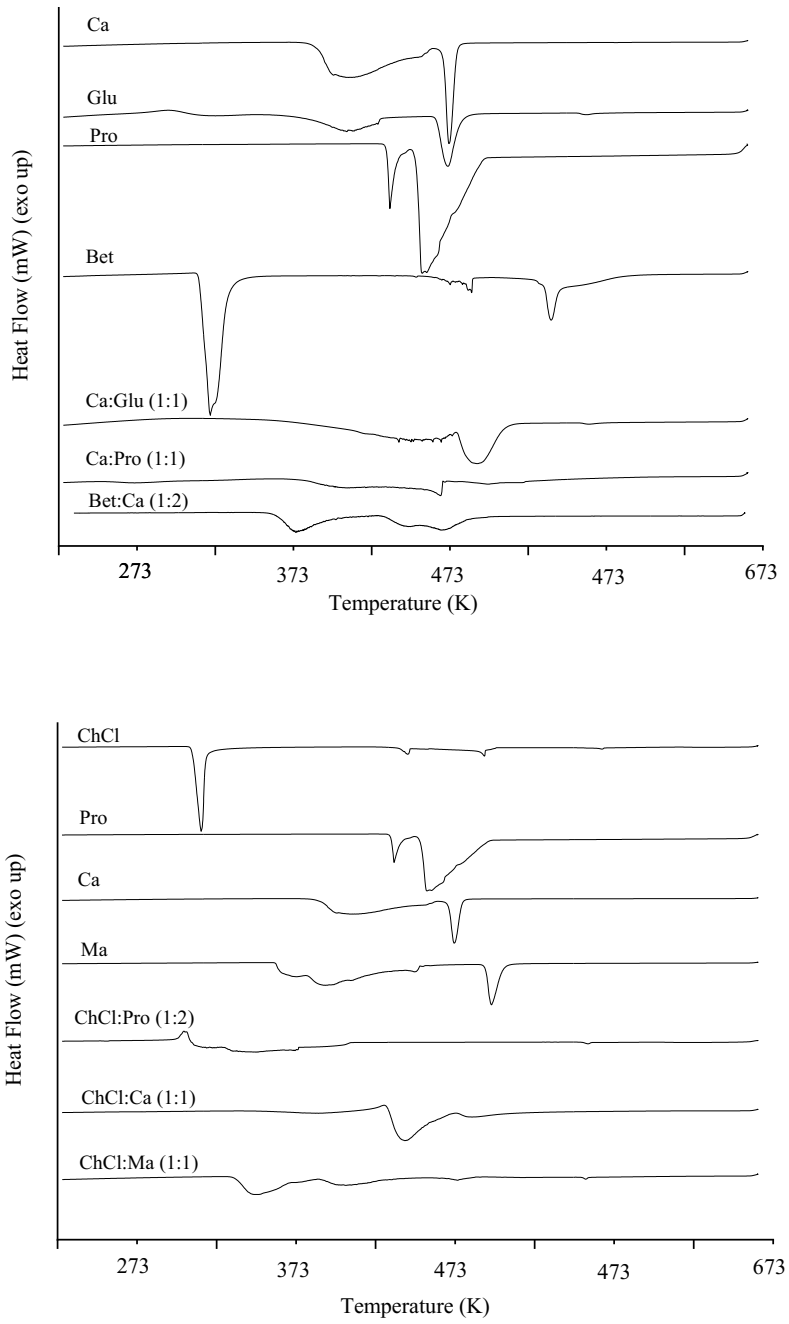

Figure 5 - DSC thermograms of six different DES and their starting components.
Note: ChCl – choline chloride, Pro – propylene glycol, Ca – citric acid, Ma – malic acid, Glu – glucose, Be – betaine, Wa – water

2.3.2.3 Rheological properties

The six synthesized DES presented a wide variation in the apparent viscosity (η), from 0.070 to 36.922 Pa.s (**Table 4**). In general, the DES solvents showed high viscosity values at room temperature (298 K) when compared to conventional solvents like ethanol (1.082 mPa.s) and water (0.890 mPa.s), as described in the literature (GONZÁLEZ et al., 2007). These results are according to other authors, which reported that the high density and viscosity is a drawback for the application of DES in the extraction process (DAI et al., 2016). The high viscosity of NADES is related to strong molecular interactions between HBA and HBD such as H-bond, van der Waals and electrostatic which result in low molecular mobility. In general, the functional groups involved in these molecular interactions are carboxyl, hydroxyl, and amine groups (DAI et al., 2013b; YANG, 2018). The DES viscosity also can be explained by "hole theory" since empty spaces are formed during the melting of their starting constituents. Thus, the viscosity is related to the probability of the molecule finding a hole with adequate size for moving into empty spaces (RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017).

Among the different evaluated DES, Bet:Ca (36.922 Pa.s) showed the highest apparent viscosity, followed by ChCl:Ca (14.480 Pa.s) and Ca:Pro (2.188 Pa.s). It can be observed that DES containing citric acid results in a high viscosity value. This fact can be explained by the structure of citric acid which presents three carboxyl groups, resulting in stronger molecular interactions than those formed by glucose or malic acid. These last components only include in their structure one and two carboxyls groups, respectively. On the other hand, the lowest apparent viscosity value was observed for ChCl:Pro (0.070 Pa.s), since the hydroxyl group of propylene glycol result in weaker interactions than the

carboxyl group. Besides, we can conclude that the low viscosity of Ca:Glu:Wa is due to the presence of water (added during synthesis, as commented in section 3.1), which decreases this rheological property (SAVI et al., 2019).

Table 4 - Apparent viscosity of six deep eutectic solvents (DES) at 298, 323, and 353 K and the Arrhenius model adjusted.

DES *	Apparent viscosity (Pa.s)			Arrhenius model adjusted				
	298 K	323 K	353 K	ln (η_0)	E_a (kJ/mol)	R^2	R^2 adj	RSME
ChCl:Pro (1:2)	0.070 ± 0.001	0.019 ± 0.001	0.009 ± 0.001	15.71 ± 1.74	32.14 ± 4.66	0.96	0.92	0.12
ChCl:Ca (1:1)	14.480 ± 0.055	2.242 ± 0.012	0.331 ± 0.004	21.57 ± 0.08	60.07 ± 0.21	0.99	0.99	0.01
ChCl:Ma (1:1)	2.082 ± 0.030	0.610 ± 0.006	0.146 ± 0.001	16.30 ± 0.64	42.28 ± 1.72	0.99	0.99	0.04
Ca:Glu:Wa (1:1:3)	0.159 ± 0.001	0.038 ± 0.006	0.014 ± 0.002	17.31 ± 1.64	38.12 ± 4.39	0.97	0.95	0.11
Ca:Pro (1:2)	2.188 ± 0.032	0.571 ± 0.003	0.088 ± 0.001	19.78 ± 1.75	51.17 ± 4.68	0.98	0.97	0.12
Be:Ca (1:2)	36.922 ± 0.532	2.775 ± 0.480	0.471 ± 0.002	24.52 ± 2.88	69.34 ± 7.73	0.98	0.95	0.20

Note: * - molar ratio, ChCl – choline chloride, Pro – propylene glycol, Ca – citric acid, Ma – malic acid, Glu – glucose, Be – betaine, Wa – water, R^2 adj – R^2 adjusted.

Regarding the effect of the temperature on the apparent viscosity, an increment from 283 to 353 K reduced the DES η values, as expected. This occurs because the internal resistance between the molecules decreased with the temperature increases, increasing the solvent fluidity (CASTRO et al., 2018). To validate this tendency, the η values were evaluated as a function of temperature by the Arrhenius model (**Table 4**), which presented a good fit ($R^2 > 0.97$ and R^2 adjusted > 0.92). Moreover, the Arrhenius fit predicted the activation energy (E_a) value, which is related to the sensibility of η variation with temperature changes. The highest E_a in the present work were obtained by the solvents Bet:Ca, ChCl:Ca and Ca:Pro and this data are in accordance to Teixeira, Ávila, Silveira, Ribani, & Ribani (2018) which affirmed that the highest value of E_a indicated a high variation rate of η with the temperature variations.

The rheological behavior of DES were adequately fitted to the Oswald-de-Waele (OW) model with RMSE values between 0.47 to 19.17 and a regression coefficient of 0.99 (**Table 5**). The predicted consistency coefficient (K) followed the same trend of apparent viscosity, and the fluid type of the six evaluated NADES can be considered Newtonian ($n \sim 1$) at room temperature. Thus, variations in flow behavior index (n) of different DES are related with K values, where the less consistent DES - Ca:Glu:Wa and ChCl:Pro - presented lower n values and high variations with temperature increase, showing Pseudoplastic flow behavior ($n < 1$) at 323 and 353 K. That is, the less consistent DES presented decreasing on apparent viscosity with the increase of shear stress.

Table 5 - Oswalt-de-Waele model fitted NADES at 298, 323, and 353 K.

NADES *	Oswald-de-Waele model											
	298K				323K				353K			
	K(Pa.s^n)	*n*	R^2	RMSE	K(Pa.s^n)	*n*	R^2	RMSE	K(Pa.s^n)	*n*	R^2	RMSE
ChCl:Pro (1:2)	8.33 ± 0.15	0.95 ± 0.01	0.99	0.47	2.62 ± 0.15	0.94 ± 0.01	0.99	1.44	2.35 ± 0.15	0.81 ± 0.01	0.99	1.41
ChCl:Ca (1:1)	1449.07 ± 2.11	1.01 ± 0.01	0.99	3.28	228.46 ± 0.98	0.98 ± 0.01	0.99	5.94	35.74 ± 0.72	0.98 ± 0.01	0.99	4.58
ChCl:Ma (1:1)	215.64 ± 0.88	0.97 ± 0.01	0.99	2.06	156.36 ± 0.45	0.98 ± 0.01	0.99	1.25	14.77 ± 0.12	0.99 ± 0.01	0.99	0.81
Ca:Glu:Wa (1:1:3)	16.49 ± 0.17	0.98 ± 0.01	0.99	1.15	5.30 ± 0.40	0.91 ± 0.02	0.99	9.62	3.19 ± 0.25	0.82 ± 0.01	0.99	2.48
Ca:Pro (1:2)	224.94 ± 0.93	0.98 ± 0.01	0.99	2.2	56.61 ± 0.26	1.01 ± 0.01	0.99	1.21	10.01 ± 0.12	0.96 ± 0.01	0.99	0.72
Be:Ca (1:1)	3781.92 ± 26.13	1.01 ± 0.01	0.99	6.26	273.97 ± 1.97	1.01 ± 0.01	0.99	19.17	47.65 ± 0.24	0.99 ± 0.01	0.99	1.51

Note: * - molar ratio, ChCl – choline chloride, Pro – propylene glycol, Ca – citric acid, Ma – malic acid, Glu – glucose, Be – betaine, Wa - water, K - consistency coefficient, n flow behavior index, and RSME – root mean square error.

Therefore, the viscosity and flow behavior of DES is associated with their starting components. Among the six evaluated DES, it would be expected that the lesser viscous - Ca:Glu:Wa and ChCl:Pro - exhibited high solvation and extraction ability of target compounds because it facilitates the transport phenomenon (CAO et al., 2019). Besides, other properties of solvents related to the molecular structure must be taken into account during the selection of the more adequate DES. These molecular contributions can be evaluated by computed-aid tools like COSMO-RS (BENVENUTTI; ZIELINSKI; FERREIRA, 2019), as described below.

2.3.3 Anthocyanin and pectin experimental solubility in DES and their σ-profile and σ-potentials by COSMO-RS

The solubility of target compounds in DES is strongly associated with the molecular interactions between solute and solvent (CAO et al., 2019; DAI et al., 2013b). Therefore, it is essential to study the molecular structure of DES constituents as well as those of anthocyanin and pectin (target compounds selected) (FU et al., 2020). According to the literature, the DES structures present an extensive hydrogen-bonds network between their components which form a supermolecule. Thus, DES structure shows a high capacity of interaction with biomolecules, especially phenolic compounds (DAI et al., 2013b, 2016; RUESGAS-RAMÓN; FIGUEROA-ESPINOZA; DURAND, 2017).

Anthocyanins – the glycosylated form of anthocyanidins (aglycones) – present a long chromophore with eight conjugated double bonds with positive charges which confer intense color in acidic conditions. The aglycones more commonly known are cyanidin, peonidin, pelargonidin, malvidin, delphinidin, and petunidin, which differ by

the position of hydroxyl and methoxyl groups (HE; GIUSTI, 2010). Glycosylation of anthocyanidins affects the polarity and molecular size, making them more soluble and stable due to the intramolecular H-bonds network (HE; GIUSTI, 2010). In *Myrciaria cauliflora* fruit by-product evaluated, especially in the peel, the main anthocyanins are cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside (QUATRIN et al., 2019). Likewise, the other selected target compound, pectin, is a type of soluble fiber found between the cell wall and the middle lamella of plant cells. Chemically is a heterogeneous polysaccharide, with the D-galacturonic acid (D-galA) as the primary constituent, and part of their carboxyl groups are esterified with methyl groups (MOHNEN, 2008).

According to the above and to perform the *in silico* solvent screening, the authors selected cyanidin 3-*O*-glucoside and D-galacturonic acid as representative molecules for anthocyanin and pectin purified extracts, respectively. The previous molecular considerations were useful to understand the charges in molecule configuration and also, for a better interpretation of COSMO-RS solubility predictions.

The σ-profile (**Figure 6**a, c and e) and σ-potential (**Figure 6**b, d and f) were used as a support for the understanding affinity of solvents and target compounds. The σ-profile, noted p(σ), provides information on the polarity distribution of molecules and their normalized distribution function describe the σ-potential [μ(σ)]. Thus, this approach allows estimating the probability of compounds that interact with DES according to polarity and hydrogen bonds (AISSOU et al., 2017). As stated by Hayyan et al. (HAYYAN et al., 2016), when the screening charge density in the σ-profile is lower than -0.0084 eÅ^{-2} or exceeds + 0.0084 eÅ^{-2}, the molecule can be considered polar enough to induce hydrogen bonding. **Figure 6** is separated in three regions according to affinity of

molecule: H-bond donor region ($\sigma < -0.0084$ eÅ^{-2}), the non-polar region (-0.0084 eÅ$^{-2} < \sigma < 0.0084$ eÅ^{-2}), and H-bond acceptor region ($\sigma > +0.0084$ eÅ^{-2}).

For anthocyanin and pectin, there is a distinctive peak residing near the H-bond donor region, which indicates the contribution of H atoms (**Figure 6**a, c, and e). However, most peaks are localized in the non-polar region, due to the carbon chain contribution. Besides, there is a notable peak for anthocyanin and pectin in the H-bond acceptor region, representing the O atoms from the hydroxyl groups of the molecules.

On the other hand, the trend for DES $p(\sigma)$ is similar between them. Two peaks can be distinguished, one near -0.005 eÅ^{-2} (non-polar region) and the other around 0.012 eÅ^{-2} (**Figure 6**a, c and e). This last peak mainly comes from Cl atoms from the ChCl and CaCl or the carboxyl group of organic acids, such as citric or malic acids. According to **Figure 6** (a, c and e), the HBD components of DES evaluated in the present work exerted higher influence than HBA in $p(\sigma)$ values. The DES containing citric or malic acid as the HBD component showed higher values of $p(\sigma)$ than DES containing propylene glycol or glucose.

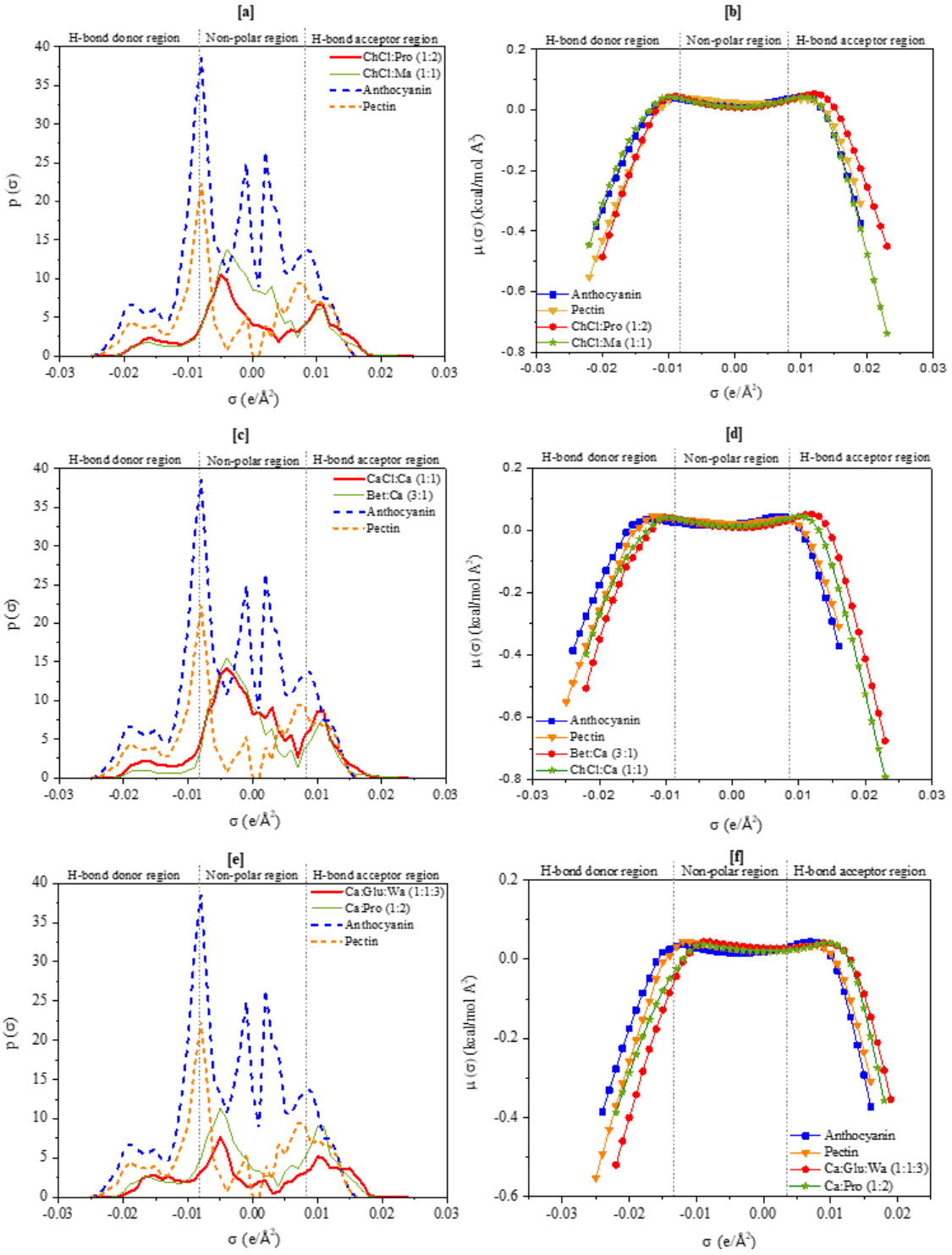

Figure 6 - Sigma potential [a, c, and e] and sigma profile [b, d, and f] of six different DES and target compounds, anthocyanin and pectin.

The previous analysis is important for the next step of this study related to the interactions between DES and target compounds, as is better shown in the σ-potentials

[μ(σ)] curves (**Figure 6**b, d, and f). Following the rule "like dissolves like", the similarity in μ(σ) curves is indicative of affinity between solute and solvent (KURNIA; PINHO; COUTINHO, 2014). All synthesized DES and the target compounds evaluated in the present work showed negative values in both H-bond donor and H-bond acceptor regions. The more negative values of μ(σ) represent high affinities in this region, which means, that all solvents and the target compounds present an amphoteric character.

All DES investigated presented weaker affinities in the H-bond donor region [μ(σ) from -0.52 to -0.40 kcal/molÅ^2] compared to water (-0.70 kcal/molÅ^2), according to data reported by Sicaire et al. (2018). Among all evaluated DES, the higher affinities in the H-bond donor region were presented by Ca:Glu:Wa and Bet:Ca [μ(σ) = -0.52 and -0.51 kcal/molÅ^2]. This fact can be explained by the water contribution in Ca:Glu:Wa and the high H-bond acceptor capacity of the betaine molecule.

On the other hand, ChCl:Ca (-0.79 kcal/molÅ^2), ChCl:Ma (-0.73 kcal/molÅ^2) and Bet:Ca (-0.67 kcal/molÅ^2) presented stronger affinity than water (-0.65 kcal/molÅ^2) in the H-bond acceptor region. This behavior can be explained by the presence of citric and malic acid as the HBD component, whose character presents affinity to H-bond acceptors compounds. These values are similar to those reported by Hayyan et al. (2016) for the NADES composed of ChCl as HBA and malonic acid as HBD [μ(σ) about -1 kcal/molÅ^2].

Likewise, all DES presented low affinity to non-polar molecules [μ(σ) values near to zero], being similar to the behavior of water and the aforementioned DES evaluated by Hayyan et al. (HAYYAN et al., 2016). Therefore, some relevant differences in affinity behavior of DES were observed, which are dependent on the molecular

structure of starting components. Among the σ-potentials estimated for NADES, Ca:Pro and Ca:Glu:Wa affinities are the most similar to anthocyanin and pectin, respectively. To corroborate the COSMO predictions the experimental solubility of the target compounds in DESs solutions were measured (**Table 3**).

Regarding anthocyanin, ChCl:Pro showed better solubilization (2.48 μmol/mol or ppm), followed by ChCl:Ca (1.75 μmol/mol or ppm) and ChCl:Ma (1.40 μmol/mol or ppm). Likewise, the ChCl:Ma (1.29 mmol/mol or 1,290 ppm), Ca:Pro (1.18 mmol/mol or 1,180 ppm), and Bet:Ca (1.13 mmol/mol or 1,130 ppm), were the best solvent to solubilize the pectin. Despite that some properties such as viscosity are not taken into account in COSMO-RS prediction, the experimental results can be related to μ(σ). The best DES for anthocyanin solubilization, ChCl:Pro, presented close values of μ(σ). Besides, the second-best DES for pectin solubilization, Ca:Pro, showed similarities with the μ(σ) curve of pectin. Therefore, the solvent screening predicted by COSMO-RS afforded a satisfactory understanding of the affinity between DES and target compounds.

2.3.4 Extraction ability and costs of DES

The yield is not just related to the solubility of target compounds in the solvents but also is very dependent on the capacity of penetrating in the solid matrix. In this sense, the recovery efficiency of a target compound is related to some properties such as viscosity of the solvent, morphology and cellular structure of solid material, and diffusion of solute in the solvent phase (SICAIRE et al., 2018). For this reason, the extraction ability was evaluated.

Comparing the extraction yields of anthocyanin and pectin from BP, there were significant differences ($p < 0.05$) between the NADES studied and conventional solvents (**Figure 7**). Ethanol and water were chosen such as conventional solvents for being the main GRAS solvents used. Besides, acidified ethanol generally results in good anthocyanin yield since the pH is one of the main factors related to solubility and stability of this molecule (CASTAÑEDA-OVANDO et al., 2009), and the citric acid solution is commonly used for pectin obtaining (MARAN et al., 2017).

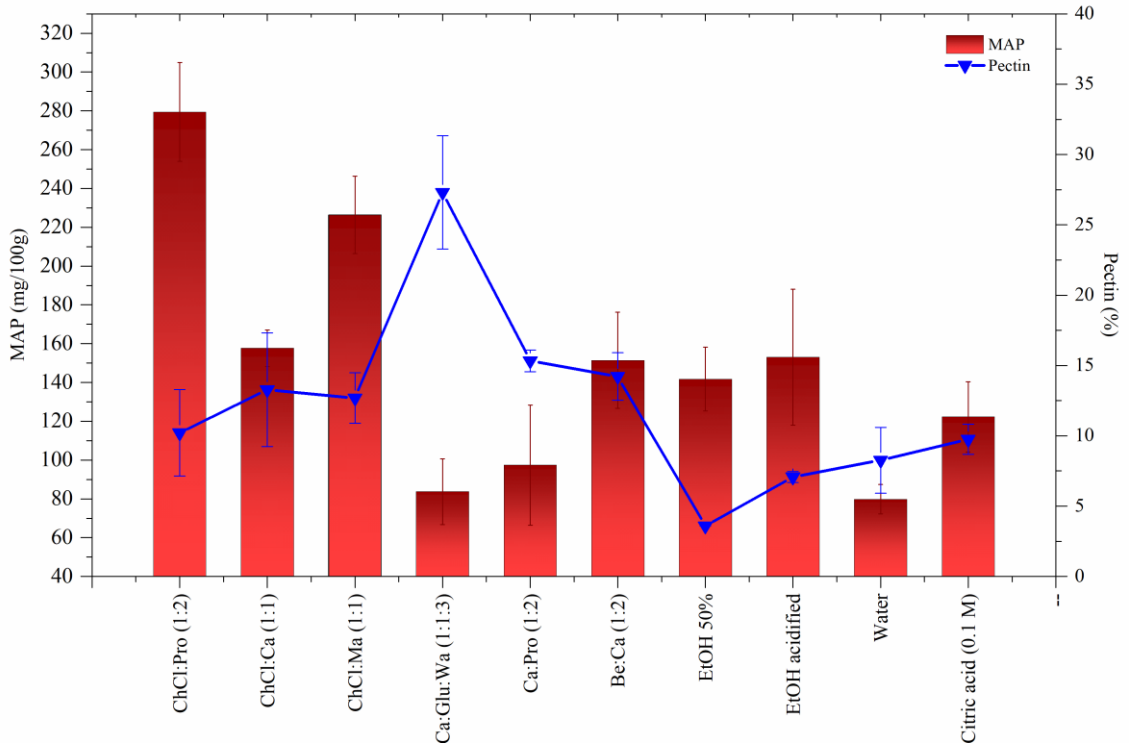

Figure 7 - Extraction yields of anthocyanin (expressed as Monomeric Anthocyanin Pigment - MAP) and Pectin (g/100 g JP) using different deep eutectic solvents (DES) and conventional solvents. Note: ChCl – choline chloride, Pro – propylene glycol, Ca – citric acid, Ma – malic acid, Glu – glucose, Be – betaine, Wa – water, EtOH 50% - ethanol/water solution 50/50 (v/v), EtOH acidified – acidified ethanol/water solution [50/50 (v/v) with 0.1 M of citric acid].

All DES showed higher anthocyanin levels than water, and only Ca:Glu:Wa and Ca:Pro had a lower performance of extraction than solutions of 50% ethanol and 50%

acidified ethanol. According to Gurak et al. (GURAK et al., 2014), the total anthocyanin level from dry *Myrciaria cauliflora* residue is 573 mg/100g, which is obtained by sequential extraction using methanol: water (0.8:0.2 v/v) and acetone: water (0.7:0.3 v/v). In this work, the highest recovery obtained by using DES: water solution (1:1 w/w) was obtained by ChCl:Pro with values of 279.45 ± 25.44 mg/100g. Therefore, these results showed that DES diluted solutions have prominent extraction ability. Regarding the conventional solvents evaluated in this work, the bests DES solutions presented recovery yields from 1.6 to 3.5 times higher. Besides, it is important to highlight that ChCl:Pro presented the shorter preparation time, i.e. less energy expenditure, and the lowest apparent viscosity, contributing to the mass transfer. This result can be successfully related to those predicted by COSMO-RS as the best solvent according to the $\mu(\sigma)$ curves, as discussed before (section 3.3).

According to the literature, the DES formed by organic acid as HBD, mainly citric and malic acids, presented a high performance for anthocyanin recovery due to high polarity and acidity (BOSILJKOV et al., 2017; PANIĆ et al., 2019). An acidic medium improves the anthocyanin yield since its conformation as flavylium cation, the more colorful anthocyanin form, is stabilized in an acidic medium (CASTAÑEDA-OVANDO et al., 2009). Besides that, ChCl:Pro was appointed as a better solvent for the extraction of anthocyanin from *Lycium ruthenicum* fruit due to molecular interactions between DES and the target molecule (SANG et al., 2018). According to Panić et al. (2019), the DES viscosity is another parameter that exerted a high influence on the anthocyanin extraction yield. Therefore, the lowest apparent viscosity of ChCl:Pro favored the extraction of this target compound.

Regarding the pectin extraction yield, all DES: water solutions (1:9 w/w) presented a higher extraction ability than conventional solvents. Among all evaluated solvents, Ca:Glu:Wa provided the highest extraction yield (**Figure 6**). This result allowed us to verify that this maximum yield (27.3%) was 3-folds higher than the citric acid solution (9.7%), which has been commonly used for this purpose (DRANCA; OROIAN, 2018; MARAN et al., 2017). Besides, the pectin yield from this work can be compared to yields obtained from the citrus peel (25-30%), sugar beet pulp (15-30%) and apple pomace (15-20%), the main matrix used for commercial pectin production (DRANCA; OROIAN, 2018).

