Universidade Federal do Rio Grande do Norte
Centro de Tecnologia
Departamento de Engenharia Química
Programa de Pós-Graduação em Engenharia Química

TESE DE DOUTORADO

Estratégias de processamento e revalorização de matérias-primas alimentares para a produção de ingredientes funcionais e sustentáveis

Edilene Souza da Silva

Orientador (a): Prof. Dra. Roberta Targino Hoskin

Natal/RN
Agosto/2023

EDILENE SOUZA DA SILVA

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A Deus, ao meu pai e avó (in memoriam) ...

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“The roots of study are bitter, but its fruits are sweet.”

Aristotle

Edilene Souza da Silva: Estratégias de processamento e revalorização de matérias-primas alimentares para a produção de ingredientes funcionais e sustentáveis. Tese de Doutorado, UFRN, Programa de Pós-Graduação em Engenharia Química. Área de concentração: Engenharia Química. Linha de pesquisa: Tecnologia e Engenharia de Alimentos. Natal/RN, Brasil.

Orientação: Profª. Drª. Roberta Targino Hoskin.

RESUMO: Um volume considerável de resíduos agroindustriais são produzidos diariamente de Norte a Sul do país. Estimativas apontam que resíduos de frutas (constituídos principalmente por peles, sementes e polpa residual) podem representar até 50% do peso da fruta original. Apesar de já ter sido amplamente demonstrado que os resíduos são fontes valiosas de compostos fitoquímicos residuais com potenciais aplicações na produção de alimentos, ainda existem poucos produtos no mercado a partir dessa matéria-prima abundante, especialmente em países em desenvolvimento como o Brasil. Segundo a Organização das Nações Unidas para Agricultura e Alimentação, a fome e a desnutrição podem ser combatidas através da inovação, a qual diz respeito não apenas ao surgimento de novas tecnologias, mas também ao uso racional de produtos e procedimentos existentes. Dessa forma, a presente tese de Doutorado tem como objetivo estudar estratégias de revalorização de matérias-primas para produzir ingredientes alimentícios derivados de frutas e vegetais e avaliá-los no que diz respeito às propriedades físico-químicas, funcionais e bioativas, bioacessibilidade, assim como a viabilidade ambiental. Especificamente, técnicas de obtenção de extratos aquosos ricos em polifenois recuperados de resíduos agroindustriais de frutas (acerola e jambolão) mediante aplicação de técnicas de extração convencional (extração sólido-líquido) e emergentes (ultrassom) foram avaliados e comparados no que diz respeito a performance e viabilidade ambiental através da ferramenta Avaliação do Ciclo de Vida (ACV). Além disso, a produção de ingredientes alimentícios do tipo proteína-polifenol foi conduzida por *spray drying* usando diferentes estratégias: a) polifenois recuperados de resíduo e polpa do sabugueiro (*elderberry*) americano encapsulados com isolado de proteína de soja (SPI) e amido de tapioca (TS) e b) polifenois extraídos de folhas de alecrim ou recuperados do resíduo da uva muscadine usando a nova proteína de inseto (*insect protein*) ou a mistura de proteína de inseto e proteína de ervilha (*pea protein isolate*) como coadjuvantes de secagem. O método UAES (Extração assistida por ultrassom e agitação) após 90 min apresentou o maior teor de polifenois, e a ACV revelou que o UAES teve o menor impacto ambiental entre todos os protocolos de extração. Para as partículas do elderberry, aquelas produzidas com SPI tiveram maior solubilidade e fluidez, bem como foram mais

bioacessíveis, enquanto as com o TS apresentaram maiores teores de compostos bioativos (TPC, ANC e PAC). As partículas protéina de inseto-polifenol apresentaram boas características funcionais. O melhor desempenho para a secagem em spray foi obtida para as partículas derivadas da mistura de proteína de inseto e proteína de ervilha, ao passo que as partículas derivadas do alecrim foram mais bioacessíveis. Esse trabalho aborda assuntos de grande interesse mundial na área de ciência e tecnologia de alimentos e produz resultados científicos importantes para avanços na área de processamento de alimentos alternativos, novas matérias-primas e revalorização de resíduos alimentares. Sobretudo apresenta novas aplicações para a produção de ingredientes alimentícios bioacessíveis, com propriedades funcionais desejáveis usando estratégias “amiga” do meio ambiente.

Palavras-chave: revalorização, alimentos funcionais, meio ambiente, proteínas alternativas.

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Orientação: Profª. Drª. Roberta Targino Hoskin.