During the pectin extraction, the acidic medium (pH value near to 2) associated with high temperatures (≥ 80 °C) favors the hydrolysis needed to break the cell wall where pectin is located (SHAFIE; YUSOF; GAN, 2019). Thus, citric acid-based DESs, which present low pH values, were appointed as good solvents for pectin extraction by other authors (LIEW et al., 2018; SHAFIE; YUSOF; GAN, 2019). In this work, Ca:Glu:Wa, using citric acid as HBA provided the highest pectin recovery as estimated by $\mu(\sigma)$ from the computed-aided tool employed. Besides, this solvent complies with the pectin isolation requirements, such as low pH value (1.01 ± 0.03) and low apparent viscosity (0.159 Pa.s at 298K), and still presented a short synthesis time (50 min).

For industrial applications, the costs should be taken into account for solvent selection. Regarding ionic liquids (IL) and conventional solvents, DES costs are acceptable and their preparation is considered sustainable (BI; TIAN; ROW, 2013; BOSILJKOV et al., 2017). According to **Table 6**, the DES costs followed the order: Ca:Glu:Wa < Ca:Pro < ChCl:Pro < ChCl:Ca < ChCl:Ma < Bet:Ca. As can be observed

DES containing citric acid as HBA, which are the best solvents for pectin recovery, showed low solvents costs, even below ethanol. The use of DES in water solutions did not block the DES from being good solvents for anthocyanin and pectin due to the affinity between these compounds and water. Also, the dilution facilitates the extraction due to the reduction of viscosity and reduces the costs of the solvent by about 48% in proportion 1:1 (w/w) and 92% in proportion 1:9 (w/w).

Therefore, the assessment of the physicochemical properties of synthesized DES leads to very relevant information about their potential as an extraction solvent. Besides, the use of *in silico* solvent screening tool was able to relate the molecular affinities to the experimental solubility. Those results associated with the extraction ability allowed us to carry out a specific selection of the NADES for high anthocyanin and pectin recoveries. This work shows a sequential process for the valorization of the Brazilian berry by-product, using new green solvents as an alternative to petrochemical solvents.

Table 6 - NADES costs per 100 mL of solution.

Solvents *	**Costs/100mL (USD)**		
	Pure	**50%**	**10%**
ChCl:Pro (1:2)	10.26	5.27	0.77
ChCl:Ca (1:1)	14.17	7.22	0.97
ChCl:Ma (1:1)	14.39	7.33	0.98
Ca:Glu:Wa (1:1:3)	1.01	0.64	0.31
Ca:Pro (1:1)	1.98	1.13	0.36
Bet:Ca (3:1)	36.12	18.20	2.06
Ethanol	5.32	2.79	0.52
Water	0.27		

Note: * - molar ratio, ChCl – choline chloride, Pro – propylene glycol, Ca – citric acid, Ma – malic acid, Glu – glucose, Be – betaine, Wa – water.

On the other hand, Chemat et al. (CHEMAT et al., 2019) recently established a guideline to define a green extraction process based on six principles. The first principle

is related to the raw material, which must be renewable and, if possible, from a low exploited local food crop. The second concerns the use of alternative solvents, of a natural origin, safe for the environment, operator, and consumer, with the possibility of recovery and reuse, suitable for industrial use at an affordable cost. The reduction of energy consumption is the third principle, improved by the use of innovative technologies that provide higher extraction yields, reducing the time and amount of solvent. A green extraction also considers the co-products generation, instead of waste, following the biorefinery concept, as the fourth principle. The fifth is related to reducing or intensifying the unit operations, improving the process control. Last, the sixth principle concerns the recovered extract, which must be safe, free from contaminants, and with quality and functionality.

Then, following the green extraction principles, the renewable raw material is an agro-industrial by-product from unique Brazilian fruit (jaboticaba). Also, the new solvents are easily prepared from natural starting components, present low volatility, no-toxicity, affordable costs, and the possibility of recovery and reuse. Sequential and selective extraction enable the recovery of anthocyanin and pectin-rich fractions, with different technological and functional attributes. Combining these alternative green solvents with innovative technologies (e.g. microwave, ultrasound, pulsed electric field, instant controlled pressure drop, sub, and super-critical fluid processing, high pressure, ohmic, among others) could lead to an authentic green extraction process. At the end of sequential extractions the soli material, rich in non-soluble dietary fiber, may be evaluated for incorporation as a co-product into functional foods, nutraceuticals, supplements, or animal feed (CHEMAT et al., 2019, 2020).

2.4 CONCLUSION

The sequential recovery of anthocyanin and pectin from *Myrciaria cauliflora* by-product using green solvents is relevant due to the bioactive and technological functions of these target compounds. In this work, the first step for the utilization of NADES in the valorization of this residue was the assessment of their physicochemical properties. The wise choice of DES must take into account the low values of viscosity and pH to improve the anthocyanin and pectin extraction. Besides, the solvent screening predicted by COSMO-RS afforded a satisfactory understanding of the molecular contributions for the affinity between NADES and target compounds. The combination of *in silico* contributions with experimental solubility and extraction capacity data was crucial to ensure the best solvent choice.

Gathering all the information, ChCl:Pro [1:1 (w/w) water solution] was the most promising DES for anthocyanin extraction, and Ca:Glu:Wa [1:9 (w/w) water solution] for pectin extraction. It is important to highlight that, for these target compounds, the dilution did not preclude the DES from being good solvents and reduced the costs. Therefore, the obtained results justified the use of the aqueous solution of DES as an alternative environment-friendly, acceptable cost, and very efficient for the selective obtention of compounds with the potential of application in food, supplements, medicines, and cosmetics. Besides, this way for the selection of starting constituents of NADES can be employed for other vegetable matrices and target compounds, spreading its applicability.

CHAPTER 3

Pressurized aqueous solutions of deep eutectic solvent (DES): a green emergent extraction of anthocyanins from a Brazilian berry processing by-product

This chapter includes the obtaining and characterization of the anthocyanin-rich fraction from jaboticaba processing by-product, employing a green approach combining pressurized liquid extraction (PLE) and DES aqueous solutions selected in Chapter 2. The content of this chapter was published as a full research article "Pressurized aqueous solutions of deep eutectic solvent (DES): a green emergent extraction of anthocyanins from a Brazilian berry processing by-product" in **Journal of Food Chemistry: X** (2022). (https://doi.org/10.1016/j.fochx.2022.100236).

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Pressurized aqueous solutions of deep eutectic solvent (DES): a green emergent extraction of anthocyanins from a Brazilian berry processing by-product

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ABSTRACT

Deep eutectic solvents (DES) are emergent solvents with high extractability of bioactive compounds. Therefore, in this study, anthocyanin-rich fractions were recovered from the Brazilian berry (jaboticaba) peels by combining aqueous solutions of DES and pressurized liquid extraction (PLE). The extraction occurred at 10 MPa for 12 min, with conditions optimized through response surface methodology (RSM): 47% DES concentration, 90 °C, and flow rate of 5.3 mL/min. PLE with different DES (choline chloride combined with propylene glycol or malic acid) solutions were compared to conventional solvents (water and acidified water) concerning yield, phenolic profile, antioxidant, anti-diabetic, and anti-obesity activities, besides the anthocyanin thermostability. Both DES solutions presented anthocyanin yields up to 50% higher than conventional solvents. The use of the different solvents resulted in significant differences among the phenolic profile. However, in general, anthocyanin was the main phenolic compound, and ellagic acid was the main phenolic acid. ChCl:Ma provided the highest anthocyanin stability (E_a = 77.5 kJ.mol^{-1}), and was a promising solvent concerning color, anti-diabetic and anti-obesity potential. Therefore, DES solution associated with PLE method is a green and efficient approach to recovering anthocyanin from the jaboticaba peel, providing an extract with potential applicability.

Keywords: green solvent; pressurized liquid extraction; natural colorant; antioxidant; anti-diabetic; anti-obesity.

3.1. INTRODUCTION

The relation between diet and human health is increasingly evident, which has been affecting the food choice of consumers. Additionally, the development of sustainable food systems is still an industrial challenge, associated with the necessity to decrease food wastage, and with better use of the underutilized resources. Therefore, natural food ingredients or additives have been evaluated as substitutes for synthetic ones due to their nutritional and technological effects associated with health benefits (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; MARTINS et al., 2016). For that purpose, bioactive compounds obtained from plants and their vegetable by-product have been widely evaluated, stimulating the biorefinery and circular economy concepts (GARCIA-MENDOZA et al., 2017; SORITA; LEIMANN; FERREIRA, 2020).

Jaboticaba (*Myrciaria cauliflora)* is an underutilized Brazilian berry, unknown to the international trade market, although it presents a high nutritional value and an appreciated taste. Its cultivation is mainly by small-scale agriculture, or extractive form, however, formal data related to jaboticaba are rising, being registered commercialization of 2,460 tons in 2017 only in the State of São Paulo, Brazil (BENVENUTTI; ZIELINSKI; FERREIRA, 2021). The high perishability of this fruit justifies the industrial obtention of juices, jams, syrups, liquors, and fermented beverages, among others. The natural consumption and the industrial process generate the jaboticaba processing by-product, representing about 40% of the whole fruit. This processing by-product consists mainly of peel and seeds, where the peel is about 25% of the whole fruit, and the seeds reach 15%. The jaboticaba fruit and its peel show rich phytochemical composition including ascorbic acid (vitamin C), β-carotene, tocopherol, and phenolic

compounds, mainly anthocyanins (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; INADA et al., 2015).

Anthocyanins are water-soluble compounds belonging to the flavonoids class and are naturally present in a wide variety of flowers, leaves, vegetables, fruits, and grains, showing color from reddish to purplish (DE MEJIA et al., 2020). These compounds have application potential in food, cosmetic and pharmaceutical products as a natural colorant, but they also present antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, cardiovascular protection, neuroprotection, and anticarcinogenic potential (DE MEJIA et al., 2020; TEIXEIRA et al., 2021).

The anthocyanin and other phytochemicals can be recovered by Deep Eutectic Solvents (DES), a new generation of solvents with potential application in various industrial fields. These solvents are formed by one hydrogen bond acceptor (HBA) and one or more hydrogen bond donors (HBD), which form a mixture, through molecular interactions, with a lower melting point than an ideal eutectic mixture, which mostly presents high performance for bioactive compounds extraction (Benvenutti, Zielinski, & Ferreira, 2019; Dai, Rozema, Verpoorte, & Choi, 2016).

The above-mentioned molecular interactions form a supramolecular structure that increases the solubility of target compounds. Therefore, the components used in the HBA:HBD mixture, as well as their molar ratio, define the DES solvation capacity and their environmental characteristics, such as toxicity and biodegradability (AHMADI et al., 2018; BENVENUTTI; ZIELINSKI; FERREIRA, 2019; DAI et al., 2016). Also, when the formers HBA and HBD are natural components, the resulting DES has been widely evaluated as harmless and non-toxic solvents compared to conventional ones.

Some DESs present water in their composition, which precipitates the supramolecular structure of these solvents, decreasing their viscosity. As a result, the analytes dissolution rate increases, while the solvent costs decrease. However, excessive water concentration weakens the hydrogen bonds between HBA and HBD, reducing the solvation ability of this solvent (BAJKACZ; ADAMEK, 2017; LIU et al., 2018). Nevertheless, the recovery of polar compounds from the solid matrix is more efficient with DES-water solutions, compared with only DES, since the water can form hydrogen bonds with the target compound, contributing to its dissolution (BENVENUTTI et al., 2020; SHISHOV et al., 2021).

To associate DES-water systems with genuine green extractions, besides factors such as safe solvents, and efficient performance, it is also necessary to combine innovative and environmentally-friendly technology, aiming to intensify the process, reducing time, energy, and solvent consumption (CHEMAT et al., 2019). Then, within the emergent technologies, the pressurized liquid extraction (PLE) applies high pressure to improve the solvent diffusion, promoting high solvation power, besides facilitating the rupture of the cells from the solid matrix, which results in high yields at low processing time and solvent amount (Rodrigues, Mazzutti, Vitali, Micke, & Ferreira, 2019; Zielinski et al., 2021).

Therefore, the aims of this study were: (*i*) to optimize the recovery of anthocyanin-rich fractions from jaboticaba by-product using DES aqueous solutions associated with PLE method, (*ii*) to evaluate the effect of choline chloride-based DES on the thermostability of the recovered anthocyanins, as well as on the antioxidant, anti-diabetic, and anti-obesity potentials from the extracts and (*iii*) to investigate this approach as a green extraction alternative for the recovery of valuable bioactive compounds.

3.2. MATERIAL AND METHODS

3.2.1 Materials

Myrciaria cauliflora industrial by-product (10 kg) was acquired from *Sítio do Bello* (Piraibuna, SP, Brazil). Malic acid P.A. was purchased from Êxodo Científica (Sumaré, SP). 2-Hydroxyethyltrimethylammonium chloride (Choline Chloride – ChCl) >98% obtained from Sigma-Aldrich (Steinheim, Germany), propylene glycol (>99.5) from Neon Commercial (Suzano, SP, Brazil). Other solvents such as 99.5% ethanol, 99.5% acetone, and ethyl acetate 99.5%, from Êxodo Científica (Sumaré, SP, Brazil). The reagents Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), ABTS (2,2'-azino bis-3-ethylbenzothiazoline-6-sulfonic acid), *p*NPG (*p*-nitrophenyl-α-D-glucopyranoside), *p*NPB (*p*-nitrophenol-butyrate), and DNS (3,5-dinitro salicylic acid) and the enzymes α-amylase from porcine pancreas, α-glucosidase from *Saccharomyces cerevisiae* and lipase from porcine pancreas were acquired from Sigma Aldrich (Steinheim, Germany). Distilled water (Milli-Q® Direct 8, Merck, Darmstadt, Germany) was used in the preparation of solutions.

3.2.2 Preparation of natural deep eutectic solvent (DES)

Two DES, choline chloride and propylene glycol at 1:2 molar ratio (ChCl:Pro), and chlorine chloride and malic acid (ChCl:Ma) at 1:1 molar ratio, were prepared according to described in section 2.2.2 (Chapter 2). To decrease the DES viscosities, enabling the handling by PLE unit and improving dissolution rates, the solvents were diluted in distilled water at concentrations from 5 to 55%.

3.2.3 Solid Sample preparation and characterization

Firstly, the peel of *Myrciaria cauliflora* processing by-product was prepared according to section 2.2.5.1 (Chapter 2). The centesimal composition in terms of moisture, fixed mineral residue (ashes), total fat, protein, and crude fiber of the prepared sample was performed according to AOAC methodologies (AOAC, 2005). The content of non-fibrous carbohydrates was calculated by difference.

3.2.4 Pressurized liquid extraction (PLE)

The recovery of anthocyanin-rich extracts was performed by pressurized liquid extraction (PLE) using the DES ChCl:Pro as solvent. The extraction was performed in a self-assembled apparatus described by Rodrigues, Mazzutti, Vitali, Micke, & Ferreira (2019) (**Figure 8**). The assays were made at continuous mode, with pressure fixed in 10 MPa and time of 12 min, which was defined through an extraction kinetic based in monomeric anthocyanin pigment (MAP) content. The assays were made at continuous mode, with pressure fixed in 10 MPa and time of 12 min, which was defined through an extraction kinetic (performed with 5g of sample, at 90°C, 30% of DES solution as the solvent and flow rate of 4mL/min) based in monomeric anthocyanin pigment (MAP) concentration, according to section 2.5.1. The kinetic data were fitted to a three straight lines model using a Statistica v.13.5 software (TIBCO Software Inc., Palo Alto, CA, USA).

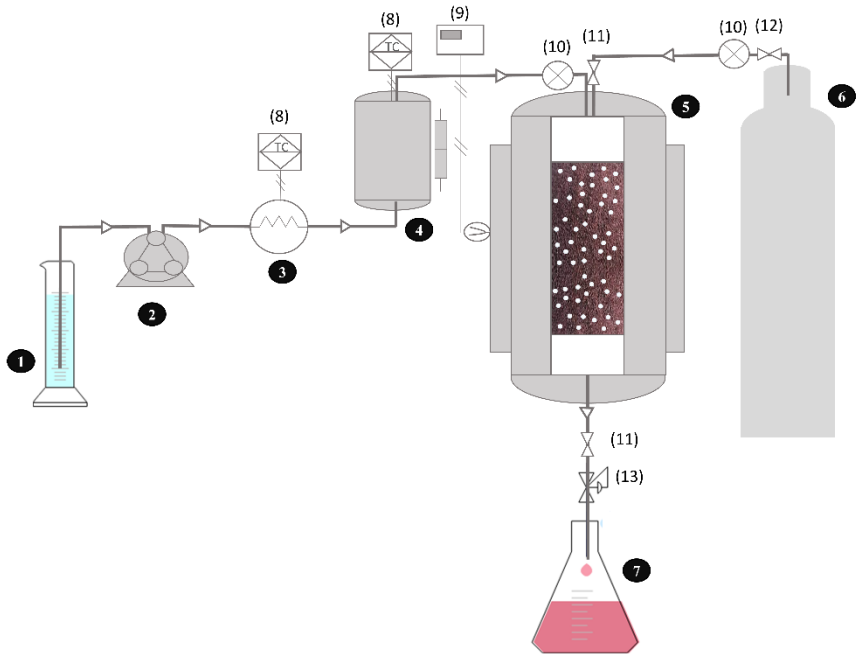


Figure 8 - Schematic design of pressurized liquid extraction (PLE) unit: 1) solvent reservoir, 2) pump, 3) heating exchanger, 4) pre-heater, 5) extractor vessel, 6) nitrogen cylinder, 7) extract, 8) automatic temperature controller, 9) temperature indicator, 10) manometer, 11) block valve, 12) regulator valve 13) regulator needle valve.
Source: The author.

For this study, the independent variables were temperature (60, 90, and 120 °C), DES concentration in water (15, 30, and 45%), and flow rate (3, 4, and 5 mL/min). The PLE process was optimized using the variable conditions (factors) combined through a Central Composite Rotatable Design (CCRD), as shown in **Table 7**. The design method allows evaluating the main effects and their interactions, providing a nonlinear response function. To CCRD study was systematically organized by the Statistica v.13.5 software, and consisted of 2^3 factorial designs, 6 factorial points [$\pm\alpha = (2^k)^{1/4}$, where k is the number of variables, and 3 central points, to evaluate the assays repeatability and the pure error of the nonlinear model (Rodrigues & Iemma, 2014).

Table 7 - Central Composite Routable Design (CCRD) including the independent variables.

Assays	Independent Variables		
	NADES concentration (%)	**Temperature (°C)**	**Flow rate (mL/min)**
1	15 (-1)	60 (-1)	3 (-1)
2	15 (-1)	120 (1)	5 (1)
3	45 (1)	60 (-1)	5 (1)
4	45 (1)	120 (1)	3 (-1)
5	30 (0)	90 (0)	4 (0)
6	15 (-1)	60 (-1)	5 (1)
7	15 (-1)	120 (1)	3 (-1)
8	45 (1)	60 (-1)	3 (-1)
9	45 (1)	120 (1)	5 (1)
10	30 (0)	90 (0)	4 (0)
11	4.90 (-1.68)	90 (0)	4 (0)
12	55.09 (+1.68)	90 (0)	4 (0)
13	30 (0)	39.80 (-1.68)	4 (0)
14	30 (0)	140.19 (+1.68)	4 (0)
15	30 (0)	90 (0)	2.33 (-1.68)
16	30 (0)	90 (0)	5.67 (+1.68)
17	30 (0)	90 (0)	4 (0)

3.2.5 Chemical analysis of the anthocyanin-rich extracts

3.2.5.1 Monomeric Anthocyanin Pigment (MAP) content

Monomeric Anthocyanin Pigment (MAP) was quantified by the pH differential method described by Giusti & Wrolstad (2001). 20 µL of sample and 280 µL of 0.025 M potassium chloride buffer (pH 1.0) or 0.4 M sodium acetate buffer (pH 4.5) were added in 96-well microplate and absorbances were measured at 520 and 700 nm in a microplate reader (Multileader Infinite M200 TECAN, ZH, Switzerland) after 15 min. The concentrations of MAP were calculated in mg of cyanidin 3-*O*-glucoside equivalent per 100 g of dry sample (mgC3GE/100g), according to Equations (5) and (6). The data were expressed in cyanidin-3-*O*-glucoside because is the most abundant anthocyanin in jaboticaba peel (QUATRIN et al., 2019).

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH4.5} \quad (5)$$

$$\mathrm{M}AP(mg/kg) = \frac{(A\ x\ MW\ x\ DF\ x\ 1000)}{l\ x\ \varepsilon} \quad (6)$$

where A is the absorbance calculated of the diluted sample, MW is the molecular weight of the cyanidin 3-*O*-glucoside (449.2 g/mol), DF diluted factor (20 µL of extract in 280 µL of the buffer, DF = 15), ε is extinction coefficient for cyanidin-3-*O*-glucoside (26900) and l is the pathlength (cm). The final results obtained from triplicate were expressed in mg of C3G equivalent per gram of dry waste (mgC3GE/100g dw), which were calculated from the final ratio solid-to-solvent in each assay.

3.2.5.2 Percentage of anthocyanin recovery (%AR)

The total anthocyanin content (TAC) from the jaboticaba peel, was determined after five sequential extractions using 50 mL of 80% methanol solution acidified with 0.1M of HCl for 0.5g of the sample under magnetic agitation at room temperature for 30 min, followed by five extractions using 50 mL of 70% acetone solution at the same conditions (GURAK et al., 2014). The percentage of anthocyanin recovery (%AR) was calculated by monomeric anthocyanin pigment (MAP) content obtained for each assay concerning TAC, according to Equation (7).

$$\%AR = \left(\frac{MAP}{TAC}\right) \cdot 100 \quad (7)$$

3.2.5.3 Polymeric color (PC)

According to the method described by Giusti & Wrolstad (2001), the extracts obtained in each assay of RCCD were diluted in potassium chloride buffer (pH 1.0) in an appropriate dilution factor (DF) for a linear range of UV-Vis spectrophotometer absorbance at 520 nm. In sequence, 280 µL of extract and 20 µL of potassium metabisulfite (0.9 M) were packed in a 96-well microplate, which was called blanched sample, while the control sample was performed

by adding 20 μL of distilled water in 280 μL of extract. After 15 min, the sample and control samples were read at 700, 520, and 420 nm. Then the color density (DC) and polymeric color (PC) of the extracts were calculated through Equation (8) using the absorbances (A) of the control sample and blanched sample, respectively.

$$DC\ or\ PC = [(A_{420} - A_{700}) + (A_{520} - A_{700})] \cdot DF \quad (8)$$

The polymeric color (PC) also was quantified employing Equation (8) but using the bleached sample data. Finally, the percentage of polymeric color (% PC) was determined by Equation (9):

$$\%PC = \left(\frac{PC}{DC}\right) \cdot 100 \quad (9)$$

3.2.5.4 In vitro antioxidant activity

The antioxidant potential of the anthocyanin-rich extract was determined by 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging capacity, according to (RE et al., 1999), with adaptations for microplate reader, and by ferric reducing antioxidant power (FRAP) assays according to Benzie and Strain (1996) adapted method. Antioxidant activity was calculated concerning a TROLOX curve ($ABTS = 0.36 \cdot x\ absorbance, R^2 = 0.97,$ and $FRAP = 30.00 \cdot x\ absorbance;\ R^2 = 0.99$). The final results were calculated from a triplicate and expressed in μmol Trolox equivalent per gram of sample (μmolTE/g dw).

3.2.6 Optimization Process

The independent factors of the PLE operating conditions, DES concentration in aqueous solution (%, x_1), temperature (°C, x_2), and solvent flow rate (mL/min, x_3), conducted each of them at three levels, were analyzed by multiple regression analysis and response surface

methodology (RSM) using Statistica v.13.5 software. For this, a generalized second-order polynomial equation was used to fit the experimental data, according to Equation (10):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{j \leq i \leq 1}^{k} \beta_{ij} x_i x_j \quad (10)$$

where, *y* represents the predicted response, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for linear, quadratic, and interaction terms, respectively, x_i e x_j are the independent variables, and k is the variable number (k = 3) (BRUNS; SCARMINO; BARROS NETO, 2006). The quality of each model fitted was verified by ANOVA, and the non-significant terms were removed from the mathematical models. Thus, the models were re-fitted using only the significant terms ($p < 0.05$). The fitting adequacy was verified by $p_{lack\ of\ fit}$, and the quality by regression coefficient (R^2) and its adjusted R^2. Then, the response surfaces were plotted.

After obtaining the mathematical models, the simultaneous optimization of all responses was performed employing the desirability function, according to Derringer & Suit (1980). The aim was to maximize the responses MAP, AR, ABTS, and FRAP and minimize the PC (since it is related to anthocyanin degradation). Finally, external validation of the suggested optimal PLE conditions was performed to verify the repeatability of models, comparing the predicted values with the experimental data using relative error (RE), Equation (11).

$$RE(\%) = \frac{observed\ value - predicted\ value}{predicted\ value} \times 100 \quad (11)$$

3.2.7 Comparing aqueous solution of DES with other solvents for PLE method

The effect of the type of solvent on PLE yield and extracts quality was evaluated using the PLE optimized conditions. Two choline chloride-based DES, ChCl:Pro and ChCl:Ma, were selected according to a previous study (BENVENUTTI et al., 2020), and compared with water and acidified water (pH 1.5) used as control solvents. The final residues of these extractions were dried at 60 °C (Lucadema, Model 82/27, SP, Brazil) and stored at -18 °C, for further pectin recovery (**Figure 9**).

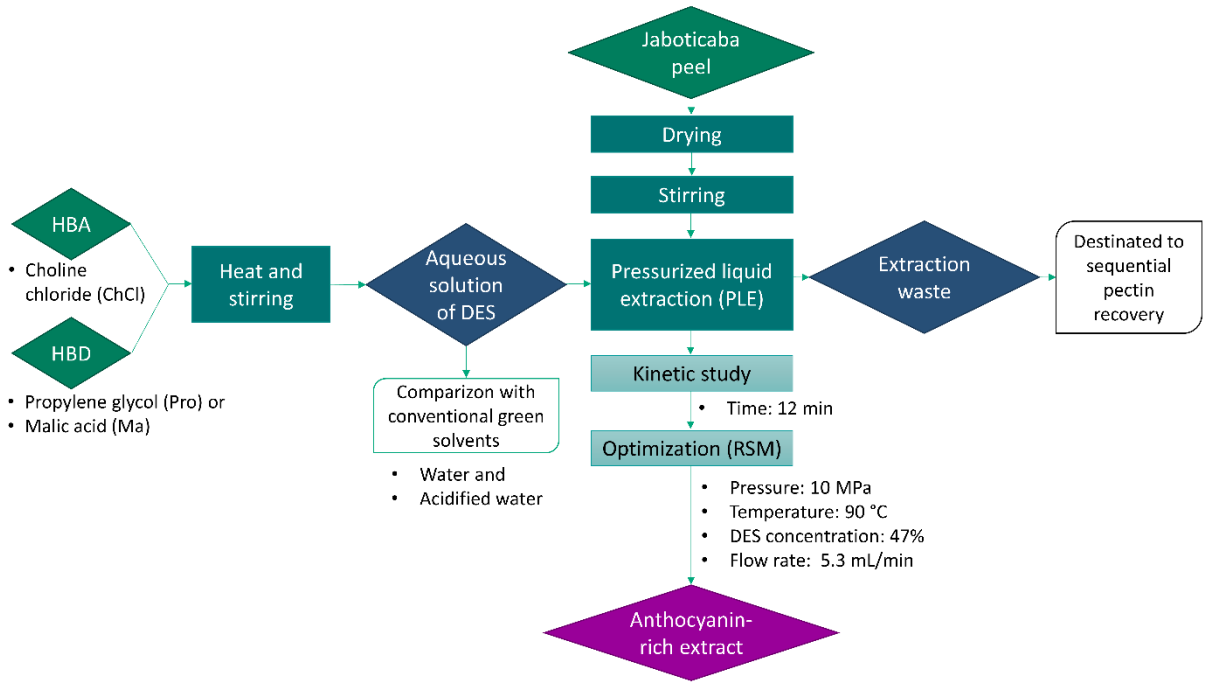


Figure 9 - Schematic diagram illustrating the extraction process performed. Note: DES – deep eutectic solvent, PLE - pressurized liquid extraction, RSM – response surface methodology.
Source: The author.

Besides the extract quality attributes, defined by MAP, PC, AR, and the antioxidant activity by ABTS and FRAP (performed as described in section 3.2.6), the following analyses were also conducted to evaluate the quality of the extracts recovered by PLE using different solvents:

3.2.7.1 Total phenolic content (TPC)

The TPC values of the extracts obtained by PLE using different solvents (ChCl:Pro, ChCl:Ma, water, or acidified water) were quantified by the Folin-Ciocalteu method according to Singleton and Rossi (1965). The values were obtained from triplicate and expressed in mg of gallic acid equivalent per g of sample (mg GAE/g dw) using a standard curve ($TPC = 4.49 \cdot x\ absorbance;\ R^2 = 0.99$) previously prepared.

3.2.7.2 Identification and quantification of phenolic compounds by LC-ESI-MS/MS

Firstly, the samples were prepared according to described by Lima et al. (2019). Briefly, 20 mL of freeze-dried (Liotop,model LD101, SP, Brazil) extract were subjected to acid hydrolysis with hydrochloric acid (6 mol/L), followed by partition using diethyl ether. The solvents were removed by rotary evaporation under vacuum (Fisatom, model 801, SP, Brazil) and the dried samples were suspended in 1 mL of chromatographic grade methanol.

The phenolic compounds identification was performed in a high-performance liquid chromatography (HPLC) system (1200 Series, Agilent Technologies, Waldbronn-BW, Germany). The HPLC system was coupled to a mass spectrometry system, which consists of a hybrid triple quadrupole/linear ion trap mass spectro- meter (Q Trap 3200 Applied Biosystems/MDS Sciex, Concord-ON, Canada). The mass spectrometer was operated in negative electrospray (TurboIonSpray Applied Biosystems/MDS Sciex, Concord-ON, Canada) ionization mode.

The samples were 10-folds diluted with methanol:water (70:30. v/v) and aliquots of 10 μL were injected into the equipment. To separation of the compounds, a Synergi column (4.0 μm, 2.0×150mm d.i.; Phenomenex, Torrance-CA, USA) was used under gradient elution

conditions. Mobile phases used were mixtures of methanol 95% and water 5% (v/v), channel A, and water and formic acid 0.1% (v/v), channel B. The separation was carried out at 30 °C using a segmented elution gradient as follows: 0–5 min, 10% A; 5–7 min, 90% A; 7–10 min, 90% A; 10–17 min, 10% A. Between the analyses, the column was conditioned for 5 min with the proportion of the initial mobile phase of the separation. The running flow rate was 150 $\mu L.min^{-1}$. The mass spectrometer was operated in negative electrospray (TurboIonSpray Applied Biosystems/MDS Sciex, Concord-ON, Canada) ionization mode. The MS/MS parameters were: capillary needle maintained at −4500 V; curtain gas at 10 psi; the temperature at 400 °C; gas 1 and gas 2 at 45 psi; and CAD gas, medium. The HPLC–ESI-MS/MS system control and data analysis were accomplished in the software Analyst version 1.6.2. The quantification of identified compounds was performed through calibration curves previously prepared (**Table 8**).

Table 8 - Chromatographic parameters of phenolic compounds analyzed by HPLC.

Analytes	Linearity range (mg/L)	Requession equation	LOD (mg/L)	LOQ (mg/L)	R^2
Cinnamic acid	0.8-6.0	y=62920x+13341	0.24	0.73	0.9915
Cyanidin*	0.09-1.8	y = 7E+09x - 154744	0.4	1.34	0.9882
Protocatechuic acid	0.1 - 6.0	y=262159x+13392	0.05	0.16	0.9976
p-coumaric acid	0.8-6.0	y=703824x+516917	0.003	0.01	0.9802
Gallic acid	0.1-6.0	y=342648x+23145	0.03	0.1	0.9974
Cafeic acid	0.03-1.0	y=2E+06x+35690	0.01	0.03	0.9930
Ferulic acid	1.0-6.0	y=43358x+64446	0.006	0.01	0.9590
Syringic acid	0.04-6.0	y=73197x-2488.8	0.01	0.04	0.9976
Synapic acid	0.05-1.0	y=75971x+869.51	0.02	0.05	0.9574
Chrysin	0.02-1.0	Y=305646x-3102.6	0.005	0.02	0.9963
Naringerin	0.01-1.0	y=375378+571.49	0.004	0.01	0.9956
Eriodictyol	0.1-2.5	y=452867x+15603	0.04	0.1	0.9948
Aromadendrin	0.03-3.0	y=301615x-3442	0.01	0.03	0.9993
Catechin	0.09-7.0	y=35424x+739.62	0.03	0.09	0.9965
Epicatechin	0.01-1.6	y=211020x+5869.5	0.003	0.01	0.9922
Ellagic acid	0.2-6.0	y=10809x-502.09	0.06	0.2	0.9913
Quercetin	0.3-6.0	y=41196x+4118.9	0.1	0.3	0.9946
Taxifolin	0.04-6.0	y=73013x+6622.2	0.01	0.04	0.9925

Miricetrin	1.0-6.0	y=10720x-9342.1	0.33	1.00	0.9663
Carnosol	0.01-0.6	y=2e+06-2627.6	0.002	0.01	0.9985
Chlorogenic acid	0.03-6.0	y=235903x-5819.5	0.01	0.03	0.9973
Isoquercetrin	0.02-6.5	y=175534x+3876.4	0.008	0.02	0.9965
Rutin	0.03-6.2	y=258612x+11011	0.01	0.03	0.9966

Note - *: mg/mL.

3.2.7.3 Identification and quantification of anthocyanins by LC-MS

The anthocyanin profiles were identified and quantified according to Teixeira (2021) with adaptations to LC-MS. The four recovered extracts, obtained by PLE with different solvents (ChCl:Pro, ChCl:Ma, water, and acidified water) were semi-purified to obtain a concentrated fraction in monomeric anthocyanin using a glass column (1.0 cm x 30 cm) filled with 10 g of Amberlite XAD-7HD resin, as described by Benvenutti et al. (2020). The freeze-dried semi-purified extracts were resuspended in methanol and filtered through a 0.22 μm nylon syringe filter. The samples (10μL) were analyzed by high-performance liquid chromatography (model LCMS-2020, Shimadzu, Kyoto, Japan), coupled with a quadrupole mass spectrometer with an electrospray ionization source in positive mode. The separation was carried out using a reverse C18 column (5 μm, 4.6×250 mm) (NST, Santos, Brazil) at 25 °C. The mobile phase flow was 1.2 mL/min and was composed of A (0.1% formic acid, v/v) and B (0.1% formic acid in methanol, v/v). The gradient elution system was programmed as: 0-16 min,14%-55% B, 16-27, 55-100% B, 27-30, 100-14% B, 30-32, 14%B. The temperature of the rotary spray spectrum interface was 350 °C, nebulizer gas flow 1.5 L/min, heat block 200 °C, and drying gas flow 15 L/min. The interface voltage was 4.5 kV and the RF-beam voltage was 60V. The identification of cyanidin-3-*O*-glucoside was performed comparing its retention time with the reference standard and by its mass spectra based on the values of mass-to-charge ratio (*m/z*) and quantification was performed by an external standard curve of cyanidin-3-*O*-glucoside and the results expressed in mg per g of sample (mg/g dw).

3.2.7.4 Color determination

For color determination, aliquots of 300 µL of the extracts obtained by PLE using different solvents (ChCl:Pro, ChCl:Ma, water, and acidified water) were placed in a 96-well microplate and the absorbance was measurement, in the range of 400 to 700 nm, in multi-reader (Tecan, Model Infinite M200, ZH, Switzerland). After reading, the results were analyzed by *ColorBySpectra* software (Far & Giusti, 2017), which converts the absorbances in color values using the CIE (Commission Internationale de l'éclairage) L* a* b* and L* C* h° scales according to 1964 standard observatory, using Illuminant D65 spectral distribution and 10° view angle. Finally, the CIELAB values were converted into images using an online converter (nixsensor.com/free-color-converter/).

3.2.7.5 Anthocyanin stability

The thermostability of the anthocyanin-rich extracts recovered by PLE with different solvents (ChCl:Pro, ChCl:Ma, water, and acidified water) was evaluated through a model system, by heating, according to Peron, Fraga, & Antelo (2017) with minor changes. The samples were placed in tubes, sealed, and immersed in a thermostatic bath (TECNAL, model TE-2005, SP, Brazil) containing water for temperatures of 60, 70, and 80 °C and glycerin solution for temperatures of 90 and 100 °C. At the exact heating times of 0, 30, 60, 90, 120, and 240 min, a tube was removed from the system and immediately cooled in an ice bath. Each tube was a degradation point quantified in terms of MAP content (section 2.5.1), and the isothermal degradation of the anthocyanins was fitted in a first-order kinetic model, Equation (11) (PERON; FRAGA; ANTELO, 2017; TEIXEIRA et al., 2021).

$$\frac{C_t}{C_0} = e^{-k_d \times t} \qquad (11)$$

where C_t is MAP concentration with tempo t (mgC3G/100g), C_0 is the MAP initial concentration (mgC3G/100g), k_d is the degradation rate (min^{-1}) and t is the time (min).

The time of half-live ($t_{1/2}$) of anthocyanin degradation was given by Equation (12):

$$t_{1/2} = \frac{\ln 2}{k_d} \tag{12}$$

The activation energy (E_a) was determined through the Arrhenius equation model, according to Equation (13):

$$lnk_d = \ln A - \frac{E_a}{R \times T} \tag{13}$$

where A is the frequency factor (min^{-1}), R is the ideal gas constant (8.314 $Jmol^{-1}\ K^{-1}$) and T is the temperature (K).

Additionally, other thermodynamic parameters as activation enthalpy (ΔH), the free energy of inactivation (ΔG), and activation entropy (ΔS) were calculated by Equations (14) to (16).

$$\Delta H = E_a - R \times T \tag{14}$$

$$\Delta G = -R \times T \ln \frac{k_d \times h}{k_B \times T} \tag{15}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{16}$$

where E_a is the activation energy for the degradation reaction (J/mol), R is the ideal gas constant (8.314 J/molK), T is the temperature (K), k_d is the kinetic rate constant (s^{-1}), k_B is the Boltzmann constant (1.2806×10^{-23} J/K), and h is Planck's constant (6.6262×10^{-34} J/s).

3.2.8 Anti-glycemic and anti-lipolytic potential

3.2.8.1 α-glucosidase inhibition assay

The extract samples were diluted in phosphate buffer (0.1 M, pH 6,8) forming a set of concentrations from 2 to 20 μg/mL for the partially purified extracts and 0.01 to 0.1 μg C3GE/mL for raw extracts, i.e before the purification step. The assays followed the method described by Barik et al. (BARIK et al., 2020). 20 μL of the diluted sample, 20 μL of phosphate buffer, and 20 μL of yeast α-glucosidase solution (0.5 U/mL) were placed in a 96-well microplate and incubated at 37 °C for 15 min. After this period, 40 μL of *p*-nitrophenyl-α-*D*-glucopyranoside (pNPG) solution in phosphate buffer was added, followed by incubation at 37 °C for 15 min. The *p*-nitrophenol release from pNPG was measured by absorbance at 405 nm in a microplate reader. The % of inhibition was calculated according to Equation (17).

$$\%\ inhibition = \left[1 - \left(\frac{\Delta A\ control}{\Delta A\ sample}\right)\right].100 \quad (17)$$

where the control is the reaction without sample and ΔA is the absorbance at 405 nm after being subtracted from the blank.

The results were expressed in mean inhibitory concentration (IC_{50}) through inhibitory activity calculated from the curves obtained in triplicate from the set of concentrations of each sample.

3.2.8.2 α-amylase inhibition assay

The extracts were also diluted in phosphate buffer in a concentration range from 0.5 to 10 mg/mL for partially purified extracts and from 1 to 50 μg C3GE/mL for raw extracts. In the assays, 40 μL of the diluted sample, 150 μL of water, 400 μL of the substrate (0.5% potato starch), and 200 μL of enzymatic solution (0.5 mg/mL) in phosphate buffer were placed in

microtubes. The mixture was incubated at 25 °C for 15 min. Sequentially, the reaction was stopped by the addition of 400µL of DNS (3,5-dinitrosalicylic) reagent followed by incubation at 90 °C for 15 minutes. After this period, the absorbance was measured in a spectrophotometer (800 XI, Femto, Brazil) at 540 mn (ALI; HOUGHTON; SOUMYANATH, 2006). The final results were expressed in IC_{50} according to described in section 3.2.8.1.

3.2.8.3 Porcine pancreatic lipase inhibition assay

According to Zhang, Wu, Wei, & Qin (2020a), the assays were performed in a microplate by addition of 50 µL of diluted sample and 50 µL of porcine pancreatic lipase (PPL)solution (1mg/mL) in Tris-HCl buffer (pH 8). After the incubation period at 37 °C for 10 min, was added 50 µL of substrate *p*-nitrophenol-butyrate (*p*NPB, 2mg/mL). The microplates were incubated again at 37 °C for 20 minutos and the *p*-nitrophenol released was measured by absorbância a 405 nm in a microplate reader and the inhibition activity, calculated according to Equation (17). The IC_{50} was estimated with the results obtained for the set of dilutions from 100 to 1000 µg/mL for partially purified extracts and 0.06 to 15 µgC3GE/mL for raw extracts.

3.2.9 Statistical analysis

Six replicates were performed in analyses using a microplate reader and triplicate for the other analyses thus, the data sets were presented as average and standard deviation. Firstly, was verified the homogeneity of variance by Levene's test ($p \geq 0.05$). Significant differences between the samples were evaluated by one-way ANOVA ($p \leq 0.05$), followed by Ficher's LSD test. The statistical significance of the models used was also determined by ANOVA and the quality and adequacy of the adjustments were assessed by the determination coefficient (R^2),

adjusted R^2, and the root-mean-square error (RMSE). All statistical analyses were performed using Statistica v.13.5 software (TIBCO Software Inc., Palo Alto, CA, USA).

3.3. RESULTS AND DISCUSSION

3.3.1 Centesimal composition of the jaboticaba by-product

The centesimal composition of the dry and ground jaboticaba processing by-product is presented in the **Table 9**. Inada et al. (2015) reported the chemical composition of different parts of jaboticaba fruit on a dry weight basis, including jaboticaba peel (JP). These data presented similar total carbohydrate content (86.9%), but higher protein (8.5%) and ash (0.6%) and lower lipid content (4%) than the present study.

Table 9 - Centesimal composition of the jaboticaba peel.

Components	Jaboticaba peel * (%)
Moisture	14.58 ± 0.4
Fixed mineral residue (Ash)	1.98 ± 0.2
Total fat (Soxhlet)	4.16 ± 0.5
Protein (N x 6.25)	5.53 ± 0.6
Crude fiber	33.01 ± 0.1
Non-fibrous carbohydrates	40.76 ± 0.2

*dry and ground sample used for extraction methods.

The range in compositions is mainly related to fruit cultivar since that author studied the *M. jaboticaba* while the *M.cauliflora* was used in the present work. Other factors as the ripening stage, climate conditions, and soil also influence the chemical composition of jaboticaba fruit (BENVENUTTI; ZIELINSKI; FERREIRA, 2021).

3.3.2 Optimization of extraction conditions using ChCl:Pro solution as the solvent

A kinetics curve from PLE, conducted at 10 MPa, 90 °C, and using 30% ChCl:Pro aqueous solution as the solvent, with a flow rate of 4 mL/min, enabled the definition of the

extraction time, and then, this time was applied for all solvents studied. The extraction kinetics provides the visualization of the mass transfer mechanisms present in the process, analogous to that defined for Supercritical Fluid Extraction (SFE) (FERREIRA; MEIRELES, 2002). This analogy to SFE was applied by Ferro et al. (2020) for the PLE from *Sida rhombifolia* leaves. Then, the analysis of the kinetics behavior for PLE from JP samples identified the following mass-transfer mechanisms: constant extraction rate period (CER), falling extraction rate period (FER), and diffusion-controlled period (DC) (**Figure 10**). Therewith, the extraction time was fixed at 12 min for all assays, because it represents the beginning of the DC period, where most part of soluble solute was already recovered by the solvent. Then, all PLE assays were performed according to CCRD plan and considering the fixed conditions of pressure, 10 MPa, and time, 12 min, for the recovery of extract samples rich in anthocyanins.

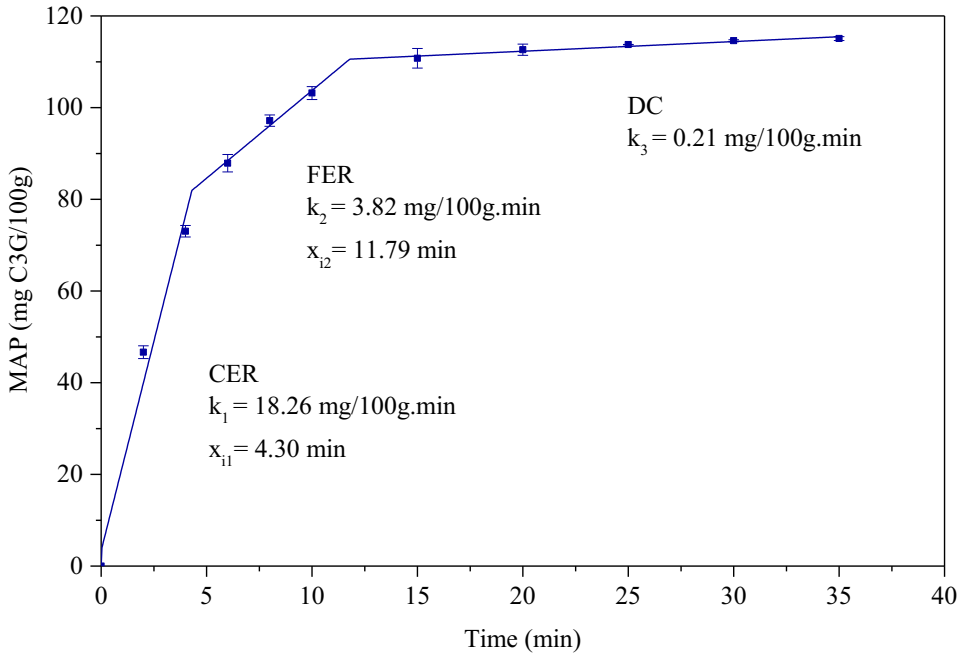


Figure 10 - Extraction kinetic of monomeric anthocyanin pigment (MAP) content.

The recovered extracts were analyzed according to MAP, AR, PC, and antioxidant activity by ABTS and FRAP methods, and the results are presented in **Table 10.** The results showed significant differences ($p < 0.05$) among the results from all assays. Therefore, this design detects the effect of DES concentration, temperature, and solvent flow rate on the anthocyanin content and antioxidant capacity of the extracts recovered from JP samples. Then, the parametric data were fit by multiple regression analysis coupled to RSM, providing mathematical models represented by Equations (18) to (22), which were significant ($p_{\text{model}} < 0.01$) and did not show a lack of fit ($p_{\text{lack of fit}} > 0.05$). Furthermore, the determination coefficients varied from 0.88 to 0.97 with R^2_{adj} from 0.83 to 0.95, which explains at least 83% of the variations in responses (**Appendix D**).

$$MAP = 164.63 + 20.85x_1 - 49.55x_2 - 34.94x_2^2 + 19.86x_3 - 19.86x_3^2 \quad (18)$$

$$PC = 27.47 + 2.76x_1 + 9.75x_2 + 4.68x_2^2 + 7.20x_3 + 3.49x_2.x_3 \quad (19)$$

$$AR = 65.97 + 8.36x_1 - 19.86x_2 - 14.00x_2^2 + 7.96x_3 - 6.82x_3^2 \quad (20)$$

$$ABTS = 208.59 + 11.03x_1 + 60.06x_3 - 13.60x_1.x_3 + 12.73x_2.x_3 \quad (21)$$

$$FRAP = 537.36 + 54.55x_1 - 36.12x_1^2 - 58.56x_2^2 + 66.83x_3 + 63.18\,x_1.x_2 \quad (22)$$

Table 10 - Responses variables of each extraction assay by PLE method.

Assays	Independent Variables			Responses				
	DES concentration (%)	Temperature (°C)	Flow rate (mL/min)	MAP (mgC3GE/100g dw)	PC (%)	AR (%)	ABTS (µmolTE/g dw)	FRAP (µmolTE/g dw)
1	15 (-1)	60 (-1)	3 (-1)	$99.17^{d} \pm 3.01$	$17.24^{j} \pm 1.29$	$39.74^{d} \pm 1.21$	$145.67^{l} \pm 0.14$	$403.80^{gh} \pm 32.96$
2	15 (-1)	120 (1)	5 (1)	$26.43^{f} \pm 4.30$	$49.09^{c} \pm 1.28$	$10.59^{f} \pm 1.72$	$265.29^{d} \pm 0.28$	$333.84^{i} \pm 8.61$
3	45 (1)	60 (-1)	5 (1)	$214.05^{a} \pm 14.37$	$31.70^{e} \pm 0.87$	$85.38^{a} \pm 5.76$	$277.06^{b} \pm 0.12$	$571.14^{bc} \pm 34.98$
4	45 (1)	120 (1)	3 (-1)	$81.41^{e} \pm 4.77$	$33.28^{e} \pm 3.19$	$32.63^{e} \pm 1.91$	$159.01^{k} \pm 0.32$	$443.17^{f} \pm 53.46$
5	30 (0)	90 (0)	4 (0)	$152.25^{c} \pm 8.83$	$26.65^{gh} \pm 1.99$	$61.02^{c} \pm 3.54$	$203.98^{i} \pm 0.17$	$532.46^{d} \pm 34.18$
6	15 (-1)	60 (-1)	5 (1)	$182.72^{b} \pm 2.27$	$25.86^{h} \pm 2.11$	$72.82^{b} \pm 0.91$	$267.22^{c} \pm 0.11$	$534.53^{d} \pm 58.88$
7	15 (-1)	120 (1)	3 (-1)	$10.65^{g} \pm 1.41$	$29.20^{f} \pm 1.00$	$4.27^{g} \pm 0.56$	$86.94^{n} \pm 0.09$	$243.69^{j} \pm 19.18$
8	45 (1)	60 (-1)	3 (-1)	$149.19^{c} \pm 3.38$	$25.90^{h} \pm 1.61$	$59.79^{c} \pm 1.36$	$204.01^{i} \pm 0.15$	$395.60^{gh} \pm 1.29$
9	45 (1)	120 (1)	5 (1)	$97.09^{d} \pm 2.87$	$55.74^{b} \pm 0.75$	$38.91^{d} \pm 1.15$	$277.08^{b} \pm 0.07$	$668.20^{a} \pm 1.32$
10	30 (0)	90 (0)	4 (0)	$174.30^{b} \pm 9.30$	$26.77^{gh} \pm 1.07$	$69.85^{b} \pm 3.73$	$204.48^{h} \pm 0.13$	$541.16^{cd} \pm 19.07$
11	4.90 (-1.67)	90 (0)	4 (0)	$148.24^{c} \pm 3.09$	$21.38^{i} \pm 0.36$	$59.41^{c} \pm 1.24$	$209.03^{f} \pm 0.11$	$373.17^{h} \pm 3.97$
12	55.09 (+1,67)	90 (0)	4 (0)	$184.62^{b} \pm 17.03$	$28.76^{fg} \pm 1.39$	$73.99^{b} \pm 6.83$	$207.86^{g} \pm 0.36$	$480.51^{e} \pm 3.32$
13	30 (0)	39 (-1.67)	4 (0)	$147.97^{c} \pm 1.43$	$18.93^{j} \pm 1.25$	$59.30^{c} \pm 0.57$	$204.51^{h} \pm 0.15$	$402.31^{gh} \pm 27.82$
14	30 (0)	140 (+1.67)	4 (0)	$0.80^{g} \pm 0.01$	$58.36^{a} \pm 1.35$	$0.32^{g} \pm 0.01$	$203.02^{j} \pm 0.10$	$325.68^{i} \pm 37.63$
15	30 (0)	90 (0)	2.33 (-1.67)	$96.97^{d} \pm 13.37$	$14.50^{k} \pm 0.64$	$38.86^{d} \pm 5.36$	$111.57^{m} \pm 0.08$	$424.87^{fg} \pm 17.54$
16	30 (0)	90 (0)	5.67 (+1.67)	$152.10^{c} \pm 27.90$	$39.12^{d} \pm 2.02$	$60.95^{c} \pm 11.18$	$306.25^{a} \pm 0.69$	$596.95^{b} \pm 48.36$
17	30 (0)	90 (0)	4 (0)	$175.86^{b} \pm 16.65$	$28.16^{fg} \pm 0.95$	$70.48^{b} \pm 6.67$	$213.17^{e} \pm 0.23$	$576.35^{b} \pm 9.60$

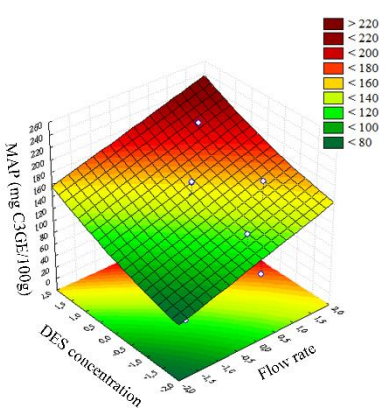
Note: MAP – monomeric anthocyanin pigment content, PC - polymeric color, AR – anthocyanin recovery, ABTS – antioxidant activity by ABTS radical discoloration, FRAP – antioxidant activity by iron ion reduction, C3GE – cyanidin 3-O-glucoside equivalent, TE – Trolox equivalent, dw – dry weight, ^{abc} – different letters in the same column indicate significative difference among the assays by Fisher test ($p < 0.05$), - the besties essays for each answer.

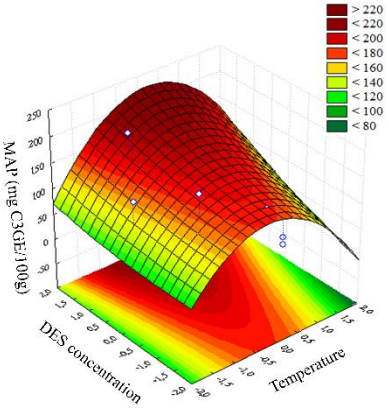
According to proposed models and three-dimensional (3D) response surfaces (**Figure 11**), the DES concentration (x_1) has a significant and positive effect on all responses. In general, the increase in DES concentration improved the extraction performance. However, PC values, a parameter related to anthocyanin degradation, also increased with the enhancement in DES concentration. This fact, combined with the negative quadratic effect of x_1 from the FRAP model (Equation 18) and the negative synergic effect between x_1 and x_3 (flow rate) from the ABTS model (Equation 17) indicates that intermediate DES concentrations improve the antioxidant potential. The use of aqueous solutions of DES instead of pure DES, increases the extraction ability, mainly due to the decrease in solvent viscosity, facilitating the matrix penetration and the analyte dissolution. However, excessive water content impairs the hydrogen bond between HBA and HBD, reducing the DES solvation capability and the ability to stabilize the analyte, and consequently decreasing the extraction ability of the solvent (BENVENUTTI; ZIELINSKI; FERREIRA, 2019).

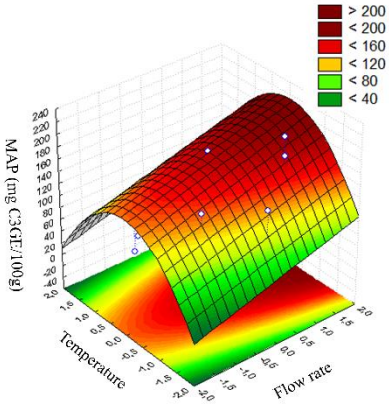
The temperature (x_2) presented the highest effect on anthocyanin extraction performance, evaluated by MAP, PC, and AR values. The linear coefficients had a higher influence, compared to the quadratic ones, in these three responses, with negative values for MAP and AR, which indicates that the lowest temperatures evaluated resulted in higher anthocyanin yield. According to the results from **Table 10**, the temperatures of 60 and 90 °C provided the highest MAP and AR values. Besides, the positive effect of temperature on PC model (Equation 15) is probably related to the increase in anthocyanin degradation at high temperatures. This effect can be observed from assays conducted at the highest temperature: assays 2 (15% DES, 120 °C and 5 mL/min), 9 (45% DES, 120 °C and 5 mL/min), and 14 (30% DES, 140 °C and 4 mL/min), which provided high PC values of 49.09, 55.74 and 58.36%,

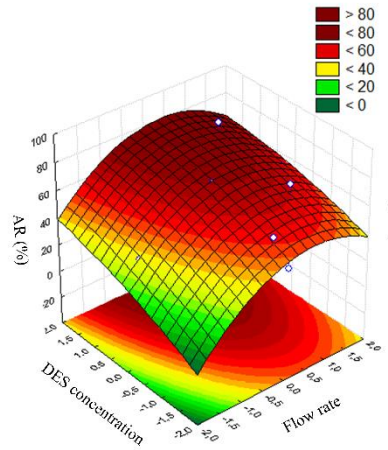
respectively (**Table 10**). According to the literature, the increase in PLE temperature accelerates anthocyanin degradation by oxidation, hydrolysis, and polymerization reactions (GARCIA-MENDOZA et al., 2017; TEIXEIRA et al., 2021). Corroborating with our results, Santos, Veggi & Meireles (2012), presented a positive effect of temperature in anthocyanin recovery from jaboticaba peel by PLE method, in the range from 40 to 80 °C, but a negative effect in the range from 80 to 120°C. Therefore, we could conclude that there is a limiting maximum temperature for the recovery of thermosensitive compounds like anthocyanins. This temperature limit depends on several factors, mainly the extraction time (SANTOS; MEIRELES, 2011).

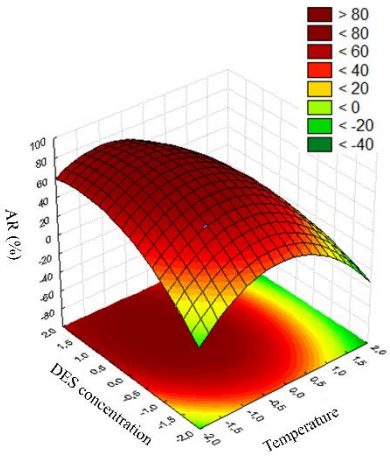
The temperature (x_2) also presented an interactive (linear and positive) effect with flow rate (x_3) and with DES concentration (x_1), for the antioxidant activity by ABTS and FRAP, respectively. This occurred probably because the increase in temperature improves the diffusion and the solubility of total analytes, disrupting solute-matrix bonds, and decreasing viscosity and surface tension of the solvent mixture, which improves their penetration in the solid matrix, increasing surface area (RODRIGUES et al., 2019; TEIXEIRA et al., 2021). This synergic effect can be observed from assays 1 and 2, with temperatures varying from 60 to 120 °C, which presented ABTS values of 145.67 μmolTE/g dw and 265.29 μmolTE/g dw, respectively. Also, assays 6 and 9 for FRAP values in the same temperature variation provided results of 534.53 μmolTE/g dw, and 668.20 μmolTE/g dw, respectively.

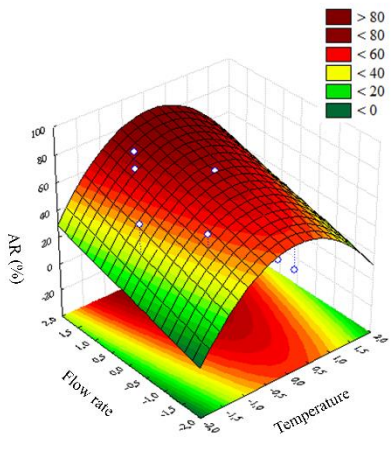

MAP (mg C3GE/100g)
DES concentration
Flow rate
> 220
< 220
< 200
< 180
< 160
< 140
< 120
< 100
< 80

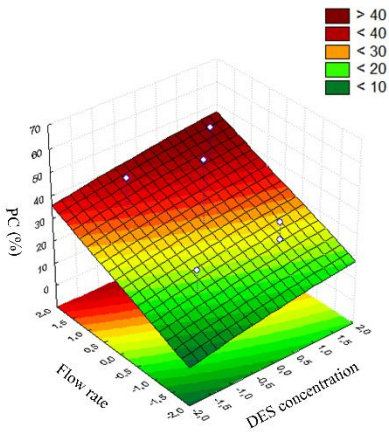

MAP (mg C3GE/100g)
DES concentration
Temperature
> 220
< 220
< 200
< 180
< 160
< 140
< 120
< 100
< 80

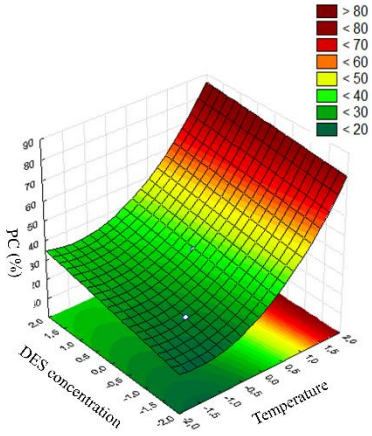

MAP (mg C3GE/100g)
Temperature
Flow rate
> 200
< 200
< 160
< 120
< 80
< 40

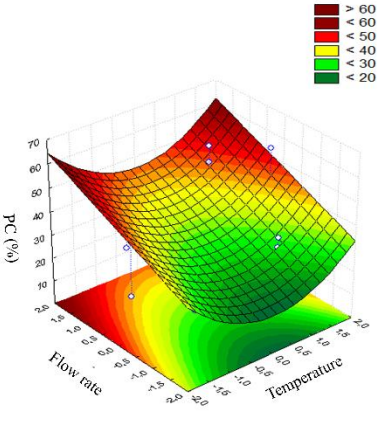

AR (%)
DES concentration
Flow rate
> 80
< 80
< 60
< 40
< 20
< 0


AR (%)
DES concentration
Temperature
> 80
< 80
< 60
< 40
< 20
< 0
< -20
< -40


AR (%)
Flow rate
Temperature
> 80
< 80
< 60
< 40
< 20
< 0


PC (%)
Flow rate
DES concentration
> 40
< 40
< 30
< 20
< 10


PC (%)
DES concentration
Temperature
> 80
< 80
< 70
< 60
< 50
< 40
< 30
< 20


PC (%)
Flow rate
Temperature
> 60
< 60
< 50
< 40
< 30
< 20

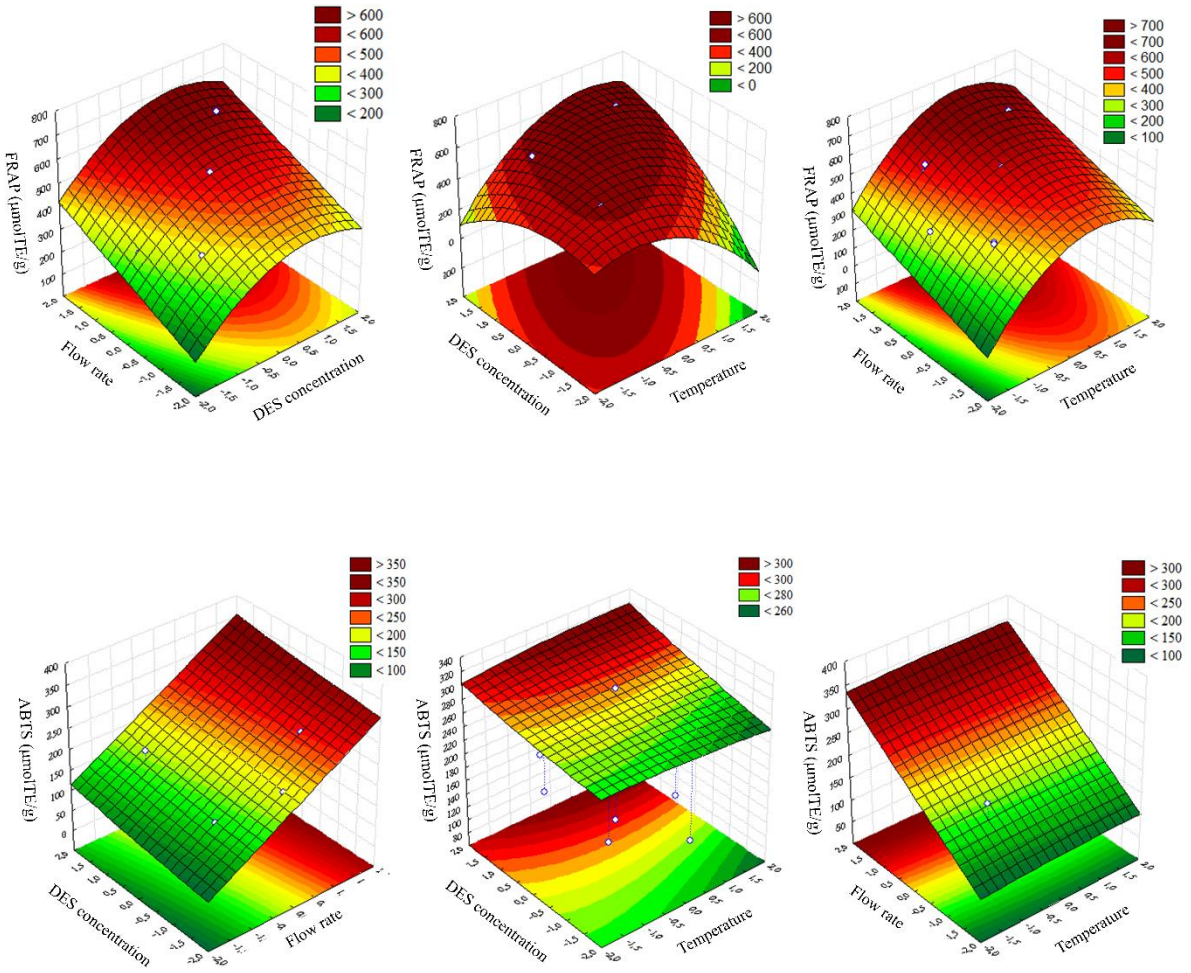


Figure 11 - Three-dimensional (3D) response surfaces showing the effect of independent variables (temperature, DES concentration, and flow rate) on the dependent variables (MAP, AR, PC, FRAP, and ABTS) for anthocyanin-rich extraction by PLE using aqueous solution of DES (ChCl:Pro) as the solvent.

The solvent flow rate (x_3) is an important process variable that affects the extraction yield, which maximum value is the equilibrium condition of solute content in the solvent phase and solid matrix. When PLE is performed at continuous mode, generally, the flow rate exerts a positive influence on the extraction rate, allowing higher solvent flux throughout the solid material (ESSIEN; YOUNG; BAROUTIAN, 2020; TEIXEIRA et al., 2021). However, the flow rate influence depends on the extraction period, observed through the kinetics curve, i.e., the

flow rate effect is not relevant at the diffusion-controlled (DC) period (ESSIEN; YOUNG; BAROUTIAN, 2020). All response models (Equations 14 to 18) had a positive linear effect on the flow rate, especially ABTS and FRAP models, where the flow rate was the main effect, which was also a positive effect for PC value.

A multiresponse optimization was provided by modeling all process variables and conducted using the desirability function (*d*) (**Figure 12**). This optimization, performed considering all responses simultaneously, suggests the best extraction performance is achieved using 47% DES solution, 90 °C, and flow rate of 5.3 mL/min, with a *d* value of 0.80038. This value represents 80% of all desired responses, a satisfactory result since *d* value ranges from 0, completely undesirable, to 1, totally desirable (DERRINGER; SUICH, 1980)

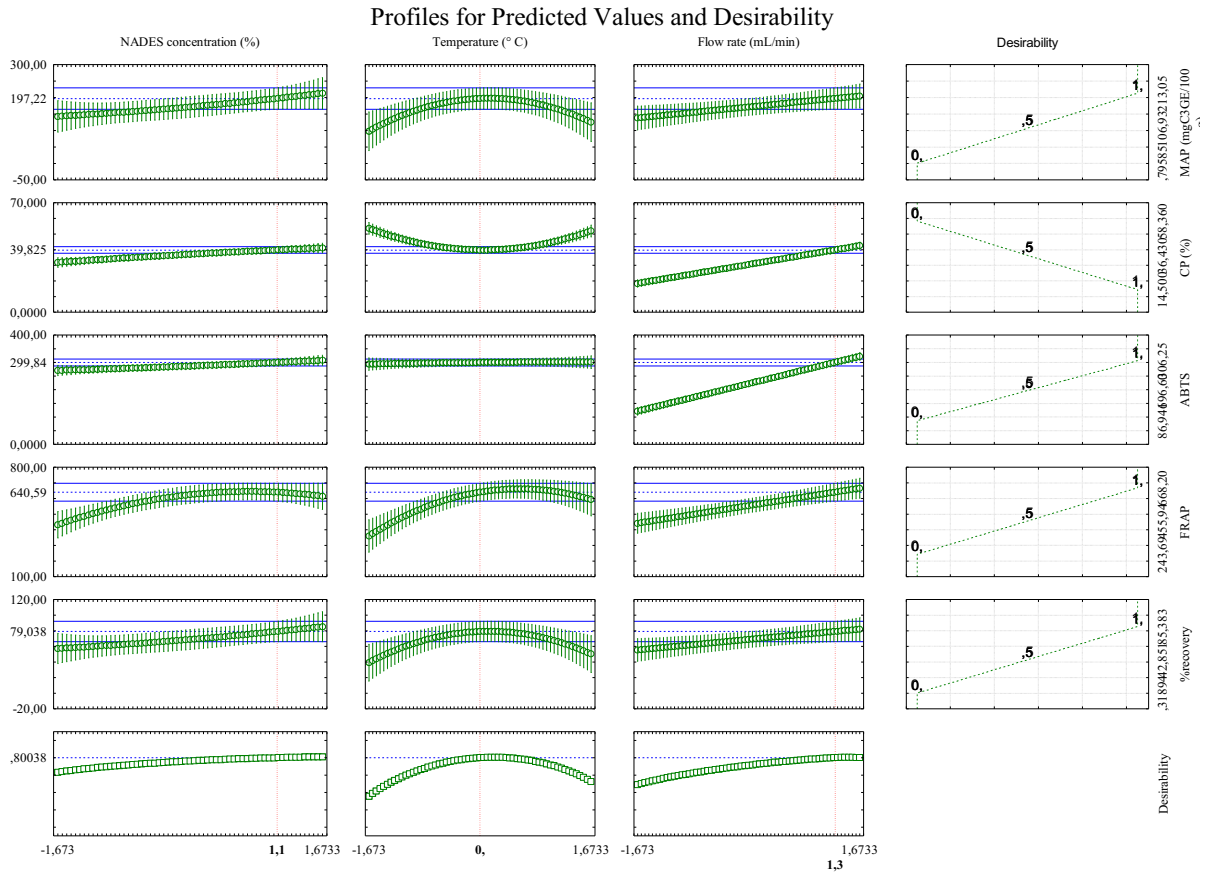


Figure 12 - Profiles for predicted values and desirability from anthocyanin-rich extraction models by PLE using aqueous solution of DES as the solvent.
Note: MAP – monomeric anthocyanin pigment, PC – polymeric color, ABTS and FRAP – antioxidant activity, PLE – pressurized liquid extraction, DES – deep eutectic solvent, ChCl – choline chloride, Pro – propylene glycol, C3GE – cyanidin-3-*O*-glucoside equivalent, TE – Trolox equivalent.

This optimization was externally validated by conducting a PLE assay at the optimized condition suggested by the desirability function (*d*). The observed and predicted values, with relative error (RE), for each variable, were:

(1) MAP (mgC3GE/100g dw): 172.70 ± 9.25 (observed), 183.33 (predicted), and RE = 5.80%;
(2) PC (%): 15.29 ± 0.10 (observed), 15.021 (predicted), and RE = 13,97%;
(3) AR (%): 69.08 ± 2.62 (observed), 73.98 (predicted) and RE = 9.61%;
(4) ABTS (mmol/g ds): 289.27 ± 7.84 (observed), 279.37 (predicted) and RE = 3.35%;
(5) FRAP (mmol/g ds): 685.99 ± 59.65 (observed), 640.59 (predicted), and RE = 6.94%.

All observed values are within the predicted interval of 95% confidence level. Therefore, the optimized conditions were validated, i.e., the proposed models can be used to predict the responses following the desirability function.

3.3.3 Solvent effect in the anthocyanin-rich extracts obtention by PLE method

Anthocyanin-rich extracts were obtained by PLE at optimum condition (section 3.2) using different solvents (water, acidified water, and ChCl:Pro and ChCl:Ma solutions). The solvent type affected the process yield and the quality of the extracts since significant differences ($p < 0.05$) were observed for TPC, MAP, antioxidant, and color parameters (**Table 11)**.

The extraction yield, presented in terms of TPC values, ranged from 72.97 to 85.68 mg GAE/g dw, with the highest value obtained by ChCl:Pro solution, followed by ChCl:Ma solution. Santos, Veggi & Meireles (2012) optimized the extraction of the phenolic from jaboticaba peel by PLE method, reaching a maximum TPC of 18.7 mg GAE/g dw using ethanol 99% as solvent at 120°C, 5 MPa for 15 min. However, Paludo et al. (2019) showed that TPC from jaboticaba varies according to cultivar and year of harvest, and for jaboticaba peel, the TPC ranged from 55.27 to 147.88 mg GAE/g dw for six different jaboticaba cultivars, harvested between 2014 and 2015.

Regarding the MAP content, the ChCl:Pro and ChCl:Ma solutions provided 57% and 42% higher values than obtained by water as the solvent, respectively. When compared to acidified water, the MAP values from DES aqueous solutions were 48% and 35% higher, respectively.

Table 11 - Comparison among different solvents used in the extraction.

Analysis	Solvents			
	Water (pH 6.7)	**Acidified water (pH 1.5)**	**ChCl:Pro solution (pH 4.5)**	**ChCl:Ma solution (pH 1.5)**
TPC (mg GAE/g dw)	$74.47^{b} \pm 7.15$	$72.97^{b} \pm 7.74$	$85.68^{a} \pm 8.29$	$78.99^{ab} \pm 2.59$
MAP (mgC3GE/g dw)	$1.10^{c} \pm 0.03$	$1.16^{c} \pm 0.01$	$1.70^{a} \pm 0.06$	$1.60^{b} \pm 0.09$
PC (%)	$13.75^{b} \pm 0.02$	$12.00^{c} \pm 0.25$	$15.29^{a} \pm 0.10$	$11.56^{d} \pm 0.27$
AR (%)	$44.06^{c} \pm 0.97$	$46.50^{c} \pm 0.41$	$69.08^{a} \pm 2.62$	$62.90^{b} \pm 3.85$
Antioxidant Activity				
ABTS (mmol TE/g dw)	$219.72^{b} \pm 2.87$	$169.05^{c} \pm 1.71$	$286.47^{a} \pm 3.06$	$288.16^{a} \pm 4.88$
FRAP (mmol TE/g dw)	$695.56^{a} \pm 22.62$	$517.12^{b} \pm 13.18$	$685.99^{a} \pm 59.65$	$727.98^{a} \pm 42.82$
Color				
L*	$78.33^{b} \pm 0.09$	$86.17^{a} \pm 0.08$	$59.67^{d} \pm 0.86$	$64.49^{c} \pm 0.07$
a*	$13.04^{c} \pm 0.14$	$9.12^{d} \pm 0.25$	$17.49^{b} \pm 0.35$	$32.49^{a} \pm 0.02$
b*	$10.63^{c} \pm 0.14$	$6.29^{d} \pm 0.07$	$19.88^{a} \pm 0.19$	$17.22^{b} \pm 0.01$
C*	$16.82^{c} \pm 0.20$	$11.08^{d} \pm 0.25$	$26.48^{b} \pm 0.37$	$36.77^{a} \pm 0.01$
°h	$39.20^{b} \pm 0.06$	$34.60^{c} \pm 0.46$	$48.65^{a} \pm 0.30$	$27.93^{d} \pm 0.03$

Note: C3GE – cyanidin-3-glucoside equivalent, dw – dry weight, PC – polymeric color, AR – anthocyanin recovery, ChCl: chlorine chloride, Pro: propylene glycol, Ma: malic acid.

The efficiency of pressurized DES aqueous solutions for the recovery of extracts rich in MAP and C3G, compared to control solvents, evidences the selectivity towards anthocyanin components. This valuable selectivity, previously evidenced by *in silico* and experimental studies, indicated ChCl:Pro and ChCl:Ma as promising eutectic solutions for anthocyanins recovery due to their high affinity with these natural colorants (BENVENUTTI et al., 2020).

The high extraction ability of DES can be explained by the hole or liquid crystal theory or the binding theory. The arrangement of HBA, HBD, and water, form a polymer-like matrix where the solute can dissolve into the space (or holes) of this molecular network. From the binding theory, significant intermolecular interactions, mostly hydrogen bonds among HBA, HBD, and target molecules make the solute part of the supramolecular structure of DES (BENVENUTTI; ZIELINSKI; FERREIRA, 2019; DAI et al., 2016; LIU et al., 2018). Despite

that water weakens molecular interactions, probably the HBA and HBD (from DES) are partly dissolved in water, while the remaining amount forms the supramolecular structure, as suggested by Liu et al. (2018). In general, polar compounds such as anthocyanin are better recovered by DES solutions, compared to pure DES, since water also forms hydrogen bonds with these target compounds (SHISHOV et al., 2021).

However, the extract recovered by ChCl:Pro solution presented the highest percentage of polymeric color (PC), of 15.29 ± 0.10 %, suggesting lower maintenance of the anthocyanin integrity, while the extract recovered by ChCl:Ma solution presented the lower PC value (11.56 ± 0.27 %), probably due to low pH (1.5) of the malic acid as HBD. According to Mejia et al., (2020), anthocyanins recovery and stability are favored in the acid medium due to the stabilization of favynium ion. The significant differences in the MAP and PC values, from samples recovered by ChCh:Ma and acidified water, both with pH 1.5, also suggest the influence of the acid chemical structure on anthocyanins recovery and stability.

High-pressure DES solutions recovered extracts with higher antioxidant activity compared to control solvents (**Table 11**). This behavior is probably associated with the DES selectivity towards bioactive compounds, as previously discussed. A high correlation between the two antioxidant methods, FRAP and ABTS was observed, with a correlation coefficient (r) of 0.81 ($p < 0.05$). However, only the antioxidant activity by ABTS assay presented correlation with TPC (r = 0.60, $p < 0.05$), MAP content (r = 0.87, $p < 0.05$) and C3G content (r = 0.83, $p < 0.05$). The antioxidant activity by ABTS presents a higher correlation with anthocyanin content (MAP and C3G), compared to TPC, because anthocyanins are the main phenolic compounds from the extracts, with high antioxidant ability. For instance, C3G presents eight hydroxyls groups, which are the main functional group related to antioxidant activity efficiency.

Besides, anthocyanins are more potent antioxidants than proanthocyanidins and other flavonoids due to their chemical structure (DE MEJIA et al., 2020).

Concerning the color attributes, which affect consumers' acceptance and selection (MARTINS et al., 2016), significant differences ($p < 0.05$) were detected for color parameters among the anthocyanin-rich extracts (**Table 11**). Extracts obtained by DES solutions, compared to controls, presented higher redness (a*), yellowness (b*), and Chroma values (C*), and lower luminosities (L*). According to Mojica, Berhow, & Gonzalez de Mejia (2017), higher anthocyanins concentration, from anthocyanin-rich samples from black beans, increases the C*, a parameter related to color intensity, and decreases the hue angle value (h°), related to color tone. Therefore, the lowest h°, and highest C* and a* (red color intensity) from the sample by ChCl:Ma is probably due to its high MAP content and low solvent pH, which maintains the conformation of anthocyanin color, predominantly flavyliun cation (DE MEJIA et al., 2020; GIUSTI; WROLSTAD, 2001; MOJICA; BERHOW; GONZALEZ DE MEJIA, 2017). Highest yellowness (b*) and lowest L*, from ChCl:Pro sample, can be related to high PC value, which indicates the anthocyanin degradation. The PC value involves the presence of colorless anthocyanins, according to pH, and the formation of melanoidin pigments at high process temperatures (JIANG et al., 2019).

3.3.4 Phenolics profile of extracts obtained by PLE

Table 12 presents the phenolics profile of the extracts obtained by PLE using aqueous solutions of DES (ChCl:Pro and ChCl:Ma), and compared to water or acidified water as solvents. In general, flavonoids are the main class of phenolics from jaboticaba extracts, and anthocyanin is the main compound, representing 57 to 86% of total phenolic compounds from the evaluated samples. Anthocyanin is one of the main phenolics present at jaboticaba that are

related to antioxidant, antimicrobial, anti-diabetic, anti-obesity, and effect against degenerative diseases and some types of cancer (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; LEITE et al., 2011). According to literature, the cyanidin-3-*O*-glucoside (C3G) is the main anthocyanin from jaboticaba peel (1261 to 1964 mg/100g dw), followed by delphinidin-3-*O*-glucoside (269-635.3 mg/100g dw) (INADA et al., 2015; LEITE et al., 2011). The results from **Table 12** shows that C3G was the only individual anthocyanin detected from the different extracts, with concentrations varying from 950.34 to 2940.05 mg/g dw. Inada et al. (2015) evaluated the phenolic composition of different jaboticaba parts, with 1261 mg/g dw of C3G detected for jaboticaba peel after sequential stirring extractions using methanol 50% and acetone:water:acetic acid (70:29.5:0.5, v/v/v), at ambient temperature.

Most of the other compounds are phenolic acids, with the highest concentration of hydroxycinnamic acids, mainly ellagic (9.28 to 26.25%) and gallic acid (1.39 to 16.32%). These results follow literature reports on the phenolic composition of jaboticaba, which appoint anthocyanin as the main phenolic compound and ellagic acid as the main phenolic acid (INADA et al., 2015; PALUDO et al., 2021). Additionally, hydroxybenzoic acids such as cinnamic, *p*-coumaric, and ferulic were also quantified. In general, phenolic acids are widely studied and applied due to their antioxidant properties and other health benefits (KUMAR; GOEL, 2019; RASHMI; NEGI, 2020). Ellagic and gallic acids can result from the hydrolyzation of the tannin components ellagitannins and gallotannis (LARROSA et al., 2010), which already were reported in jaboticaba extracts (BENVENUTTI; ZIELINSKI; FERREIRA, 2021). Both ellagic and gallic acids have been extensively investigated by *in vitro* and *in vivo* due to their antioxidant, anti-inflammatory, and anti-cancer potentials, besides hepatoprotective and

vascular health effects (GUPTA et al., 2021; KAUR et al., 2021; LARROSA et al., 2010; OJEABURU; ORIAKHI, 2021).

Table 12 - Phenolic profile of extracts obtained by pressurized liquid extraction (PLE) method using water, acidified water and the aqueous solution of DES, ChCl:Pro, and ChCl:Ma as the solvents.

Phenolic compound	Water (mg/100g dw)	Acidified water (mg/100g dw)	ChCl:Pro solution (mg/100g dw)	ChCl:Ma solution (mg/100g dw)
Phenolic acids				
Hydroxycinnamic acids				
Cinnamic acid	$4.29^{b} \pm 0.13$	$6.14^{a} \pm 0.65$	$5.85^{a} \pm 0.01$	$3.26^{c} \pm 0.01$
p-coumaric acid	$3.11^{b} \pm 0.24$	$3.87^{a} \pm 0.44$	$4.43^{a} \pm 0.33$	$1.80^{c} \pm 0.18$
Ferulic acid	$1.65^{b} \pm 0.02$	$1.86^{b} \pm 0.01$	$2.74^{a} \pm 0.28$	$0.72^{c} \pm 0.11$
Cafeic acid	$0.30^{b} \pm 0.02$	$0.55^{a} \pm 0.03$	$0.47^{a} \pm 0.04$	$0.07^{c} \pm 0.02$
Chlorogenic acid	$0.26^{b} \pm 0.02$	$0.33^{a} \pm 0.02$	$0.02^{c} \pm 0.01$	$0.03^{c} \pm 0.01$
Synapic acid	$0.12^{b} \pm 0.01$	< LOQ	$0.24^{a} \pm 0.01$	$0.05^{c} \pm 0.01$
Hydroxybenzoic acids				
Ellagic acid	$270.24^{a} \pm 80.28$	$346.38^{a} \pm 16.31$	$317.97^{a} \pm 32.30$	$276.88^{a} \pm 19.23$
Protocatechuic acid	$54.70^{b} \pm 3.34$	$79.71^{a} \pm 5.73$	$34.31^{c} \pm 2.05$	$37.34^{c} \pm 1.71$
Gallic acid	$273.23^{b} \pm 7.31$	$270.20^{a} \pm 11.69$	$169.27^{c} \pm 8.42$	$41.68^{d} \pm 1.32$
Syringic acid	$0.99^{b} \pm 0.04$	$1.22^{a} \pm 0.10$	$0.83^{c} \pm 0.05$	$0.59^{d} \pm 0.06$
Flavonoids				
Anthocyanins				
Cyanidin-3-*O*-glucoside	$950.34^{d} \pm 13.93$	$1389.16^{c} \pm 3.34$	$2940.05^{a} \pm 9.14$	$2578.02^{b} \pm 8.79$
Flavonols				
Myricetrin	$101.17^{ab} \pm 12.86$	$82.76^{b} \pm 10.91$	$122.85^{a} \pm 29.62$	$42.29^{c} \pm 5.5$
Isoquercetrin	$1.25^{a} \pm 0.12$	$1.47^{a} \pm 0.24$	$0.02^{b} \pm 0.01$	$0.02^{b} \pm 0.01$
Quercetin	$0.56^{b} \pm 0.06$	$2.12^{a} \pm 0.32$	$0.41^{b} \pm 0.01$	$0.55^{b} \pm 0.01$
Rutin	< LOQ	< LOQ	< LOQ	< LOQ
Flavanonol				
Taxifolin	$0.82^{b} \pm 0.18$	$1.24^{a} \pm 0.20$	$0.85^{b} \pm 0.36$	$0.07^{c} \pm 0.01$
Aromadentrin	$0.78^{c} \pm 0.04$	$1.41^{a} \pm 0.07$	$1.08^{b} \pm 0.18$	$0.03^{d} \pm 0.01$
Flavone				
Chrysin	$0.09^{a} \pm 0.01$	$0.10^{a} \pm 0.01$	$0.14^{b} \pm 0.02$	$0.13^{b} \pm 0.01$
Flavanones				
Naringerin	$0.18^{d} \pm 0.01$	$0.60^{a} \pm 0.02$	$0.32^{b} \pm 0.02$	$0.28^{c} \pm 0.01$

Note – dw: dry weight, ChCl: choline chloride, Pro: propylene glycol, Ma: malic acid, LOQ: limit of quantification, *abc*: different letters in the same line indicate significant differences among the samples by Fisher test, $p < 0.05$.

Furthermore, four flavonols were identified in the following concentration order: myricetrin>isoquercetrin>quercetin>rutin. Flavonols present high biological potential, with most pieces of evidence from preclinical and clinical studies about cardiovascular diseases

(CVD) and metabolic disorders (BARRECA et al., 2021). Compounds from other phenolic classes as flavanonols, flavone, flavanones also were quantified in lower quantities. All quantified compounds were already reported from jaboticaba samples (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; BETTA et al., 2018; WU; LONG; KENNELLY, 2013), except for taxifolin and aromadentrin. However, these two compounds were reported in other dark-colored fruits such as juçara (SCHULZ et al., 2020).

There were significant differences in composition ($p < 0.05$) among the extracts obtained by different solvents, indicating variation in selectivity. PLE using aqueous solutions of DES provided up to 3-folds higher C3G concentration, compared to conventional solvents (water and acidified water), and of 2.33-folds higher than values obtained by Inada (2015). The solvent selectivity also was evidenced in the recovery of phenolic acid and flavonols. In general, the acidified water and ChCl:Pro solutions resulted in the highest concentrations. Corroborating with our result, Wojeicchowiski et al. (2021) also reported that ChCl:Pro presents a high extraction ability of phenolic compounds. Besides, the same author evaluated a range of 1372 DES possibilities by COSMO-RS for antioxidant extraction from Rosemary and concluded that propyleneglycol as HBD in DES with 30% of water presented the best performance (WOJEICCHOWSKI et al., 2020). The high performance of acidified water can be related to acid hydrolysis during the extraction process since some phenolic compounds are covalently bonded to cell wall structural components such as cellulose, hemicellulose, lignin, pectin, and structural proteins. These bonded compounds can be recovered by acid or alkaline hydrolysis (INADA et al., 2015).

According to Inada et al. (2015), protocatechuic acid and trans-cinnamic acid are the main bonded phenolic compounds from jaboticaba, components such as ellagic acid and gallic

acid are found in free and bonded forms, while the glycosylated flavonoids (such as anthocyanin and some flavonols), and most of the phenolic acids are exclusively in free form, i.e. are facile recovered by solvents. These authors presented a concentration of ellagic acid facile extracted (free form) in jaboticaba peel extract of 83 mg/100 dw, 41 mg/100g dw after alkaline hydrolysis, and 120 mg/100g after acid hydrolysis, resulting in a final concentration of 244 mg/100 dw. Therefore, probably the high pressure and temperatures from PLE method (**Table 12**) were efficient to recover part of the bonded phenolic compounds. Furthermore, the high extraction ability of ChCl:Pro and acidic medium favor the recovery of bonded compounds.

3.3.5 Effect of type of solvent in the stability of anthocyanin obtained by PLE method

The stability of bioactive molecules from natural extracts is relevant to define its applications and storage conditions and aids the definition of the extractions conditions (DAI et al., 2016). Therefore, the influence of the solvent type (ChCl:Pro, ChCl:Ma, water, and acidified water) on the thermostability of anthocyanin recovered by PLE was investigated.

The thermostability of anthocyanin-rich extracts was represented by thermal degradation curves (**Figure 13**) and kinetics parameters (**Table 13**). The lowest anthocyanin degradation was observed at 60 °C (lower temperature) for the ChCl:Ma sample, followed by acidified water sample, solvents with pH close to 1.5. At pH close to 1, the flavylium cation is the only existent conformation.

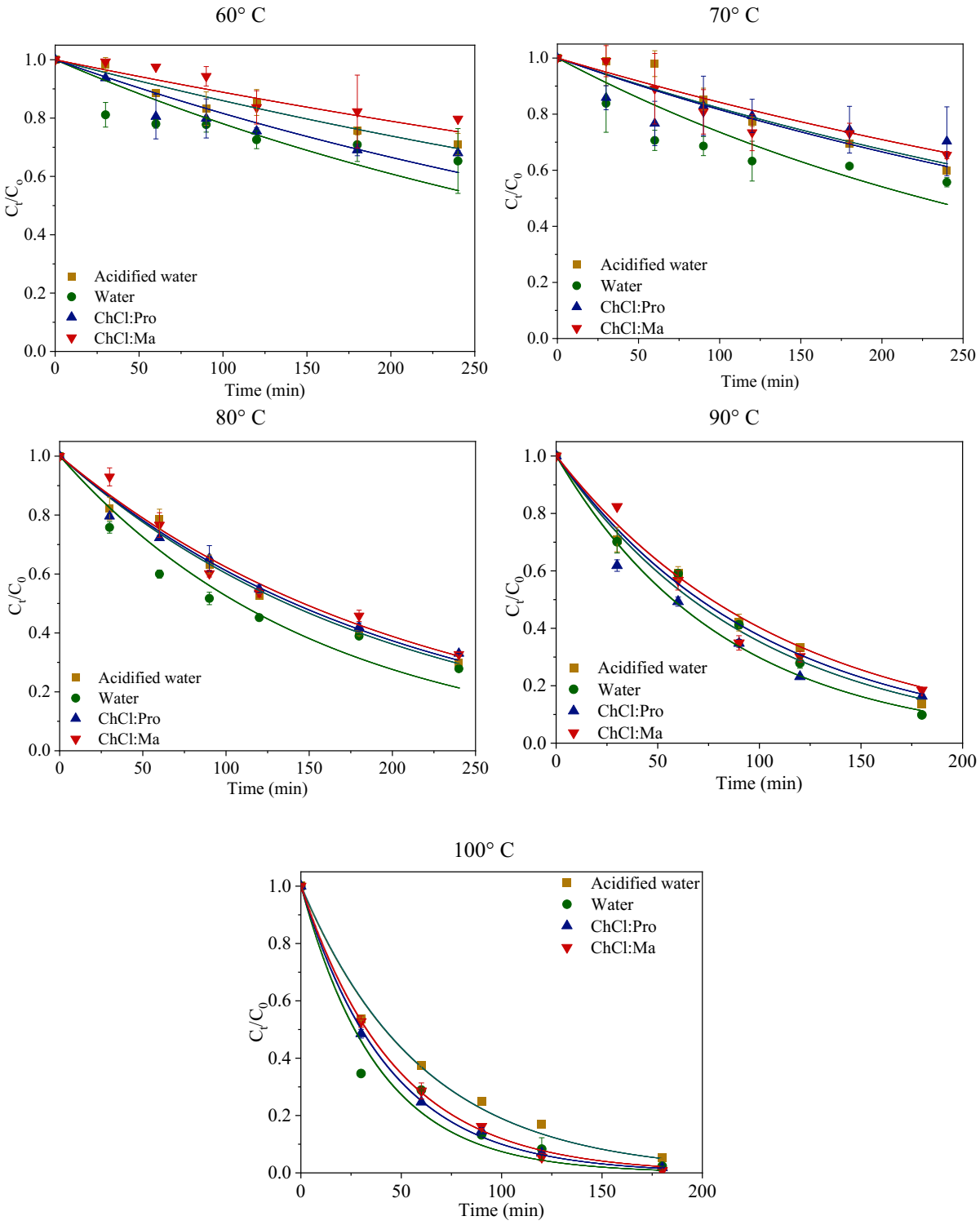


Figura 13 - Thermostability of anthocyanin-rich extracts in the different solvents evaluated (water, acidified water, and aqueous solution of DES: ChCl:Pro and ChCl:Ma) at temperatures of 60, 70, 80, 90, and 100 °C, where the lines correspond to the fit a first-order degradation model (Equation 11).
Note: ChCl: chorine chloride, Pro:propyne glycol, and Ma: malic acid.

However, with pH increase, different conformations coexist in complex chemical equilibrium, forming a color hemiketal stable form, which is related to the color fading of anthocyanins (GIUSTI; WROLSTAD, 2001). Besides, van der Waals and hydrogen bond molecular interactions between DES structure and anthocyanins improve their solubility, decreasing the molecule mobility, and the contact with oxygen at the air interface, reducing oxidative degradation, the main degradation mechanism at low temperatures (DAI et al., 2016).

At 80 and 90 °C the samples recovered by both DES solutions presented lower degradation rates (**Table 13**). The anthocyanins' thermal degradation occurs through the hydrolysis of glycosidic bond, forming aglycone (cyanidin), which is more unstable and discolors faster than the glucoside form. Another type of degradation is the opening of the heterocyclic ring, forming chalcone, the colorless structure of anthocyanin (GARCIA-MENDOZA et al., 2017). Therefore, the protective effect of DES solutions at these temperatures can also be related to the molecular interactions, which reduce the mobility and protect the anthocyanin from the nucleophilic attack of water molecules, decreasing its susceptibility to degradation reactions (DAI et al., 2016). The strength of these molecular interactions is mainly related to the number of hydroxyls and carboxyls groups, besides the size and spatial structure of the molecules (BENVENUTTI; ZIELINSKI; FERREIRA, 2019). Therefore, the better protective effect of ChCl:Ma compared to ChCl:Pro could be explained by the malic acid structure, with one hydroxyl and two carboxyl groups, resulting in more interactions with anthocyanins than propylene glycol (two hydroxyl groups). Besides, the acylation of the anthocyanin glycosyl by malic acid may have occurred at the extract recovered by ChCl:Ma because the -OH groups of the anthocyanin glycosyls are partially or fully esterified by organic acids. The acylated anthocyanin has higher color stability and higher

resistance to physicochemical and biochemical factors (e.g. digestive enzymes, light, heat, pH, among others) than the nonacylated form (ZHAO et al., 2017).

Table 13 - Parameters of kinetics thermal degradation for anthocyanin-rich extracts, recovered using different solvents (water, acidified water (pH 2) and NADES ChCl:Pro and ChCl:Ma), provided by the fit of the first-order model (equation 11).

	k(min^{-1})	**RSS**	**R^2**	**R^2_{adj}**	**$t_{1/2}$ (h)**	**D (h)**
60 °C						
Water	0.0025	0.0686	0.7746	0.7746	4.6582	15.4744
Acidified water	0.0015	0.0261	0.8829	0.8829	7.6506	25.4148
ChCl:Pro	0.0020	0.0603	0.7859	0.7859	5.6630	18.8120
ChCl:Ma	0.0012	0.0525	0.7624	0.7624	9.7902	32.5224
70 °C						
Water	0.0031	0.0932	0.7953	0.7953	3.7630	12.5005
Acidified water	0.0020	0.0393	0.9087	0.9087	5.8642	19.4804
ChCl:Pro	0.0020	0.0451	0.8257	0.8257	5.6630	18.8120
ChCl:Ma	0.0017	0.0565	0.8594	0.8594	6.7165	22.3119
80 °C						
Water	0.0064	0.0196	0.98258	0.98258	1.7939	5.9591
Acidified water	0.0051	0.07031	0.93422	0.93422	2.2741	7.5544
ChCl:Pro	0.0049	0.02183	0.97693	0.97693	2.3433	7.7843
ChCl:Ma	0.0047	0.03281	0.97062	0.97062	2.4424	8.1134
90 °C						
Water	0.0121	0.0305	0.9784	0.9784	1.1097	3.1690
Acidified water	0.0104	0.0292	0.9812	0.9812	0.9540	3.6865
ChCl:Pro	0.0098	0.0366	0.9763	0.9763	1.1764	3.9080
ChCl:Ma	0.0091	0.0354	0.9737	0.9737	1.2695	4.2172
100 °C						
Water	0.0260	0.0682	0.9647	0.9647	0.4440	1.4749
Acidified water	0.0166	0.0210	0.9878	0.9878	0.6943	2.3063
ChCl:Pro	0.0231	0.0030	0.9986	0.9986	0.5012	1.6649
ChCl:Ma	0.0212	0.0057	0.9973	0.9973	0.5439	1.8068

Note: k: rate constant RSS: residual sum of squares. R^2: determination coefficient. R^2adj: adjusted determination coefficient. $t_{1/2}$: time of half-live.

At 100 °C, the extract recovered by acidified water had the lowest degradation rate (k_d =0.0166 min^{-1}, $t_{1/2}$ = 0.69 h). The degradation rate of anthocyanins increased with temperature for all solvents because high temperatures favor the hydrolysis reactions, converting chalcones (colorless structure of anthocyanin) into brown precipitates, commonly

observed as the final product of anthocyanin degradation (Garcia-Mendoza et al., 2017). From 90 to 100 °C the increase in degradation rates was higher for the extracts recovered by DES solution, probably due to the extensive hydrogen bonds network among DES and anthocyanin molecules, favoring the formation of brown precipitates involving chalcones.

To evaluate the temperature dependence of anthocyanin degradation, the ln k_d values against 1/T were fit by the Arrhenius equation, predicting the activation energy (E_a) with good adust ($R^2 > 0.92$ and $R^2_{adj} > 0.90$) (**Table 14**). E_a is a thermodynamic parameter representing the energy needed to reach the transition state of the chemical reactions. Therefore, high E_a represents low susceptibility to degradation. As expected, ChCl:Ma presents a higher E_a, followed by acidified water, ChCl:Pro, and water.

Table 14 - Parameters of Arrhenius adjustment.

	E_a (kJ mol^{-1})	RSS	R^2	R^2_{adj}
Water	62.4420	0.13578	0.96414	0.95218
Acidified water	66.7102	0.11237	0.97374	0.96498
ChCl:Pro	65.0670	0.31771	0.92579	0.90105
ChCl:Ma	77.5523	0.10446	0.98178	0.97571

E_a = energy of activation. RSS: residual sum of squares. R^2: determination coefficient. R^2_{adj}: adjusted determination coefficient.

Activation enthalpy (ΔH), free energy of activation (ΔG), and activation entropy (ΔS) were also determined (**Table 15**). The ΔH is calculated from the E_a and measures the energy barrier that can be overcome by reactant molecules, representing the strength of the bond that must be broken and remade during the reaction. Positive ΔH values indicate endothermic reaction, then, an increase in temperature enhances degradation. Additionally, positive ΔG values indicate that the degradation reaction is not spontaneous, besides, close ΔG values from different solvents are explained by the anthocyanins from the same source, with similar degradation reactions (PERON; FRAGA; ANTELO, 2017).

Table 15 - Thermodynamic parameters obtained by the equations 14 to 16 for anthocyanin degradation of jaboticaba among 60 and 100 °C in different solvents, water, acidified water, and NADES ChCl:Pro and ChCl:Ma.

T (°C)	ΔH (kJ mol^{-1})	ΔG (Jmol^{-1}K^{-1})	ΔS (Jmol^{-1})
Water			
60	59.6720	86.9727	-81.9471
70	59.5889	89.0588	-85.8806
80	59.5057	89.5631	-85.1123
90	59.4226	90.2768	-84.9628
100	59.3394	90.4741	-83.4374
Acidified water			
60	63.9415	88.3060	-73.1668
70	63.8583	90.2838	-77.0423
80	63.7752	90.2201	-74.9148
90	63.6920	90.6948	-74.3879
100	63.6089	91.8229	-75.6409
ChCl:Pro			
60	62.2983	87.4731	-75.5999
70	62.2152	90.1843	-81.5426
80	62.1320	90.3081	-79.8188
90	62.0489	91.1007	-80.0327
100	61.9657	90.8124	-77.3368
ChCl:Ma			
60	74.7835	88.9888	-42.6583
70	74.7004	90.6709	-46.5611
80	74.6173	90.4296	-44.7942
90	74.5341	90.8709	-45.0050
100	74.4510	91.0660	-44.5443

Note: ΔH - activation enthalpy, ΔG - free energy of activation, and ΔS - activation entropy.

Finally, negative ΔS values represent that the transition molecules are more organized than that from the initial reaction. Higher negative ΔS values indicate higher distance from the equilibrium condition, resulting in a quick reaction to obtain the active complex (PERON; FRAGA; ANTELO, 2017). Therefore, these thermodynamic parameters indicate ChCl:Ma solution as the best solvent to slow the thermal degradation reaction for anthocyanin from JP samples.

3.3.6 Anti-glycemic and anti-lipolytic potentials of anthocyanin-rich extracts obtained by PLE with different solvents

The PLE extracts were semi-purified using Amberlite XAD-7HD resin to concentrate the C3G, the main anthocyanin from this study due to its bioactive and colorant potential. The crude extracts (before semi-purification) and the semi-purified extracts, obtained by PLE with different solvents, were evaluated for inhibition activity of the enzymes α-amylase, α-glucosidase, and pancreatic lipase (PL).

The α-amylase catalyzes the starch hydrolysis during human digestion while α-glucosidase catalyzes the hydrolysis of disaccharides and monosaccharides. Therefore, the inhibition of these enzymes maintains the glucose levels by reducing the rate of blood sugar absorption from the small intestine, decreasing the spread and progression of type 2 *diabetes mellitus* (T2DM) (BARIK et al., 2020; TEIXEIRA et al., 2021). The results from enzyme inhibition are presented in **Table 16**, which shows that all anthocyanin-rich extracts provided higher inhibition of α-glucosidase, compared to α-amylase. The α-glucosidase inhibition from semi-purified extracts (IC_{50} from 7.07 to 4.12 μg/mL) were better than the reported inhibition of crude bilberry extract, IC_{50}=13.2 μg/mL (ALNAJJAR et al., 2020); of semi-purified black bean extract, IC_{50}=33 μg/mL (TEIXEIRA et al., 2021), and also of the commercial inhibitor acarbose (1mg/mL inhibit 47-59% of the α-glucosidase activity) (ALNAJJAR et al., 2020; BARIK et al., 2020). However, the α-amylase inhibition of the semi-purified extracts (IC_{50} from 1.62 to 7.44 mg/mL) were less efficient than purified black bean extract, IC_{50}=0.521 mg/mL (TEIXEIRA et al., 2021), and the acarbose (0.645 mg/mL inhibit 66.8% of α-amylase activity) (MOJICA; BERHOW; GONZALEZ DE MEJIA, 2017).

Table 16 - Inhibitory activity of digestive enzymes of raw and partly purified anthocyanin-rich extracts.

Assays	Partially purified extracts				Raw extracts			
	Water	**Acidified water**	**ChCl: Pro**	**ChCl:Ma**	**Water**	**Acidified water**	**ChCl:Pro**	**ChCl:Ma**
α-Amylase (IC_{50} - mg/mL)	$7.13^{a} \pm 0.09$	$7.44^{a} \pm 0.18$	$6.36^{b} \pm 0.88$	$1.62^{c} \pm 0.39$	-	-	-	-
α-Glucosidase (IC_{50} - µg/mL)	$7.07^{a} \pm 0.69$	$6.78^{a} \pm 0.09$	$5.34^{b} \pm 0.085$	$4.12^{c} \pm 0.18$	-	-	-	-
Lipase (IC_{50} - µg/mL)	$614.29^{a} \pm 14.78$	$499.04^{b} \pm 43.80$	$171.88^{c} \pm 7.20$	$173.81^{c} \pm 23.41$	-	-	-	-
α-Amylase (IC_{50} - µg C3GE/mL)	$86.31^{b} \pm 1.11$	$92.06^{b} \pm 2.21$	$113.93^{a} \pm 15.44$	$23.7^{c} \pm 5.68$	$9.42^{de} \pm 0.08$	$11.92^{d} \pm 0.03$	$8.41^{de} \pm 0.25$	$2.51^{e} \pm 0.32$
α-Glucosidase (IC_{50} - µg C3GE/mL)	$0.08^{b} \pm 0.01$	$0.08^{b} \pm 0.01$	$0.10^{a} \pm 0.01$	$0.06^{c} \pm 0.01$	$0.04^{b} \pm 0.01$	$0.08^{d} \pm 0.01$	$0.02^{e} \pm 0.01$	$0.01^{f} \pm 0.01$
Lipase (IC_{50} - µg C3GE/mL)	$11.16^{a} \pm 0.27$	$6.17^{b} \pm 0.54$	$2.08^{d} \pm 0.08$	$2.54^{c} \pm 0.34$	$2.73^{c} \pm 0.23$	$0.85^{e} \pm 0.15$	$0.36^{f} \pm 0.01$	$0.12^{f} \pm 0.01$

Note - C3GE cyanidin 3-O-glucoside, ^{abc}: different letters in the same line indicate significant differences, $p < 0.05$.

According to Barik et al. (BARIK et al., 2020), anthocyanins are most effective to inhibit α-glucosidase than other polyphenols. This high effectivity probably is related to the anthocyanin molecular structure of flavan-3-ols, mainly of cyanidin, that presents high inhibitor potential due to the one bond in the C ring and two hydroxyl substitutions in positions 2 and 3 of the B-ring. Besides, Sui, Zhang, & Zhou (2016) reported that inhibition of α-amylase activity increased when cyanidin bounds to glucose in position 3, such as C3G. These molecular structure favors the anthocyanin occupation of the active site of α-amylase and α-glucosidase, inhibiting their activities and also, inhibiting the catalytic action of these enzymes by hydrogen bonding.

DES solutions provided PLE extracts with the best inhibitory effects, compared to other solvents, probably due to the higher anthocyanin levels due to their higher solubility in these solvents. DES solutions also improve the bioavailability of the bioactive components (RADOŠEVIĆ et al., 2016). ChCl:Ma solution provided the highest inhibitor effects for these two enzymes, probably due to the higher anthocyanin percentual (86%, **Table 12**) and integrity, expressed by lower PC value (**Table 11**). In addition, the possible anthocyanin glycosyl acylation by malic acid can increase the *in vitro* and *in vivo* chemical stability, including stronger inhibition of digestive enzymes (ZHAO et al., 2017).

Despite the high inhibitory effect of anthocyanin, their combination with other phenolic compounds from crude extracts resulted in higher inhibition compared to purified extracts, for all solvents. Flavonols, specially myricetin and quercetin, and hydrolyzable tannins are polyphenols present in jaboticaba peel which have been reported as inhibitory to carbohydrate-hydrolyzing enzymes (LACROIX; LI-CHAN, 2014).

On the other hand, the inhibition of the pancreatic lipase (PL) activity is reported as a beneficial health effect associated with anthocyanins consumption, since it decreases the absorption of fat from the diet, and attenuates cases of obesity (XIE et al., 2018). The IC_{50} values of semi-purified extracts, presented at **Table 16** (from 171.88 to 614.29 μg/mL) are in accordance with that from pure cyanidin-3-*O*-glucoside (IC_{50} = 385 μg/mL), but less efficient than Oristatic® (IC_{50} = 64 μg/mL), a potent specific lipase inhibitor (FABRONI et al., 2016). The semi-purified extracts from DES solutions presented higher PL inhibition probably due to the higher anthocyanin content, which agrees with the positive correlation between anthocyanin content and enzymatic inhibition capacity, reported in the literature (FABRONI et al., 2016). The galloyl moiety, a common structure from flavonoids, including anthocyanins, can be associated with PL inhibition capacity, probably due to a bond between the structure with the PL active site (RAHIM; TAKAHASHI; YAMAKI, 2015). However, as observed for carbohydrases, the PL inhibition capacity was higher from crude extracts compared to the semi-purified ones due to the synergic effect with other polyphenols (FABRONI et al., 2016).

Therefore, the DES solutions combined with high-pressure technology showed is a promising approach to obtain anthocyanin-rich extracts with possible biological potential. The ChCl:Ma was the more suitable solvent due to their protective effect on anthocyanins' stability, preserving their color and bioactivity.

The extraction waste from the anthocyanin obtention was submitted to sequential extractions to value this by-product (**Figure 9**). Therefore, this step of anthocyanin recovery does not generate waste from the vegetal matrix. The proposed approach can be considered a green extraction method according to Chemat et al. (2019) since it uses renewable and low

explored food crops, applies eco-friendly solvents at a high-pressure method that improves yield, reduces processing time, solvent consumption, and unit operations.

3.4. CONCLUSIONS

The optimum PLE conditions using aqueous solutions of DES as solvents were established for the valorization of a Brazilian berry by-product. The best processing conditions were 47% of aqueous solution at 90 °C and a flow rate of 5.3 mL/min. Cyanidin was the main phenolic compound present in the extracts, and ellagic acid was the main phenolic acid. The ChCl:Ma solution was the most promising DES for anthocyanin recovery from jaboticaba peel, and also presented a high protective effect against the thermodegradation of the recovered extract, which showed good bioactivities, especially high anti-glycemic potential. This protective effect is mainly related to the low pH value of the DES solution, the molecular interactions, and possible anthocyanin glycosyl acylation by malic acid. Therefore, ChCl:Ma aqueous solution coupled with PLE method is an efficient and eco-friendly approach for the recovery of anthocyanin from an underutilized Brazilian berry by-product. PLE using DES solutions as the solvent result in high yields of a natural colorant with bioactive properties and high potential application in food, nutraceutical, and pharmaceutical formulations.

CHAPTER 4

Subcritical Water Extraction (SWE) modified by Deep Eutectic Solvent (DES) for pectin fractions recovery from the jaboticaba by-product

ABSTRACT

Pectin, a soluble fiber with functional, nutritive, and technological functions, is commonly obtained from the citrus peel or apple pomace. The aim of this chapter is the recovery of pectin from jaboticaba processing by-product, as an alternative matrix, using subcritical water extraction (SWE) modified by DES. The extractions were performed following a Box-Behnken Factorial Design, using temperatures of 100, 125, and 150 °C, DES solution concentrations of 2, 6 and 10%, and flow rate of 2, 5, and 8 mL/min, as dependent variables, with the pectin yield (%) as the response variable. The effect of process variables on pectin fractions recovery was evaluated by multi regression analysis coupled to response surface methodology (RSM) to optimize the process conditions. The model suggested best performance is obtained at 122 °C, 8% of DES, and a flow rate of 2 mL/min. After optimizing the extraction conditions, the pectin quality - in terms of the degree of esterification (DE), total carbohydrate content (TCC), galacturonic acid (GalA) content, total phenolic content (TPC), antioxidant activity by ABTS, FRAP, and DPPH methods and functional properties as water and oil holding capacities (WHC and OHC) and emulsion activity (EA) - were evaluated and compared to with citric acid as SWE modifier (pH 2) and SWE without modifier at the same process conditions. Besides, the low-pressure method, heat-stirred extraction (HSE), was performed as a control. In terms of yield and GalA content, SWE was more efficient to pectin fractions recovery, with 1.5 to 1.8-fold higher values than HSE. In general, the pectins fractions obtained from jaboticaba pomace are high methoxyl (HM) and presented oil holding capacity (OHC) and emulsion activity (EA), showing potential use as stabilizers in fat formulations and emulsifiers. Compared to the control solvents and methods, SWE-DES extraction results in high GalA content and antioxidant capacity, and low DE. Therefore, the suggested approach was efficient to recover pectin fractions from the jaboticaba industrial by-product. The obtained pectin-rich extract presents application potential in food formulations due to their bioactivities and functionalities.

Keywords: Green chemistry; biorefinery; sequential extraction; jaboticaba processing by-product; heteropolysaccharide; bioactive properties.

4.1. INTRODUCTION

Pectin is a soluble fiber found between the cell wall and the middle lamella of plant cells, and present functional, nutritive, and technological properties (NAQASH et al., 2017). Chemically, pectin is a heteropolysaccharide with D-galacturonic acids (D-GalA) linked by α-1→4 galacturonosyl bonds as the main chain, which is partially esterified by methyl pr acetyl ester groups. The pectins are classified by the quantity of methyl-esterification defines the degree of esterification (DE or DM), classifying in low methoxyl pectin (LM) or high methoxyl pectin (HM) (ADETUNJI et al., 2017). Concerning their structures, the pectin is a complex heterogeneous polysaccharide presenting mainly these three domains: *homogalacturonans (HG)*, presenting a linear chain of D-GalA; *rhamnogalacturonan-I (RG-I)* which had a highly ramified structure with L-rhamnose (Rha) and α-D-GalA residues and *substituted galacturonan (SG or RG-II)* that had an HG backbone of approximately nine D-GalA residues substituted by clusters of different hetero-oligomeric side chains (CUI et al., 2021).

The main sources of pectin explored by the industries are citrus peel and apple pomace (ADETUNJI et al., 2017; CUI et al., 2021), although, matrixes such as sugar beet pulp (ABOU-ELSEOUD; HASSAN; HASSAN, 2021) and sunflower seed (SAHARI; AKBARIAN M; HAMEDI, 2003) have also been considered. Nevertheless, new alternative sources such as by-products from grapefruit (CUI et al., 2020), banana (OLIVEIRA et al., 2016), mango (MUGWAGWA; CHIMPHANGO, 2019), among other food residues have been evaluated due to energetic and economic demands. In this context, the jaboticaba processing by-product can be considered as a new alternative source of pectin. The processing by-product of this Brazilian berry is the non-edible part of the fruit, mostly consumed as fresh fruit, but also industrially

processed, generating a by-product that can represent 40% of the whole jaboticaba fruit (GURAK et al., 2014).

For the pectin recovery from the vegetal matrix, besides solubilizing the heteropolymer, the extraction solvent must be able to hydrolyze the protopectin (the polysaccharides bounded to the cell wall) and cellulose (ADETUNJI et al., 2017). Therefore, hot acid wash, using acetic, citric, lactic, hydrochloric, nitric, phosphoric, sulphuric acids, or other mineral and organic acids, are the conventional solvents applied for pectin extraction (KUMAR et al., 2020).

Currently, one of the main industrial challenges is to protect the environment and consumers, which increases the search for green extraction processes, able to reduce or eliminate energy consumption and the use of fossil-based solvents, and ensure the quality and safety of the extract (CHEMAT et al., 2019). The subcritical water extraction (SWE), a high-pressure process, is among the emergent extraction technologies applied for pectin recovery, which consists of a fast method with low energy and solvent consumption (ADETUNJI et al., 2017; KUMAR et al., 2020). SWE, also denominated superheated water extraction (SHWE), or pressurized hot-water extraction (PHWE), uses water in a subcritical state as the solvent, with temperatures between 100 and 374 °C and pressure that maintains the liquid form, mostly between 5 and 10 MPa (GALLEGO; BUENO; HERRERO, 2019; HERRERO et al., 2013; ZIELINSKI et al., 2021). The physical and chemical properties of water are largely changed with temperatures variation, which turns the water into a viable and interesting solvent. At subcritical conditions, the dielectric constant of water decreases drastically, which decreases their polarity, making the water solvation ability close to organic solvents such as ethanol and methanol (PLAZA; TURNER, 2015; RODRIGUES et al., 2019; ZIELINSKI et al., 2021).

The extraction ability and selectivity of SWE can be improved by the addition of co-solvents or modifiers, changing water polarity. In order to contribute to the greener attributes of the SWE, alternative solvents such as some ionic liquids (IL) and deep eutectic solvents (DES) have been used (ESSIEN; YOUNG; BAROUTIAN, 2020) due to its high capacity to extract bioactive compounds from different agri-food by-products (BENVENUTTI; ZIELINSKI; FERREIRA, 2019; GULLÓN et al., 2020). DES are binary or ternary mixtures of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA), forming intermolecular hydrogen bonds (BENVENUTTI; ZIELINSKI; FERREIRA, 2019; DAI et al., 2016; GULLÓN et al., 2020). This structure is responsible for the reported efficiency of DES in the extraction process, including the pectin recovery (BENVENUTTI et al., 2020; SHAFIE; YUSOF; GAN, 2019). Generally, DES are non-toxic, have no adverse effects, and have been considered compatible for food and pharmaceutical applications (BENVENUTTI; ZIELINSKI; FERREIRA, 2019)

Thereby, this chapter aims to verify the use of DES as an SWE modifier to an innovative green approach for pectin recovery from jaboticaba processing by-product in terms of pectin fractions yield and quality.

4.2. MATERIAL AND METHODS

4.2.1 Material

Myrciaria cauliflora processing by-product (10 kg) was acquired from *Sítio do Bello* (Piraibuna, SP, Brazil). Anhydrous glucose and citric acid P.A were purchased from Êxodo Científica (Sumaré, SP). The reagents TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), ABTS (2,2'-azino

bis-3-ethylbenzothiazoline-6-sulfonic acid) DPPH (2,2-Diphenyl-1-picrylhydrazyl), and *m*-hydroxydifenyl as well the standards gallic acid, galacturonic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) were acquired from Sigma Aldrich (Steinheim, Germany). Other solvents such as 99.5% ethanol, 99.5% acetone, and sodium hydroxide >98% were purchased from Êxodo Científica (Sumaré, SP, Brazil). Distilled water (Milli-Q® Direct 8, Merck, Darmstadt, Germany) was used in the preparation of s solutions.

4.2.2 Preparation of the DES solutions

The Deep Eutectic Solvent (DES) was prepared according to section 2.2.2 (Chapter 2) using citric acid as HBA and glucose and water as HBD (Ca:Glu:Wa). The homogenous liquid obtained was used as a modifier for subcritical water in concentrations from 2 to 10% (w/w).

4.2.3 Pectin fractions extraction

The pectin fractions extraction was performed using the residual jaboticaba by-product obtained after the recovery of the anthocyanin-rich fraction (Chapter 3), i.e., after the PLE method. The SWE was performed in a self-assembled unit presented at **Figure 7** (Chapter 3), using the conditions for subcritical water extraction (SWE). The assays were conducted at continuous mode with pressure fixed at 10 MPa. The processing time was determined by a kinetics study performed at 125 °C, 6% of DES as water modifier, and flow rate of 5 mL/min based on pectin yield (%). The extraction kinetics was fitted by three straight lines using a Statistica v.13.5 software (TIBCO Software Inc., Palo Alto, CA, USA) (**Figure 13**).

The effect of process variables of temperature (100, 125, and 150 °C), DES concentration (2, 6, and 10%), and flow rate (2, 5, and 8 mL/min), on pectin yield, was evaluated by 15 assays performed following a Box-Behnken factorial design (**Table 17**). The pectin from

the extract samples was precipitated with the addition of twice the extract volume of ethanol 96% and maintained at 4 °C overnight, forming a gel. The precipitated pectin was washed with ethanol: water solution (gel-solvent ratio 1:1 w/w) using gradient concentrations (60% twice, 70% twice, 80% until the filtrated sample became colorless, and 96% twice) (YAPO; WATHELET; PAQUOT, 2007). Sequentially, the gel was filtered through a muslin filter and dried in an oven with air circulation at 45 °C for 18h (Lucadema, Model 82/27, SP, Brazil). The pectin yield (%) was obtained by gravimetry, calculated by Equation (23):

$$Yield\ (\%) = \frac{pectin\ fractions\ weight\ (g)}{initial\ sample\ weight\ (g)} \times 100 \quad (23)$$

3.2.4 Optimization Process

The effect of independent variables, called x_i, DES (Ca:Glu:Wa) concentration (%, x_1), temperature (°C, x_2), and flow rate (mL/min, x_3), were analyzed by response surface methodology (RSM) tool, associated with multilinear regression analysis. A second-order polynomial equation was utilized to fit the experimental data, according to Equation (4), cited at section 3.2.6 (Chapter 3).

The mathematical model resulting from the experimental data fit was used to select the process variable conditions that maximize the pectin recovery, defined from the responses surface and contour plots. Then, external validation was performed to verify the models' adequacy, comparing the predicted values to the experimental data, conducted at the suggested optimal conditions. The statistical analyses were performed using Statistica v.13.5 Software (TIBCO Software Inc., Palo Alto, CA, USA).

4.2.5 Effect of DES as SWE modifer

The effect of DES as an SWE modifier on pectin yield and composition was compared to citric acid as a modifier, and SWE without a modifier. The assays were conducted at optimal conditions, defined according to section 2.5 (chapter 2). The recovered pectin fractions were precipitated and isolated from the aqueous extract following the procedure from section 4.2.3.

4.2.6 Comparison to a conventional low-pressure extraction method

The SWE performance was compared to the conventional extraction method, heat-stirred extraction (HSE). This extraction (HSE) was performed in a heated water bath with intermittent shaking (Ethik technology, 304 TPA model, SP, Brazil) at 80 °C, a solid-to-solvent ratio of 1:30 (g/mL), for 2.5h (BENVENUTTI et al., 2020). The HSE solvents were: pure water, 8% of DES aqueous solution, and 8% citric acid aqueous solution. The recovered pectin fractions were precipitated and isolated from the aqueous extract according described in section 4.2.3.

4.2.7 Characterization of the pectins obtained

4.2.7.1 Fourier transform infrared (FTIR) analysis

FTIR spectroscopy (Agilent Technologies, model CARY 660, CA, USA) was used to identify the functional groups from the pectin fractions and determine the degree of esterification (DE) as described by Liew et al. (2018). The FTIR spectra were recorded from 400 to 4000 cm^{-1} and the DE was obtained by the ratio between methyl-esterified carboxyl groups and the sum of carboxyl groups, according to Equation (24):

$$DE\ (\%) = \frac{A_1}{A_1 + A_2} \cdot 100 \qquad (24)$$

where A_1 is the area of peak in 1745 cm^{-1} and A_2 is the area of peak in 1630 cm^{-1}, corresponding to methyl-esterified groups and non-methyl-esterified groups, respectively.

4.2.7.2 Total carbohydrate content (TCC)

The total carbohydrates present in the pectin fractions were determined by the phenol-sulfuric method (DUBOIS et al., 1956) with adaptation for the microplate reader. An aliquot of 100 μL of pectin solution (0.1-0.2 mg/L), 300 μL de of concentrated sulfuric acid and 80 μL of 5% phenol in water were placed in vials tubes, which are incubated at 90 °C for 5 min. After this period, 300 mL of this mixture was placed in 96-well microplates and read at 490 nm. The total carbohydrate content was expressed in percentage (%) calculated through a standard curve of glucose (0.02-0.2 mg/mL) ($TCC = 0.27 \cdot x_{absorbance}$, $R^2 = 0.98$).

4.2.7.3 Galacturonic acid (GalA) content

The galacturonic acid (GalA) content was determined by the colorimetric *m*-hydroxyphenyl method, according to Blumenkrantz and Asboe-Hansen (1973). Aliquots of 200 μL of pectin aqueous solution (0.1-0.2 mg/mL) and 6 mL of sodium tetraborate 0.0125 M in concentrate sulfuric acid were placed in assay tubes and heated in a thermostatic water bath (Unique, USC-4880, Sao Paulo, Brazil) at 95 °C for 5 min and, immediately cooled in an ice bath. Sequentially, 20 μL of *m*-hydroxyphenyl 0.15% (w/v) in NaOH 0.5% was added and completely mixed. Finally, the absorbance was measured at 520nm in UV-VIS spectrophotometer (Femto 800 XI, São Paulo/SP - Brasil). The GalA content was obtained through a calibration curve of mono-hydrate galacturonic acid ($GalA = 500 \cdot x_{absorbance}$) and expressed in miligrams of GalA equivament per 100 miligrams of sample (mgGalAE/100mg).

4.2.7.4 Antioxidant activity

The antioxidant potential was determined by the capture of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging capacities, and also by ferric antioxidant power (FRAP) according to Brand-Williams; Cuvelier; Berset (1995), Re et al. (1999), and Benzie & Strain (1996) methods, respectively, with adaptations for microplate reader. For the DPPH and ABTS methods, the reduction percentage of radical colors (%R) was calculated according to Equation (25). The results were expressed in micro mols of Trolox Equivalent (TE) antioxidant capacity per g in dry weight (μmolTE/g dw), which were calculated through the calibration curves (DPPH = $7.21 \cdot \%R$; ABTS $= 4.16 \cdot \%R$ and FRAP = $852.69 \cdot x_{absorbance}$).

$$\%R = 1 - \left(\frac{A_{sample}}{A_{control}}\right) \cdot 100 \quad (25)$$

4.2.7.5 Techno-functional properties

Water holding capacity (WHC) and oil holding capacity (OHC), emulsion activity (EA) and emulsion stability (ES) of the pectin fractions, obtained at high- and low-pressure methods and different solvents, were determined. The WHC and OHC were determined by Du et al. (2014) with minor adaptations. Briefly, the pectin fractions (3.0 g) were weighed in a pre-weighed centrifuge tube and 25 mL of distilled water or oil was added. The mixtures were shaken at 5 min intervals and kept for 30 min, followed by centrifugation at 412 x g for 15 min (Quimis, Model Q222T, SP, Brazil). The excess water from the decanted portion was removed by draining for 25 min at 50 °C (Lucadema, Model 82/27, SP, Brazil) while the tubes with oil were inverted for 25 min to drain the oil before reweighing. The samples were reweighed water and oil absorption capacities were expressed in grams of bound water or oil per gram of sample on a dry basis.

The emulsion activity (EA) was measured according to Hosseini et al. (2019) with minor adaptations. Briefly, 5 mL of canola oil, 5 mL of pectin water solution (0.5% w/v), and 0.02% of sodium chloride (NaCl) were treated in ultrasound probe (Eco-sonics, QR550W, Sao Paulo, Brazil) for 5 min followed by centrifugation (Quimis, Q222T, Diadema, Brazil) at 4000×g during 5 min. The EA was calculated according to Equation (26):

$$EA(\%) = \frac{V_E}{V_T} \cdot 100 \quad (26)$$

where V_E is the emulsion volume, and V_T is the total mixture volume.

ES was calculated using Equation (27) after the storage of emulsions at 4 °C for 30 days.

$$EA(\%) = \frac{V_R}{V_I} \cdot 100 \quad (27)$$

where V_R is the remained emulsion volume and V_I is the initial emulsion volume.

4.2.6 Statistical analysis

All data were obtained in triplicate and shown as average results followed by the standard deviation. Firstly, the homogeneity of variance was verified by Levene's test ($p \geq 0.05$). Significant differences between the samples were evaluated by the t-student test or one-way ANOVA ($p \leq 0.05$), followed by Ficher's LSD test.

4.3. RESULTS AND DISCUSSION

4.3.1 Optimization of extraction conditions

The SWE processing time was determined following the analysis of the extraction kinetics and overall extraction curve (**Figure 14**). The mass transfer mechanisms, represented

by the three straight lines fit to experimental data represent the constant extraction period (CER), followed by decreasing extraction rate period (FER), and diffusional controlled period (DC), as detailed by Ferreira et al, (1993). Considering the end of the CER period, the SWE time was fixed at 5 min, since the excessive time at SWE conditions can result in pectin degradation (CUI et al., 2021). Then, SWE assays were conducted following the conditions established at the Box-Behnken design, at 10 MPa, and 5 min.

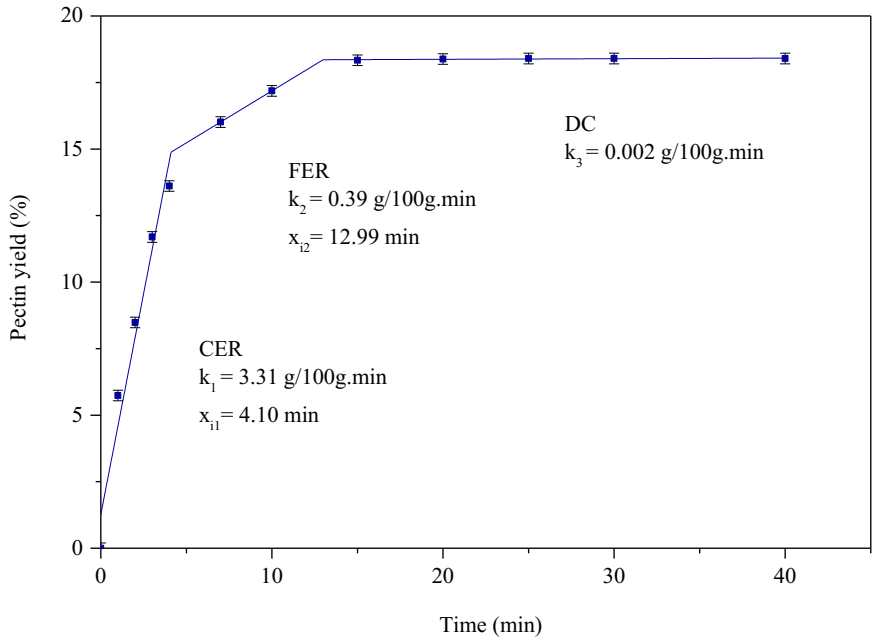


Figure 14 - Extraction kinetics of pectin from jaboticaba by-product by SWE modified by deep eutectic solvent (DES) at 125 °C, 6% of NADES, and flow rate of 5mL/min.
Note: CER - constant extraction rate period, FER - falling extraction rate period, and DC - diffusion-controlled period.

The yield values obtained for the SWE assays, conducted at different conditions of temperature, DES concentration, and flow rate, are presented at **Table 17**. The yield values show significant differences ($p < 0.05$), varying from 2.01 to 20.04 % (w/w). The data were fitted by RSM coupled to multiple regression analysis, and the mathematical model had adequacy checked by ANOVA F-value, to verify the statistical significance. The predicted

model is described by Equation (28), which was significant ($p_{model} < 0.01$), with R^2 of 0.99 and R^2_{adj} of 0.98, explaining 98% of the response variations.

$$Yield = 16.41 - 4.21x_1 - 10.21x_1^2 + 4.12\,x_2 - 1.19x_2^2 + 3.59x_1x_2^2 - 3.99x_1^2x_2 \quad (28)$$

Table 17 - Pectin yields obtained in each assay of Box-Behnken factorial design.

Assays	Temperature (°C) x_1	NADES concentration (%) x_2	Flow rate (mL/min) x_3	Yield (%)
1	100(-1)	2(-1)	5(0)	$5.87^{d} \pm 1.31$
2	150(+1)	2(-1)	5(0)	$3.24^{de} \pm 0.16$
3	100(-1)	10(+1)	5(0)	$4.76^{de} \pm 0.31$
4	150(+1)	10(+1)	5(0)	$4.88^{de} \pm 0.32$
5	100(-1)	6(0)	(-1)	$10.28^{c} \pm 0.67$
6	150(+1)	6(0)	2(-1)	$2.59^{de} \pm 0.17$
7	100(-1)	6(0)	8(+1)	$11.19^{c} \pm 2.87$
8	150(+1)	6(0)	8(+1)	$2.01^{e} \pm 0.14$
9	125(0)	2(-1)	2(-1)	$10.62^{c} \pm 0.60$
10	125(0)	10(+1)	2(-1)	$20.04^{a} \pm 1.01$
11	125(0)	2(-1)	8(+1)	$12.20^{c} \pm 0.21$
12	125(0)	10(+1)	8(+1)	$19.26^{ab} \pm 5.42$
13	125(0)	6(0)	5(0)	$16.16^{b} \pm 0.89$
14	125(0)	6(0)	5(0)	$15.91^{b} \pm 0.55$
15	125(0)	6(0)	5(0)	$15.87^{b} \pm 0.61$

Note. ^{abc}: Different letters indicate significant differences among the samples.

According to the mathematical model, temperature (x_1) was the main influential variable. The combination of linear and quadratic significant negative effects indicates that a maximum yield was provided by an intermediate temperature. In general, temperature increases until a certain point facilitate the extraction on high-pressure methods since the temperature increase reduces the viscosity and the dielectric constant of water, affecting the mass transfer rate and resulting in higher solubility of solutes (ESSIEN; YOUNG; BAROUTIAN, 2020). However, excessively high temperatures can damage the pectin structure, leading to its degradation and decreasing the extraction yield (ESSIEN; YOUNG; BAROUTIAN, 2020). Corroborating with this result, Ma et al. (2020) evaluated the temperature effect on pectin recovery from sunflower heads by SWE in a range of 100 to 140 °C, finding 120 °C as the

optimum temperature. The presented model also presented interactive effects between x_1 and DES concentration (x_2). High temperatures are necessary to disrupt the cell membrane, facilitating the solvent permeability in the matrix and promoting the extraction of compounds highly bound to the cell wall, such as the pectin (DIAS et al., 2020).

The concentration of DES as a water modifier (x_2) presented a positive linear effect on pectin fraction yield. This co-solvent alters the water polarity, increasing the efficiency of SWE. Besides, Ca:Glu:Wa solution presents a high potential for pectin recovery mainly due to the low pH value (next to 2.0), important to breaking the cell walls, and to the molecular affinity with the pectin basic structure (BENVENUTTI et al., 2020). The quadratic effect of this variable (x_2) was negative since the increase in DES concentration contribute to increase the solvent viscosity, reducing the solvent penetrability and dissolution capacity, and also increasing the process costs (BENVENUTTI et al., 2020; LIU et al., 2018).

The independent variables were defined from these model analyses combined with the response surface (**Figure 15**). Then, because the solvent flow rate (x_3) show no effect on pectin extraction yield, the lower value was selected for further assays to reduce the solvent consumption and process costs. Also, the best pectin recovery was observed using the intermediate temperature condition, just below the central point, while the DES concentration was just above the central point. Thus, the suggested optimum conditions were 122 °C, 8% of DES, and a flow rate of 2 mL/min.

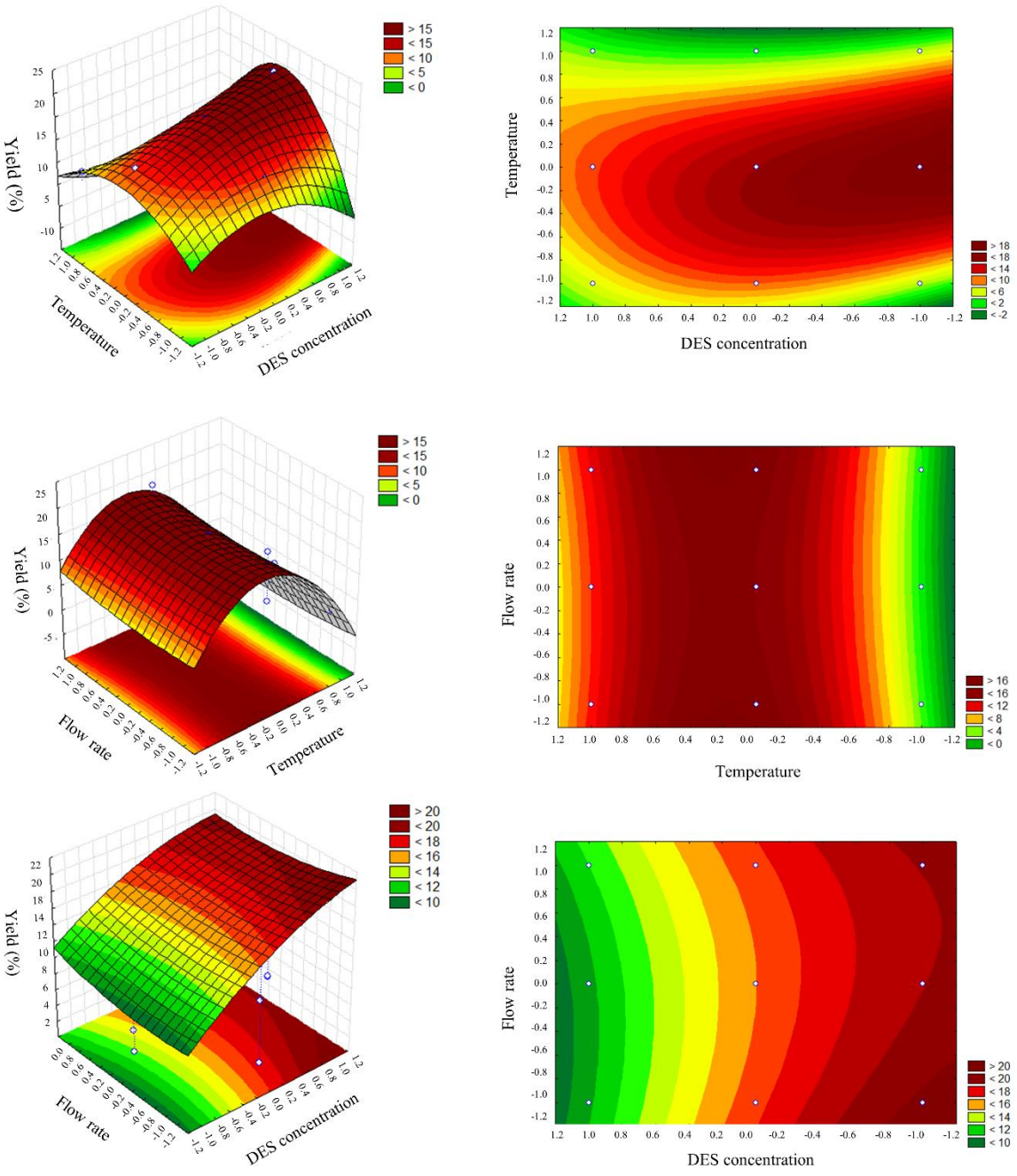


Figure 15 - Three-dimensional (3-D) response surfaces and contour plots showing the effect of independent variables (temperature, NADES concentration, and flow rate) on the pectin yield (%).

At the optimized conditions, the predicted pectin yield was 18.69%, ranging from 17.65 to 19.73, with 95% confidence interval level. These optimal conditions were externally validated by repeating the extraction at the optimized condition in triplicate. The experimental

values obtained at the optimized conditions presented a pectin yield of 17.95 % ± 1.52 %, which is within the confidence interval level, and a relative error of 3.95%. Therefore, the proposed model was able to adequately define the best extraction conditions and predict the response values.

4.3.2 DES as SWE modifier compared to low-pressure conventional solvents

The characterization of pectin fractions obtained by different methods (SWE and HSE) and solvents (water, water modified by DES, or citric acid solution) are presented in **Table 18**. The yield values presented significant differences ($p > 0.05$), with SWE as the more efficient method for the pectic fractions recovery, providing yields from 1.5 to 1.8-fold higher than HSE. Muñoz-Almagro (2019) also reported significantly higher pectin yields from cacao pod husk by SWE (performed at 121 °C, 103.4 bar, and 30 min) compared to the low-pressure method (conducted using solid to liquid ratio of 1:25 g/mL), citric acid solution as solvent at pH 3, and 95 °C for 95 min). The high effectivity of SWE, achieved at a shorter time than the low-pressure method is due to SWE advantages such as the decrease in dielectric constant and solvent viscosity, which increases the solvent vapor pressure and diffusion capacity, contributing to the mass transfer (ESSIEN; YOUNG; BAROUTIAN, 2020; MUÑOZ-ALMAGRO et al., 2019).

The best yield values were provided by SWE-DES and SWE-citric acid. The effectivity of these SWE modifiers is mainly related to pH values near 2 since this is one of the main factors that affect pectin yield (KUMAR et al., 2020).

The solvent type and the extraction method also affect the pectic polysaccharide characteristics in terms of the degree of esterification (DE), GalA acid content, and antioxidant activity, which is related to the differences in the structure and composition of the recovered pectin fraction (CUI et al., 2021). In general, SWE resulted in pectin with a higher GalA content

than HSE. Muñoz-Almagro et al. (2019) also verified higher GalA content in SWE than conventional extraction using citric acid as the solvent and indicated that SWE results in pectin with a smaller amount of components derivate from other fonts. Furthermore, probably, no hydrolysis of the pectin main chain happened at SWE optimum condition, which is one drawback of the high-pressure method, as reported in the literature (CUI et al., 2021). However, the pectin fractions obtained by SWE presented lower DE values, probably due to de-esterification reactions, since the SWE conditions can facilitate hydrolysis reactions (ZHANG et al., 2020b).

In general, SWE provided pectin with higher antioxidant activity compared to HSE. This result can be related to the high GalA content from the samples recovered by high-pressure methods, since uronic acid groups present high hydrogen-bonding capacity, providing important antioxidant contributions in polysaccharides (CUI et al., 2021).

Table 18 - Characterization of pectins obtained by SWE and SWE modified by deep eutectic solvent or citric acid in comparison to conventional low-pressure extraction methods, HSE, using the same solvents.

	High-pressure			Low-pressure		
	SWE - DES	**SWE - Citric acid**	**SWE**	**HSE - DES**	**HSE - Citric acid**	**HSE - Water**
Yield (%)	$19.39^{a} \pm 0.26$	$17.79^{a} \pm 2.94$	$12.07^{bc} \pm 0.62$	$13.79^{b} \pm 0.52$	$9.31^{cd} \pm 1.80$	$7.47^{d} \pm 2.04$
DE (%)	67.16	63.64	69.55	77.89	75.79	73.12
TCC (%*)	$65.22^{c} \pm 1.69$	$79.64^{b} \pm 1.06$	$79.66^{b} \pm 0.09$	$97.85^{a} \pm 0.12$	$45.34^{e} \pm 3.31$	$55.54^{d} \pm 3.32$
GalA (%**)	$44.94^{a} \pm 1.08$	$37.12^{b} \pm 1.01$	$49.31^{a} \pm 7.57$	$48.37^{a} \pm 2.89$	$28.37^{c} \pm 1.44$	$29.62^{c} \pm 4.33$
Antioxidant activity						
DPPH (μmolTE/g)	$472.51^{a} \pm 14.24$	$214.38^{d} \pm 12.95$	$346.76^{b} \pm 9.04$	$241.4^{c} \pm 21.48$	$213.76^{d} \pm 5.43$	$206.99^{d} \pm 9.85$
ABTS (μmolTE/g)	$260.76^{b} \pm 10.74$	$159.56^{c} \pm 19.71$	$312.35^{a} \pm 15.05$	$115.44^{d} \pm 10.15$	$122.67^{c} \pm 2.90$	$72.14^{cd} \pm 42.74$
FRAP (μmolTE/g)	$521.36^{a} \pm 26.14$	$426.19^{b} \pm 18.79$	$508.37^{a} \pm 36.13$	$235.33^{d} \pm 22.60$	$284.4^{d} \pm 22.32$	$276.73^{e} \pm 7.89$
Functional properties						
WHC (g water/g sample)	$1.01^{c} \pm 0.00$	$1.01^{bc} \pm 0.00$	$1.01^{c} \pm 0.00$	$1.02^{ab} \pm 0.00$	$1.02^{bc} \pm 0.00$	$1.03^{a} \pm 0.01$
OHC (g oil/g sample)	$1.01^{b} \pm 0.00$	$1.19^{a} \pm 0.19$	$1.01^{b} \pm 0.00$	$1.01^{b} \pm 0.00$	$1.01^{b} \pm 0.00$	$1.01^{b} \pm 0.00$
EA (%) – day 0	$53.36^{c} \pm 1.20$	$54.78^{c} \pm 0.25$	$56.43^{c} \pm 1.38$	$68.15^{a} \pm 2.94$	$62.71^{b} \pm 1.52$	$61.73^{b} \pm 4.43$
EA (%) – day 30	$52.66^{c} \pm 1.42$	$52.61^{c} \pm 0.95$	$53.95^{c} \pm 0.01$	$65.84^{a} \pm 1.17$	$60.41^{b} \pm 1.36$	$61.04^{b} \pm 0.67$
ES (%)	$98.14^{ab} \pm 0.32$	$96.04^{bc} \pm 2.37$	$95.67^{c} \pm 3.31$	$98.61^{a} \pm 0.3$	$96.34^{bc} \pm 1.12$	$98.87^{a} \pm 0.38$

Note: SWE – subcritical water extraction, HSE – heating-stirring extraction, DES – deep eutectic solvent, * glucose equivalent, ** galacturonic acid equivalent, GAE – gallic acid equivalent per g of pectin, TE – Trolox equivalent, WHC water holding capacity, OHC – oil holding capacity, EA – emulsifying activity, ^{abc} - different letters in the same line indicate significant differences ($p < 0.05$).

Concerning the pectin techno-functionalities, such as water holding capacity (WHC) and oil holding capacity (OHC), important attributes to define the pectin applications, the results from **Table 18** show significant differences among the samples. WHC and OHC are defined by the ability of 1g of sample to hold water and oil, respectively (KAZEMI; KHODAIYAN; HOSSEINI, 2019). WHC is related to the physical properties of pectin, such as density, particle size, porosity, and microstructure, which influence the mouth feeling, improving the sensorial attributes of some food systems, such as yogurt (LI et al., 2022). The pectin from jaboticaba peel presented WHC values near to 1g/g (**Table 18)**, showing lower hydration capacity than pectin from eggplant peel (6.02g/g), calyxes (4.62g/g) (KAZEMI; KHODAIYAN; HOSSEINI, 2019), and orange peel (3.10 g/g), a conventional source of pectin (HOSSEINI et al., 2019). On the order hand, the OHC values, from 1 to 1.19g/g (**Table 18**), were similar to those reported for eggplant calyxes (1.46 g/g) (KAZEMI; KHODAIYAN; HOSSEINI, 2019), orange peel (1.32 g/g) (HOSSEINI et al., 2019), and *Opuntia ficus indica* (1.23 g/g) (BAYAR; FRIJI; KAMMOUN, 2018). These OHC results suggest the potential use of the pectin fraction as stabilizer and emulsifier of fat food systems since the OHC is related to the pectin ability to decrease the oil and fat losses during food process and storage, maintaining the mouth texture and flavor (DU et al., 2014; KAZEMI; KHODAIYAN; HOSSEINI, 2019; LI et al., 2022). Furthermore, high OHC values might be related to positive effects on the maintenance of blood lipids level (LI et al., 2022).

Corroborating with WHC and OHC results, the emulsifying activity (EA) values above 53% on day 0 and emulsion stability (ES) after 30 days at 4 °C above 95.67%, highlight the potential of the pectin fractions from jaboticaba peel as emulsifiers. The emulsion properties of fractions recovered by SWE method are similar to pectin from orange peel obtained by

ultrasound-assisted extraction (UAE) at 150 W for 10 min using citric acid solution pH 1.5, with EA of 53.42%, and ES of 90.25% (after 30 days at 4 °C) (HOSSEINI et al., 2019) and higher than the obtained by conventional heat extraction method using water at 90 °C for 90 min and ratio solid-liquid 1:25 (w/v), with EA of 45% and ES of 86.6% (after 30 days at 4 °C) (HOSSEINI; KHODAIYAN; YARMAND, 2016). High pressure and temperatures result in strong changes in pectin structure, which considerably affect their physicochemical properties, including EA (EINHORN-STOLL et al., 2005). Pectins with high molecular weight (MW) are capable to form more molecular interactions, resulting in high EA; while low MW pectins are unable to stabilize the oil/water interface (CUI et al., 2021). Therefore, probably, the pectin fractions obtained by high-pressure methods resulted in lower MW and consequently lower emulsion properties than low-pressure methods (**Table 18**). Furthermore, the higher DE values in low-pressure methods contribute to EA due to the hydrophobicity of methyl esters (CUI et al., 2021). In addition, in general, the use of acidified solvents beyond increases the pectin fractions yield improves their emulsifying properties.

FTIR spectra of the pectin samples (**Figure 16**) show common refraction bands as reported in the literature, as the band near to 3400 cm^{-1}, corresponding to O-H stretching vibration, and near to 2930 cm^{-1} due to the C-H stretching of the CH_2 groups. The bands between 1200 and 950 cm^{-1} constitute the specific "fingerprint" of each polysaccharide. It was not attributed to a specific atom group vibration in this region because it is originated from complex inter-acting vibrational models (MANRIQUE; LAJOLO, 2002). However, strong absorptions peaks observed at 1101 and 1018 cm^{-1} can indicate the presence of a pyranose ring (SHAFIE; YUSOF; GAN, 2019).

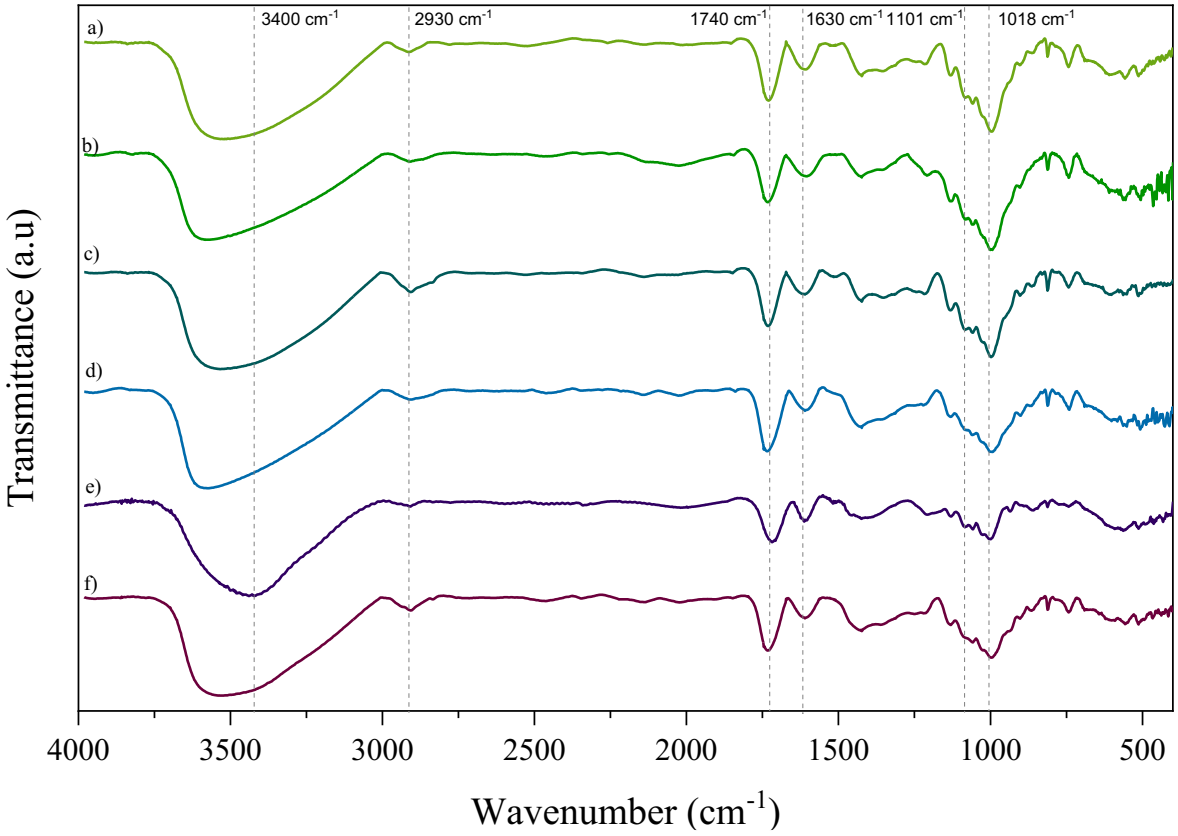


Figure 16 - FTIR spectra of the pectins obtained by a) SWE-DES, b) SWE-citric acid, c) SWE, d) HSE-DES, e) HSE-citric acid, f) HSE-water.
Note: SWE – subcritical water extraction, NADES – natural deep eutectic solvent, HSE - heating-stirring extraction.

The bands near 1630 and 1740 cm^{-1}, corresponding to symmetrical stretching vibration of COO^- group and ester carbonyl group (C=O), respectively (MANRIQUE; LAJOLO, 2002), are relevant to the degree of esterification (DE) determination. The pectin from SWE presented a lower DE value than HSE due to the hydrolysis of methyl esterified groups, as reported in the literature (CUI et al., 2021). Furthermore, the use of acid modifiers intensified the hydrolysis at SWE samples, decreasing DE values. However, HSE in the acid medium increased DE values. In general, pH values around 2 result in high-methoxylated pectin (CUI et al., 2021; LIEW et al., 2018). Nevertheless, all pectin obtained presented DE above 50%, therefore are

classified as high methoxyl pectin (HM), with gelling ability in the presence of high sugar content (60-65% w/w) and pH < 3.5 (ZHAO et al., 2015b).

4.4. CONCLUSIONS

The SWE modified by DES is an efficient and fast approach for the recovery of pectin-rich fractions from the jaboticaba by-product. Among the independent variables evaluated, the temperature and DES concentration influenced the pectin yield, and the optimized extraction conditions were suggested as 122 °C, 8% of DES, and a flow rate of 2 mL/min. The pectin samples recovered from jaboticaba peel are high methoxyl (HM) and show oil holding capacity (OHC) and emulsion activity (EA). The high-pressure extraction method modifies the pectin structure, affecting technological and bioactive properties. Compared to the control solvents and methods, SWE-DES increases the GalA content and antioxidant activity of pectin fractions, however, decreases their DE. Therefore, SWE modified by DES is a fast and efficient approach to obtaining pectin-rich extracts with application potential in functional foods.

CHAPTER 5

Sequential extraction using aqueous solutions of deep eutectic solvents (DES) at high-pressure: Towards a sustainable valorization process

ABSTRACT

The plant-based extraction process based on circular economy and biorefinery concepts with a focus on the valorization of industrial by-products is an economic, ecological, and sustainable approach to supply current demands. Thus, the chapter aimed to evaluate the sequential extraction of anthocyanin and pectin-rich fractions from jaboticaba industrial by-products using pressurized aqueous solutions of DES. Besides, the "greenness" of this method was verified to validate this high-pressure approach as an alternative for the fractionation of complex bioactive extracts from a Brazilian berry. The process combination was represented by the first step for the recovery of anthocyanin-rich fraction, conducted using 47% ChCl:Ma aqueous solution at 10 MPa, 90 °C, a flow rate of 5.3 mL/min for 12 min (optimization according to section 3.3.2, chapter 3). Then, a second solvent (water modified by 8% Ca:Glu:Wa) was used to obtain the pectin-rich fraction, at 10 MPa, 122 °C, a flow rate of 2 mL/min for 5 min (optimization according to section 4.3.1, chapter 4). The sequential extraction was evaluated by extraction yield and by a semi-quantitative green metric tool, the *Green Certificate*, to measure the sustainability of the process. Extractions using pressurized aqueous solution of DESs were performed successfully in immediate sequence, without opening the system, eliminating the drying step of the vegetal matrix after the first extraction. Both recovered fractions showed high technological and bioactive quality, with high application potential in food and pharmaceutical formulations. The proposed approach presented a *Green Certificate* value of 90.35, being considered a genuine green extraction that can be used to recover added-value compounds from other underutilized resources. For this, 6 summarized steps were pointed out: select the vegetal matrix, determinate the target compounds, define the appropriated DES by *in silico* and experimental data, optimization of extraction conditions, evaluate the quality of the extracts, and study the potential application of the recovered fractions.

Keywords: Sequential extraction; deep eutectic solvent; green metric tools; pressurized liquid extraction; subcritical water extraction.

5.1 INTRODUCTION

The growing world population demonstrates the need for the development of economically viable processes to provide for the increasing food demand. Besides, over the last decade, there was a growing interest from the global consumers for natural compounds as ingredients and additives to different products, to replace the synthetic ones, and to attend to the current health and sustainability concerns. In this sense, the *plant-based* extraction process mainly with a focus on the valorization of industrial by-products can be an economic, ecological, and sustainable solution (MORONE et al., 2019). This approach contributes to the seventeen Sustainable Development Goals (SDGs) adopted by United Nations Members States (2015), which aim to end poverty, protect the environment and ensure prosperity for all by 2030.

Based on circular economy and biorefinery concepts, available and underutilized vegetal resources can be used to recover primary metabolites such as lipids (SUI et al., 2021), proteins (TORRES et al., 2022), polysaccharides (BENVENUTTI et al., 2020; RUDKE; DE ANDRADE; FERREIRA, 2020) and secondary metabolites such as polyphenols (BENVENUTTI et al., 2020; RUDKE et al., 2019), alkaloids (PAGANO et al., 2021), among others. These *plant-based* compounds have been widely evaluated for use as nutritional, technological, and health promotion in food and pharmaceutical applications (BENVENUTTI; ZIELINSKI; FERREIRA, 2021; DA SILVA et al., 2020; HERRERO et al., 2015; MARQUETTI et al., 2018; ZIELINSKI et al., 2021).

According to Chemat et al. (2019), a green extraction process reduces energy consumption and eliminates the use of fossil-based solvents, ensuring the security and quality

of the recovered extract. Therefore, the use of aqueous solutions of deep eutectic solvents coupled with high-pressure extraction methods, an eco-friendly approach, is a potential alternative that can be applied for the fractionation of different target compounds from several vegetal matrixes. However, environmental analyses such as Life Cycle Assessment, carbon footprint, E-factor, or green metric tools must be considered to affirm the proposed method as a promising environmental strategy (CHEMAT et al., 2019; ESPINO et al., 2018; VAN AKEN; STREKOWSKI; PATINY, 2006).

Therefore, the objectives of this chapter are: (*i*) to evaluate the sequential extraction of anthocyanin- and pectin-rich fractions from jaboticaba peel using aqueous solutions of deep eutectic solvent by a high-pressure sustainable process; *(ii)* to discuss the use of DES as novel solvents and the potential application of the recovered extracts, and (*iii*) to suggest a procedure to use this approach for the recovery of other relevant fractions from different vegetal matrixes, widening the use of DES solutions at high-pressure conditions.

5.2 METHODS

5.2.1 Sample preparation

The jaboticaba peel (JP) sample was dried and stirred according to the procedure presented at Chapter 2 (section 2.2.5.1).

5.2.2 DES preparation

The aqueous solutions of DES ChCl:Ma and Ca:Glu:Wa were prepared according to section 2.2.2 (Chapter 2) and diluted following the optimized conditions determined for the

recovery of anthocyanin and pectin fractions, i.e, 47% ChCl:Ma and 8% Ca:Glu:Wa, respectively.

5.2.3 Sequential methods to obtain anthocyanin-rich and pectin-rich fractions

The two-step sequential recovery of anthocyanin-rich and pectin-rich fractions were obtained without opening the extraction vessel after the first step, as represented at **Figure 17**. Briefly, 5g of the JP sample was placed on the extractor vessel and, as the first step, the anthocyanin-rich fraction was obtained at optimized conditions, according to Chapter 3 (section 3.3.2), i.e., 47% ChCl:Ma aqueous solution, 10 MPa, 90 °C, and flow rate of 5.3 mL/min for 12 min. After this extraction, a needle valve (model 15-11AF2, HIP, USA) was used to purge the remaining solvent. Then, the second-step extraction, to obtain the pectin-rich fraction, was performed at the optimized conditions from Chapter 4 (section 4.3.1), i.e., 8% Ca:Glu:Wa aqueous solution, 10 MPa, 122 °C, and flow rate of 2 mL/min for 5 min. The extraction at both conditions was also performed using water as the control solvent.

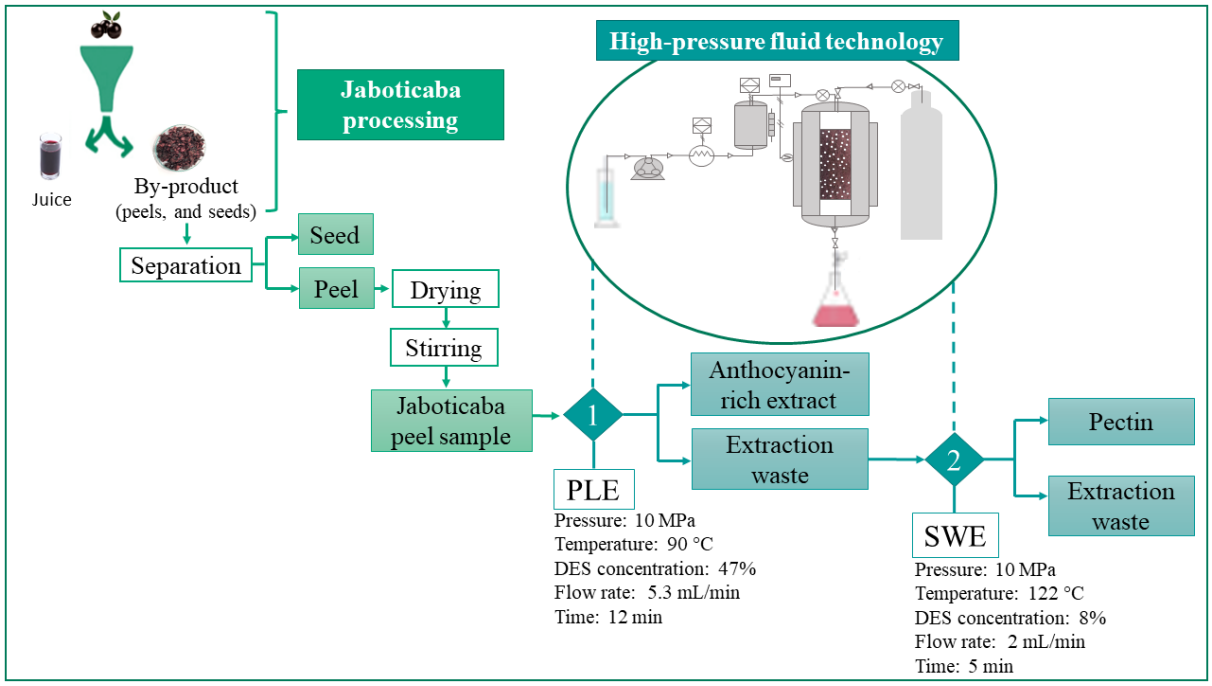


Figure 17 - Representative scheme of the sequential extraction of an anthocyanin-rich fraction and a pectin-rich fraction to valorize the jaboticaba residue.
Note: PLE – pressurized liquid extraction, SWE – subcritical water extraction, DES – deep eutectic solvent.
Source: The author.

The yield of anthocyanin was quantified by monomeric anthocyanin (MAP) content according to Giusti & Wrolstad (2001) while the pectin-fraction was precipitated using ethanol 96% and their yield was determined by gravimetry as described in Chapter 4 (section 4.2.3).

5.2.4 Green metric

The "greenness" of the sequential high-pressure extractions was semi-quantified through the *Green Certified*, according to Espino et al. (2018). This green metric tool is a semi-quantitative strategy used to measure the sustainability of the chemical process, which has been already used to compare different extraction methods (ESPINO et al., 2018). Briefly, the method considers Penalty Points (PP), applied to reduce from 100% environmentally safe process, according to various process parameters, which are tabulated (PP values). The parameters are: amount and environmental hazard of the solvents, energy, and generated waste.

The penalty points related to the solvents (PP_S) are quantified using Equation (29):

$$PP_R = 0.61 \cdot V^{0,31} \quad (29)$$

where V is the solvent volume (mL). The PP_S value should be multiplied by the penalty point for environmental and health hazards of the solvent (PP_H), which is evaluated based on the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. In this system, dangerous substances are marked with pictograms that address toxicity, physical hazards, and environmental hazards. The absence of pictograms concerns 0 penalty points, each warning pictograms concern 1 penalty point, and more several pictograms involve 2 penalty points (ESPINO et al., 2018).

Concern the energy consumption, less or equal than 0.1 kWh per sample involves 1 penalty point, between 0.1 and 1.5 kWh per sample concerns 2 penalty points, and >1.5 kWh per sample, 3 penalty points. The energy consumption was calculated through the equipaments potencies (kW) (i.e., pump (71.5 W), preheat and heat systems (200 W each), and temperature indicator (4.88 W) for high-pressure methods; and thermostatic bath (2100 W) and centrifugal (130 w) for low-pressure methods), multiplicated for their respective use times (h).

Finally, the penalty point for waste volume (PP_w) is determined by Equation (30) (ESPINO et al., 2018):

$$PP_w = 0.50 \cdot W^{0.4} \quad (30)$$

where W is the amount of waste (g or mL).

5.3 RESULTS AND DISCUSSIONS

5.3.1 Extraction yield

Two extract fractions were sequentially obtained from jaboticaba by-product, the anthocyanin- and the pectin-rich fractions, using pressurized DES aqueous solutions, without depressurizing (opening) the system (**Figure 18**). Using this approach, the cleaning of the system and the drying of the solid matrix after the first step are not necessary. As expected, the ChCl:Ma aqueous solution provided a yield of about 40% higher than water, at the same conditions (first step), as presented at Chapter 3 (section 3.3.3).

In the second step, after the anthocyanin-rich fraction recovery, the pectin fraction yield by SWE modified by DES (Ca:Glu:Wa) was 1.6-folds higher than SWE (**Figure 18**). These results are about 30% lower than the pectin fraction yield obtained at Chapter 4 (**Table 18**). This fact can be related to the absence of the drying step, which was used to preserve the vegetal matrix after the separation of the anthocyanin-rich fraction (Chapter 3, section 3.2.7), and probably concentrated the pectin fraction. Considering the dry oven power of 1200 W and the drying time of 16 h at 60 °C, the energy consumption of the drying step was 19.2 kWh, which is a disadvantage for practical purposes as discussed in the next section.

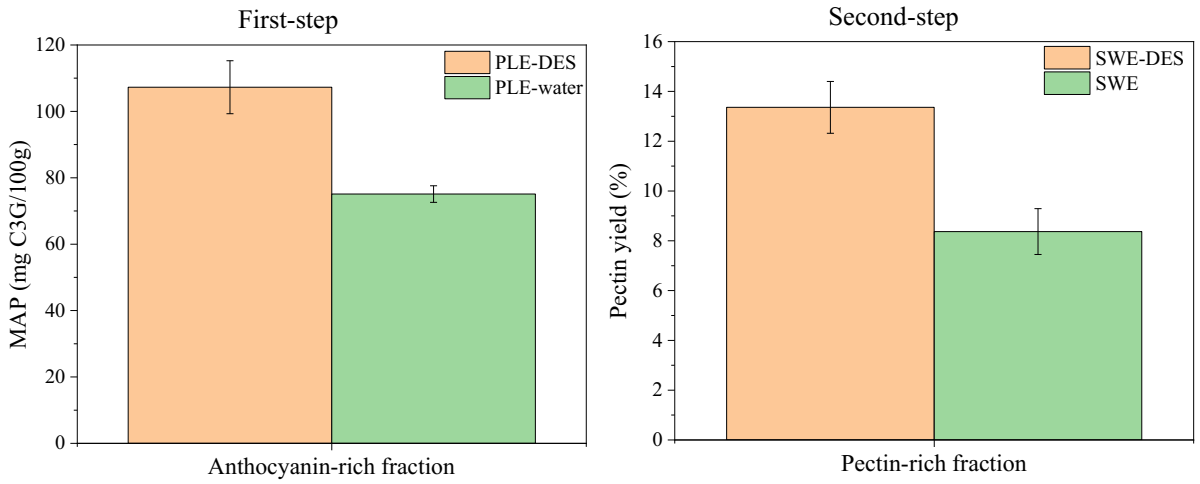


Figure 18 - Extraction yields of sequential extraction of anthocyanin and pectin-rich fractions from jaboticaba peel by high-pressure extraction techniques.
Note: MAP – monomeric anthocyanin pigment, C3G – cyanidin-3-*O*-glucoside, First-step - 47% of DES composed by choline chloride (ChCl) and malic acid (Ma) (ChCl:Ma 1:1), Second-step - 8% of DES composed by citric acid (Ca), glucose (Glu) and water (Wa) (Ca:Glu:Wa 1:1:3).

Therefore, the sequential extraction using pressurized DES aqueous solutions can be a viable approach to recover a natural colorant with bioactive properties and a polysaccharide with functional and technological characteristics for food and pharmaceutical applications.

5.3.2 Greenness of the sequential process using pressurized aqueous solutions of DES

According to the *Green Certificate* values (**Table 29**), the greenness attributes of the solvents evaluated follows this order: water > Ca:Glu:Wa solution > ChCl:Ma solution. Water is a non-hazardous substance and is considered the greenest solvent (CASTRO-PUYANA; MARINA; PLAZA, 2017). The choline chloride and glucose, substances used as DES formers, are also considered not dangerous according to the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals. On the order hand, citric acid (Ca) presents 1 pictogram, and malic acid, 2 pictograms. Therefore, solvents with Ca received 1 penalty point, and with Ma, 2 penalties points.

Regarding energy consumption, although low-pressure methods have lower energy consumption than high-pressure ones, they receive the same penalty point because their values are between 0.1 and 1.2 kWh. Despite the higher energy costs and the initial investment of the high-pressure methods, compared to low-pressure ones, the processing time and solvent consumption for PLE and SWE are lower than the evaluated low-pressure methods. These factors combined with the high yields pf PLE and SWE provide lower manufacturing costs than low-pressure methods (SANTOS; VEGGI; MEIRELES, 2012).

Although *Green Certificate* does not consider the extraction yields, it is an important factor. However, it was not taken into account to evaluate the green effectiveness of the high- and low-pressure methods because, unlike the high-pressure methods studied (Chapter 3 and 4), the conditions of the low-pressure extraction were not optimized.

After the first step, no waste was accounted for because there was no solvent removal, and the solid matrix was immediately submitted to the second step (pectin extraction). After the second step, the pectin fraction was precipitated with ethanol. The ethanol content in the liquid fraction was recovered by evaporation and used for cleaning the unit. The resultant solid material, for now, was treated as extraction waste. However, it is a fiber source and, future studies can be conducted to evaluate its potential as a functional ingredient.

Table 19 - Penalty points to calculate the *Green Certificate* for anthocyanin-rich extract obtained by high-pressure fluid techniques using different solvents compared to conventional methods.

Extraction technique	Solvent					Energy		Waste	Total PPs	Green Certificate	Category
	Type	Quantity (mL)	PP_R	PP_{RH}	Subtotal (PP)	Consumption (kWh)	PP_E	PP_W			
PLE	Water	63.6	2.21	0	0	0.43	2	0	1.00	98.00	A
	ChCl:Ma solution	63.6	2.21	2	4.42	0.43	2	0	5.42	93.58	A
HSE	Water	150	2.88	0	0.00	0.18	2	0	1.00	98.00	A
	ChCl:Ma solution	150	2.88	2	5.77	0.18	2	0	6.77	92.23	A
SWE	Water	10	1.25	0	0.00	0.37	2	0.71	1.71	97.29	A
	Modified by Ca:Glu:Wa	10	1.25	1	1.25	0.37	2	0.71	2.96	96.04	A
HSE	Water	150	2.88	0	0.00	0.27	2	0.71	1.71	97.29	A
	Modified by Ca:Glu:Wa	150	2.88	1	2.88	0.27	2	0.71	4.60	94.40	A
PLE + SWE	ChCl:Ma solution + Ca:Glu:Wa as modifier	73.6	2.31	3	6.94	0.61	2	0.71	8.65	90.35	A
Maceration + HSE	ChCl:Ma solution + Ca:Glu:Wa solution	300	2.31	3	10.72	0.44	2	0.71	12.44	86.56	B

Note: PLE – pressurized liquid extraction, SWE – subcritical water extraction, HSE – heat-stirring extraction, * - deep eutectic solvents (DES), ChCl - choline chloride, Ma – malic acid, Ca – citric acid, Glu – glucose, Wa – water, HSE – heating-stirring extraction, PPs – penalty point for solvent quantity, PP_{SH} – penalty point for solvent hazard, PP_E – penalty point for energy consumption, PP_W - penalty point for waste volume, PP – penalty points.
* $Subtotal\ (PP) = PP_s \cdot PP_{SH}$, ** $Total\ PP = PP_s + PP_E + PP_W$, $Green\ certificate = 100 - Total\ PP$

Then, the sequential recovery of an anthocyanin-rich fraction and a pectin fraction from jaboticaba peel, by pressurized aqueous solutions of DES, presented a *Green Certificate* value of 90.35. This process can be classified at the A category (values from 90-100) of green effectiveness (ARMENTA; DE LA GUARDIA; NAMIEŚNIK, 2017). Therefore, the proposed approach can be considered a genuine green extraction sequential process.

5.3.4 Application potential of the recovered fractions

After the environmental analysis, it is safe to suggest the use of PLE with different DES solutions for the sequential recovery of anthocyanin- and pectin-rich extracts with high application potential. The anthocyanin-rich fraction in an aqueous solution of DES presented good stability and can be applied as a natural colorant with bioactive properties as antioxidant, anti-diabetic, and anti-obesity (Chapter 3) with the possibility of no need for solvent removal (DA SILVA et al., 2020). The better stability of anthocyanin in DES medium corroborates with this suggestion, i.e., the use of the solvent as a delivery vehicle for the recovered bioactive compounds (anthocyanin-rich fraction), in pharmaceutical or nutraceutical applications.

Concerning the safety of the DES components, choline chloride (ChCl), malic acid, and propylene glycol are GRAS substances, when following good manufacturing practices, and are approved by FDA for use in food formulations (https://www.ecfr.gov/). Besides, ChCl:Ma has high biodegradability (> 80% after 28 days) and, according to Türker and Dogen (2021), can be considered a biodegradable green solvent. This DES also shows low cytotoxicity, with less than 50% inhibition of human cell growth (MCF - 7 and HeLe) at concentrations from 10 to 2000 mg/L (RADOŠEVIĆ et al., 2016). The cytotoxicity of ChCl-based DES was evaluated by Ahmadi et al., (2018) using *in vitro* model with human HEK-293 cells. The results show that ChCl:Pro at 1:2 molar ratio presented no cytotoxicity.

The pectin-fraction presented oil holding capacity (OHC), emulsion activity (EA), and antioxidant activity (Chapter 4). Therefore, it presents a potential application in nutraceutical and functional foods, mainly in fat food systems such as a stabilizer or emulsifiers. The pectin fractions were isolated from the aqueous extract and, therefore, are free of solvents. However, the remaining amounts of the solvents used are also not a problem for applications. According to Radošević et al. (2018) citric acid-based DES with glucose, fructose, proline, and glycerol did not present any cytotoxic effect in HeLa, MCF-7, and HEK293T cell lines. Furthermore, the ethanol used in pectin precipitation and purification is a GRAS solvent (RENARD, 2018).

5.3.5 Suggested procedure to use pressurized DES aqueous solutions to valorize different industrial by-products

The approach conducted to valorize the jaboticaba by-product using DES aqueous solutions at high-pressure conditions can be replicated for the recovery of different added-value compounds from underutilized biomasses. In order to provide a protocol for the valorization of different raw materials by using alternative solvents and extraction methods, **Figure 17** summarizes the main procedures represented by 6 sequential items.

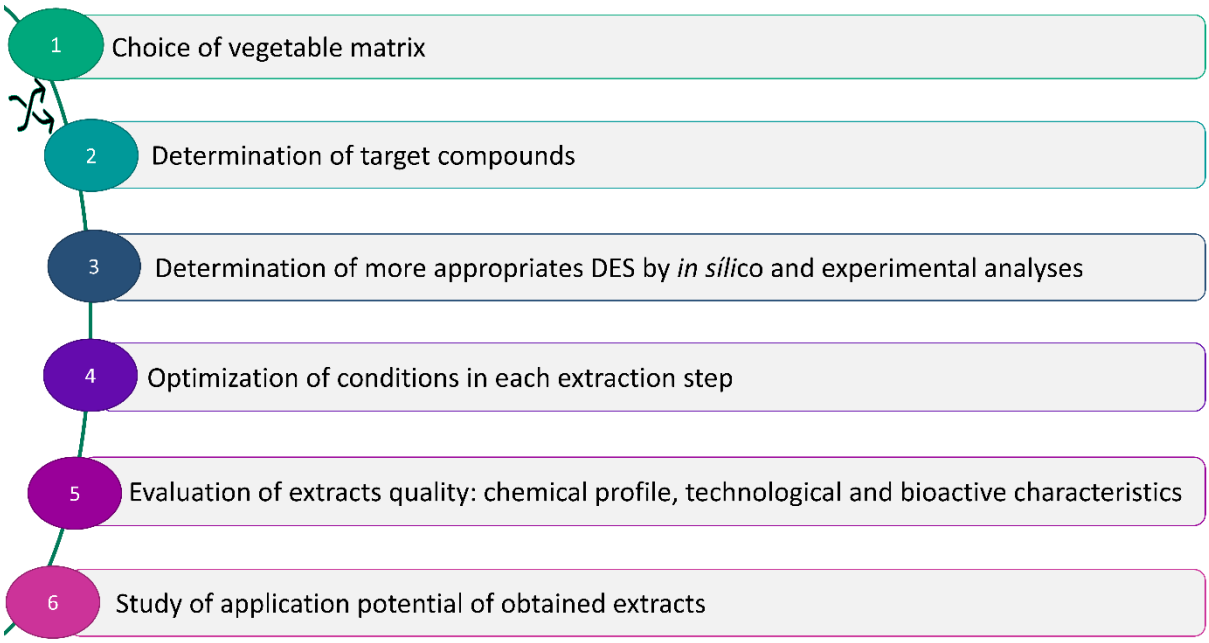


Figure 19 - Representative scheme 6 summarized steps to valorize a vegetal matrix using pressurized aqueous solutions of DES.
Note: DES – deep eutectic solvent.
Source: The author.

The first and second items, the choice of vegetal matrix and the target compounds aim the exploration of available resources, i. e. underused edible plants or industrial by-products, to supply the food demand and found new components related to human health (CHEMAT et al., 2019). Therefore, these items involve the evaluation of the composition of the materials and detect the functional, technological, and health benefits associated.

After selecting the target compounds from a specific raw material, and considering the physiological characteristics of the solid matrix, then, the next item is the definition of the most suitable DES, based on its capacity to penetrate the solid material and solubilize the target components. The solubilization of a target compound is related to the thermodynamic properties of the system and can be estimated by the probability of compounds interacting with the solvent according to their polarities and molecular interactions. Procyanidins and anthocyanins, for

example, can be recovered by acid DES since the solubility of these target components increases in acidified medium (BENVENUTTI et al., 2020; CAO et al., 2018). DES also can solubilize non-water soluble and poor water-soluble such as rutin, quercetin, cinnamic acid, carthamin, taxol, ginkgolide B, and 1,8-dihydroxyanthraquinone, with solubility capacity dependent on DES components (DAI et al., 2013a). DES composed of 1,2-propanediol-choline chloride-water (PCH), the least polar solvent evaluated by Dai et al. (2013a), show the highest cinnamic acid and ginkgolide B solubilities. On the other hand, glucose-choline chloride-water (GCH) result in higher rutin and starch solubilization. Xylitol-choline chloride-water (XoCH) was the more efficient to solubilize carthamin and quercetin. Besides the DES composition, these authors reported that water addition can improve the solubilizing capacity in a concentration-dependent on the target compound. Thus, some tools based on statistical mechanics, molecular dynamics (MD), or quantum chemistry can be used to relate molecular structures of systems with solubility capacity (BENVENUTTI; ZIELINSKI; FERREIRA, 2019). According to Chapter 2, the combination of *in silico* using COSMO-RS and experimental analysis is a good approach for the selection of appropriate DES for a selective and efficient extraction.

Sequentially, and depending on the defined target compounds (fractions from the raw materials), it is important to determine the better processing conditions for the high-pressure extraction methods that allow the recovery of target fractions with appropriated extraction yield. A multi-regression analysis associated with response surface methodology (RSM) is an efficient approach to optimize the extraction conditions, as performed at Chapters 3 and 4.

Finally, as the last item, the recovered fractions can be evaluated and characterized by observing the chemical composition (by HPLC, CG among other techniques), the bioactivities

associated (antioxidant, antimicrobial, anti-viral, anti-inflammatory, anti-diabetic, anti-obesity, anti-tumoral, among others), the functional and technological properties (such as natural colorant, substitute of fat, foaming, emulsifying and gelling capacities, among others), or nutritional quality (fatty acids and amino acid compositions in fat and protein-rich fractions, for example), to help to define their potential applications.

5.4 CONCLUSION

The sequential recovery of anthocyanin- and pectin-rich fractions from jaboticaba by-product, using pressurized DESs aqueous solutions, was successfully performed without depressurizing the system in a unique extraction unit. Both fractions showed high technological and bioactive quality, with high potential for application in food and pharmaceutical formulations. Through a green metric tool, the approach considering high-pressure methods combined with DES solutions showed a *Green Certificate* value of 90.35, being an effectively green extraction that can be adapted for other vegetal matrices and target compounds.

FINAL CONSIDERATIONS AND FUTURE STUDIES

From the obtained results so far, it is possible to conclude that the use of aqueous solutions of DES combined with high-pressure fluid technologies can be used to valorize jaboticaba processing by-products obtaining added-value products. The selection of the DES starting components through *in silico* and experimental analysis is crucial to provide high selectivity towards the target compounds. Aiming to decrease the energy consumption and process time, the combination of an aqueous solution of DES (47% ChCl:Ma) with PLE was efficient to obtain anthocyanin-rich extracts with a high yield (107.27 mgC3G/100g), besides resulting in good thermostability and high antioxidant, antidiabetic and antiobesity potentials. Therefore, its application as a natural colorant with health benefits can be a viable alternative. Besides, the protective effect of DES on anthocyanin stability, maintaining the extract characteristics, suggests the application of the recovered fraction without solvent removal, because the solvent may act as a delivery vehicle of anthocyanin in pharmaceutical or nutraceutical applications can be a viable alternative.

The sequential extraction of pectin using SWE modified by Ca:Glu:Wa results in a good yield (13.36%) and short time (5 min). Furthermore, the DES presented positive effects on pectin functionality, suggesting the application of the recovered pectin-fraction in nutraceutical and functional foods, mainly in fat food systems such as stabilizing or emulsifiers.

The sequential extraction approach, performed in one extraction unit, can be considered a genuine green extraction methodology a green metric analysis and can be adapted for by-products from other residual biomasses.

Although the optimization of extraction conditions contributes to the scale-up of the process, the definition of the process costs and a refined study of the scale-up procedures are

essential for an industrial application. Also, a viable alternative for the DES recovery from the system and its recycling is still a processing challenge. For full use of the by-product, taking into account the principles of biorefinery, it is also necessary to provide an adequate destination for the resulting solid material after pectin extraction (final residue).

Besides, future studies about the digestibility of anthocyanin-rich extracts in DES could be performed to confirm the benefits of maintaining the solvent with the extract, due to its contribution to anthocyanin stability and disponibility. Furthermore, it is necessary to continue the *in vitro*, *in vivo*, and clinical trial assays to confirm the human health benefits of both obtained fractions or attest these food ingredients as functional.

Nevertheless, the present study contributes to the advance the use of Deep Eutectic Solvent (DES) in extraction processes, especially high-pressure techniques, as an alternative green approach, and applied to increase the value of agro-industrial by-products. Furthermore, this research supports the dissemination of *jaboticaba*, a Brazilian underexplored fruit, as an alternative source of valuable components for the food and pharmaceutical industries. Therefore, this thesis contributes to the development of this research area, promotes more efficient utilization of natural resources, and agrees with international efforts to promote economic growth through inclusive industrialization and fostering innovation (Sustainable Development Goals from United Nations – SDGs/UN).

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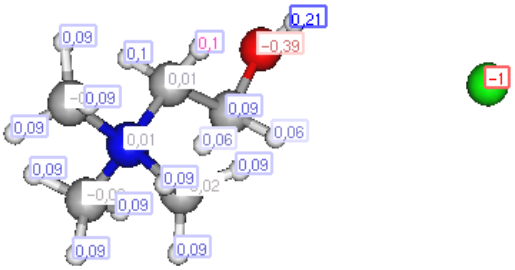
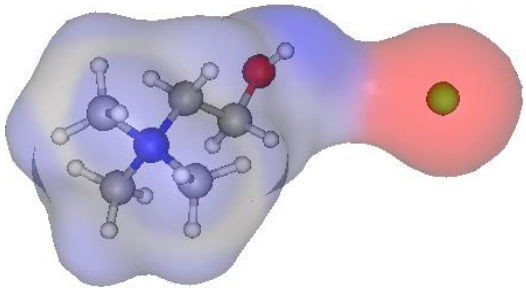
APPENDIX A - Molecular 3D lowest energy structure (left) and their σ-surface (right) of NADES components and target compounds.





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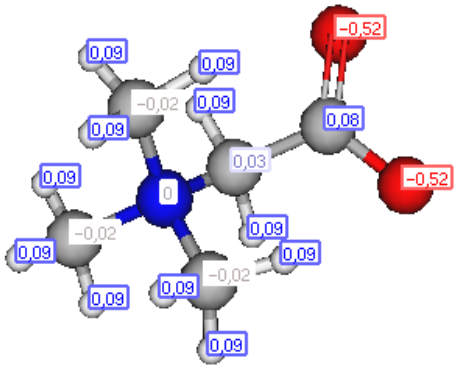
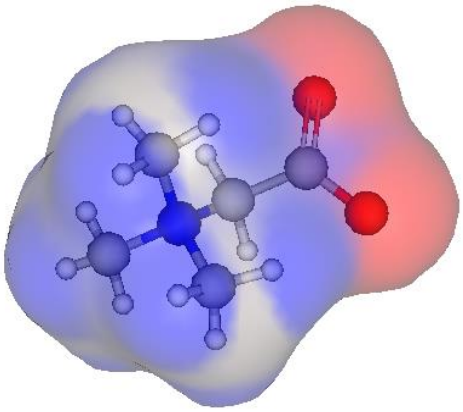
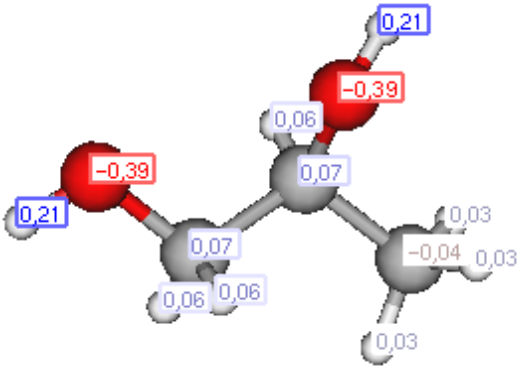
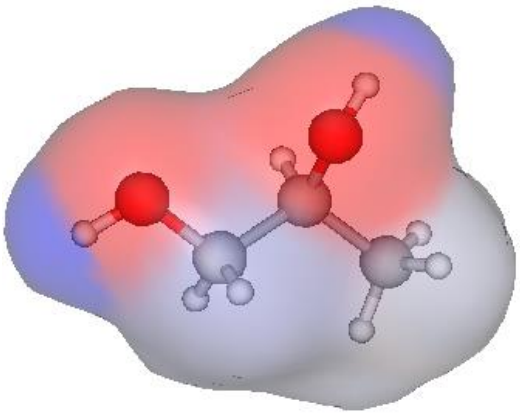


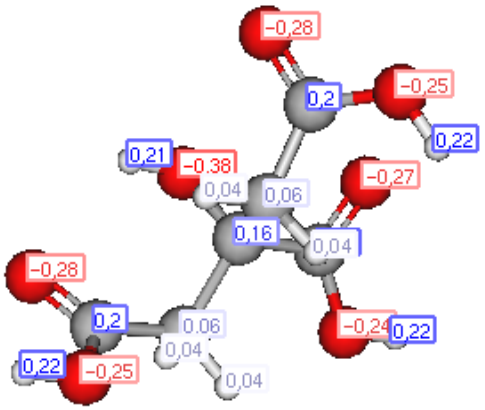


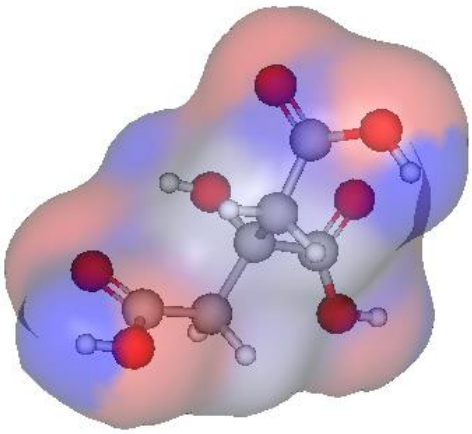
Betaine





Propylene glycol





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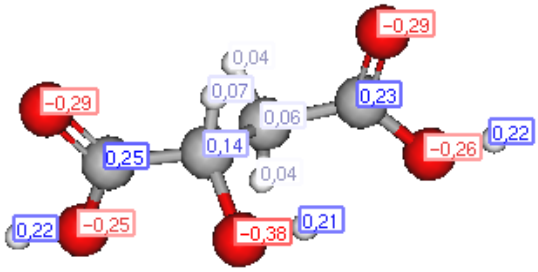

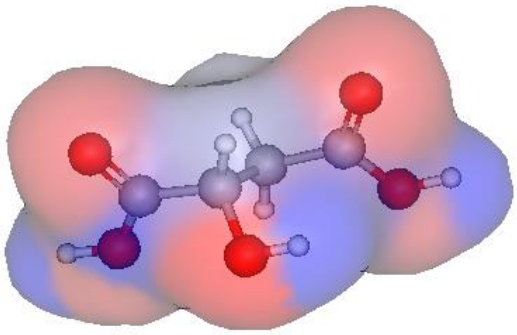

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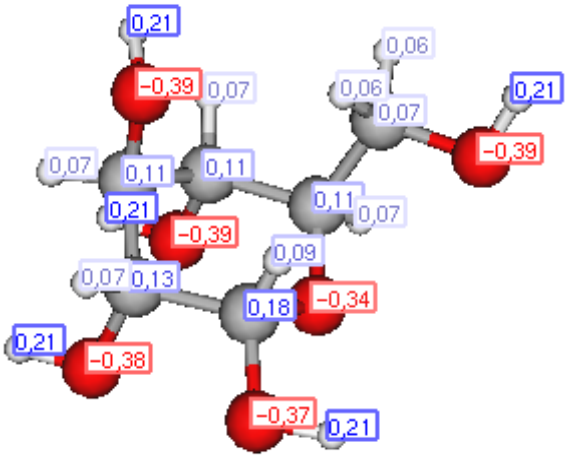
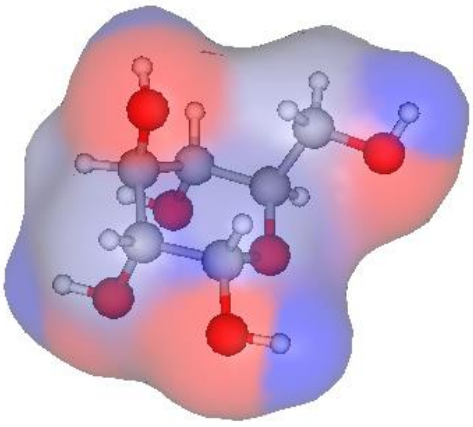
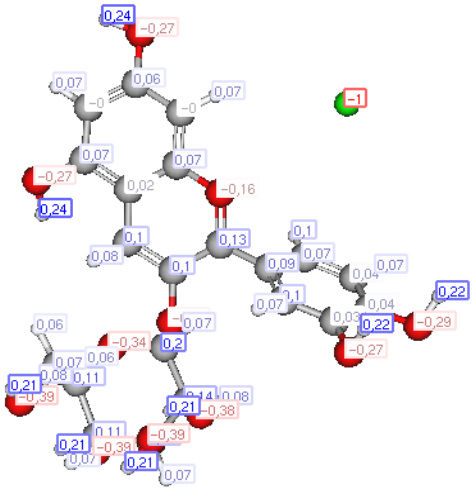
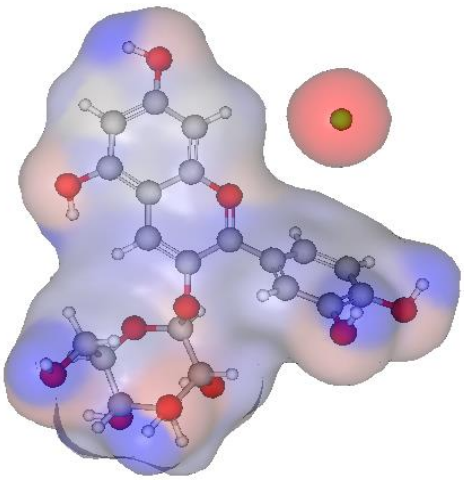


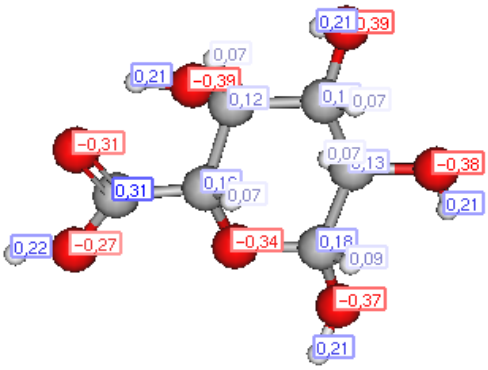


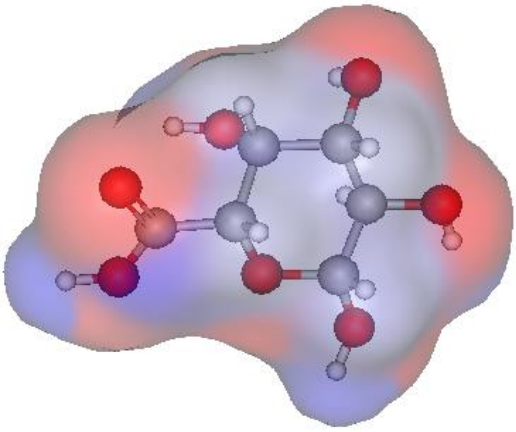
Glucose





Cyanidin-3-*O*-glucoside

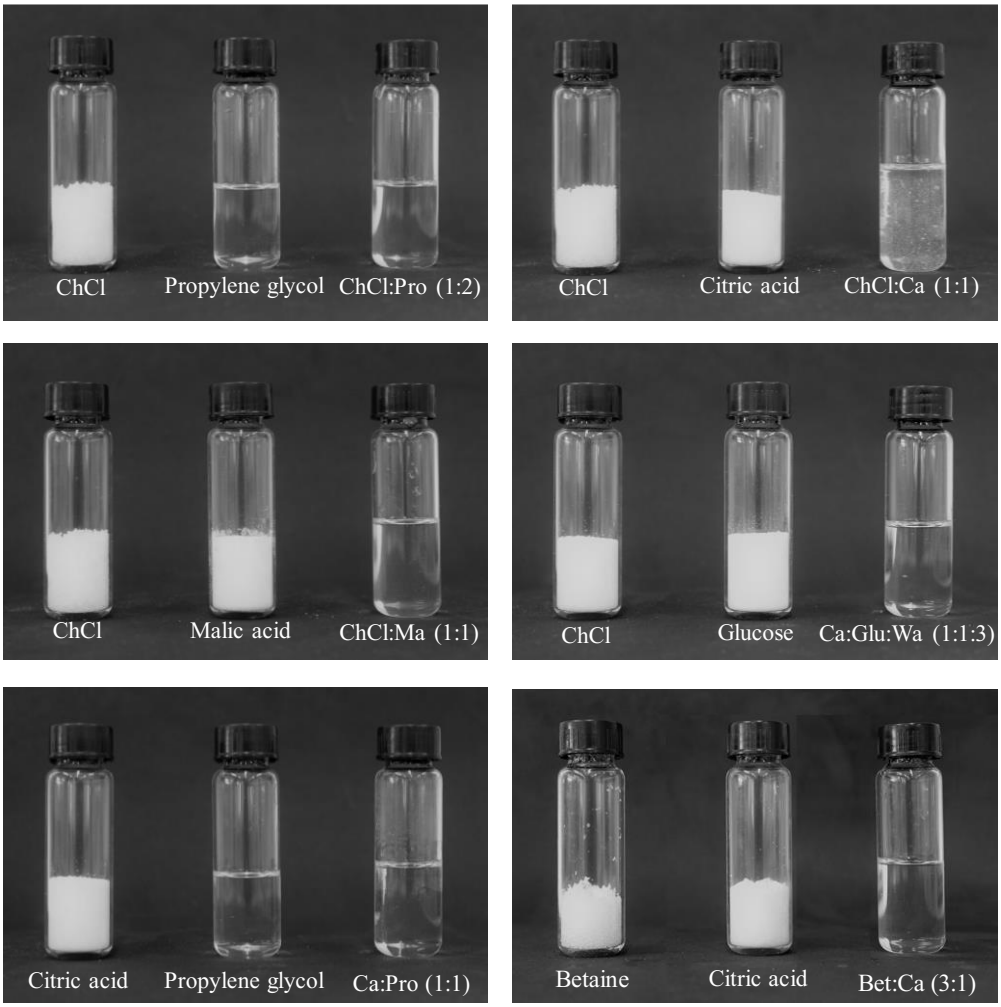




D-galacturonic acid

Note: The red regions indicate negative charge density and the blue region indicates the positive charge density

APPENDIX B - Visual appearance of deep eutectic solvent (DES) and its components



APPENDIX C - Regression coefficients and statistical parameters of multi regression adjustment of each response variable in the central composite rotatable design (CCRD).

Table 1S – Regression coefficients and statistical parameters of multi regression adjustment of each response variable in the Central Composite Rotatable Design (CCRD).

Response variables	Factors	Regression coefficient	Standart error	t-value	p-value	- 95 confidence	+ 95 confidence
MAP (mgC3GE/ 100g)	Constant	164.6259	8.648554	19.03508	0.000000	145.5906	183.6612
	x_1	20.8557	5.410803	3.85445	0.002679	8.9466	32.7648
	x_2	-49.5459	5.410803	-9.15686	0.000002	-61.4550	-37.6368
	x_2^2	-34.9405	5.724101	-6.10410	0.000077	-47.5392	-22.3419
	x_3	19.8607	5.410803	3.67056	0.003686	7.9516	31.7698
	x_3^2	-17.0304	5.724101	-2.97520	0.012627	-29.6290	-4.4317
R^2	0.93						
adjusted R^2	0.89						
p_{model}	< 0.01.						
$p_{lack\ of\ fit}$	0.31						
CP (%)	Constant	27.47083	0.911712	30.13102	0.000000	25.46416	29.47749
	x_1	2.76348	0.749423	3.68748	0.003579	1.11401	4.41295
	x_2	9.74812	0.749423	13.00751	0.000000	8.09866	11.39759
	x_2^2	4.68099	0.772487	6.05964	0.000082	2.98076	6.38123
	x_3	7.20305	0.749423	9.61146	0.000001	5.55358	8.85252
	$x_2.x_3$	3.49090	0.977128	3.57262	0.004375	1.34026	5.64155
R^2	0.97						
adjusted R^2	0.95						
p_{model}	< 0.01						
$p_{lack\ of\ fit}$	0.07						
AR (%)	Constant	65.9752	3.465979	19.03508	0.000000	58.3466	73.6038
	x_1	8.3581	2.168424	3.85445	0.002679	3.5854	13.1307
	x_2	-19.8559	2.168424	-9.15686	0.000002	-24.6286	-15.0833
	x_2^2	-14.0027	2.293981	-6.10410	0.000077	-19.0517	-8.9537
	x_3	7.9593	2.168424	3.67056	0.003686	3.1867	12.7320
	x_3^2	-6.8251	2.293981	-2.97520	0.012627	-11.8741	-1.7760
R^2	0.93						
adjusted R^2	0.9						
p_{model}	< 0.01						
$p_{lack\ of\ fit}$	0.31						
ABTS (μmolTE/g)	Constant	208.5968	3.731532	55.90113	0.000000	200.4665	216.7271
	x_1	11.0346	4.171979	2.64493	0.021378	1.9446	20.1246
	x_3	60.0572	4.171979	14.39537	0.000000	50.9672	69.1472
	$x_1.x_3$	-13.5963	5.439595	-2.49951	0.027941	-25.4482	-1.7445
	$x_2.x_3$	12.7259	5.439595	2.33950	0.037420	0.8741	24.5778

R^2	0.96						
adjusted R^2	0.95						
p_{model}	< 0.01						
$p_{lack\ of\ fit}$	0.09						
FRAP	Constant	537.3610	25.93233	19.65197	0.000000	463.6970	555.5458
(μmolTE/g)	x_1	54.5488	21.31624	2.28310	0.039889	10.9175	86.4168
	x_1^2	-36.1202	21.97228	-1.80962	0.093525	-78.6729	-0.8500
	x_2^2	-58.5635	21.31624	3.83439	0.002068	43.9852	119.4845
	x_3	66.8671					
	x_1x_2	63.1791					
R^2	0.88						
adjusted R^2	0.83						
p_{model}	< 0.01						
$p_{lack\ of\ fit}$	0.18						

Note - x_1: DES concentration in water, x_2: temperature, x_3: flow rate, MAP: monomeric anthocyanin pigment content, C3GE – cyanidin-3-glucoside equivalent, PC – polymeric color, AR – anthocyanin recovery, TE: Trolox equivalent.