ABSTRACT: A considerable volume of agro-industrial by-products are produced daily from North to South of Brazil. Fruit by-products, consisting mainly of skins, seeds, and residual pulp, constitute up to 50% of the initial weight of the fruit. Although it has been demonstrated that fruit by-products are valuable sources of residual phytochemicals with potential applications in the food industry, there are only few products in the market made from this abundant raw material, especially in developing countries like Brazil. According to The Food and Agriculture Organization (FAO), innovation is a valuable tool against hunger and malnutrition, and this includes not only the development of new technologies, but also the rational use of existing products and procedures. Thus, this PhD dissertation demonstrates strategies for the revalorization of underexplored food materials to produce plant-based ingredients, that were assessed regarding their physicochemical, functional, and bioactive properties, polyphenol bioaccessibility, and environmental viability. Specifically, extraction protocols designed to obtain aqueous polyphenol-rich extracts recovered from tropical fruit residues (acerola and jambolan) using conventional (solid-liquid extraction) and emerging (ultrasound) techniques were evaluated performance-wise. In addition, the environmental viability of each one of the extraction methods was evaluated by the LCA tool. Furthermore, the production of protein-polyphenol food ingredients was carried out by spray drying using different strategies: a) polyphenols recovered from American elderberry residue and juice encapsulated with soy protein isolate (SPI) and tapioca starch (TS) and b) polyphenols extracted from rosemary leaves or recovered from muscadine grape residue using a novel insect protein or a mixture of insect protein and pea protein isolate as drying carriers. The UAES protocol (Ultrasound Assisted Extraction and Stirring) after 90 min showed the highest total polyphenol content, and the LCA revealed that the UAES had the lowest environmental impact among all extraction protocols. For elderberry particles, those produced with SPI had greater solubility and flowability, as well as higher bioaccessibility, while those with TS had higher levels of bioactive compounds (total phenolics, anthocyanins and proanthocyanidin). The insect protein-polyphenol particles showed good functional characteristics and the best spray drying performance were observed

when insect protein and pea protein blend was used while rosemary-derived particles were more bioaccessible. This work addresses issues of great interest worldwide in food science and technology and unveils important scientific results for the advance of alternative foods processing, new raw materials and repurposing of food waste. Furthermore, new applications for bioaccessible food ingredients with desirable functional properties using environmentally friendly approaches are shown here.

Keywords: repurposing, functional food, environmentally friendly, alternative protein.

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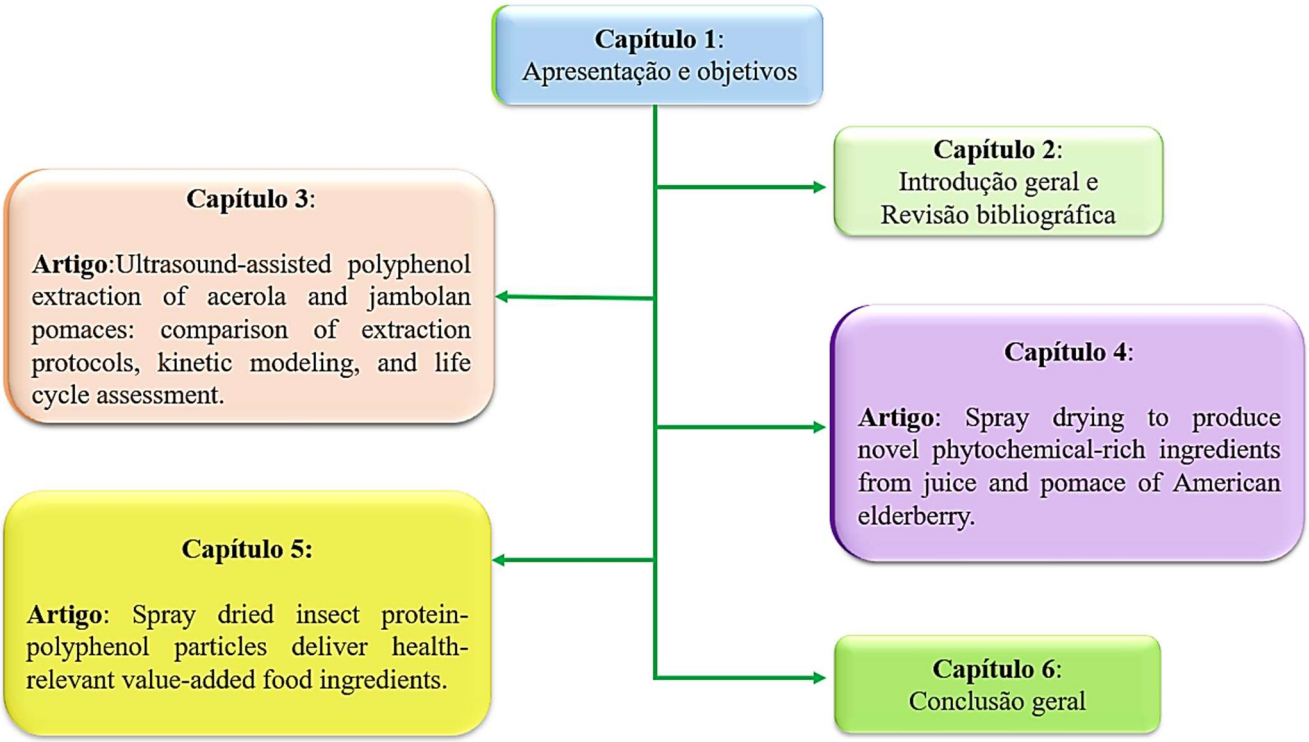
CAPÍTULO 1

APRESENTAÇÃO E OBJETIVOS

Capítulo 1. Apresentação

Esta tese é subdividida em 6 capítulos, mostrados esquematicamente na Figura 1. Inicia com o presente capítulo que trata da estruturação da tese e seus objetivos. O capítulo 2 é constituído por uma introdução geral onde a revisão bibliográfica dos principais assuntos abordados nesse trabalho é apresentada. Os capítulos 3, 4 e 5 apresentam os artigos científicos que fazem parte dessa tese, todos relacionados com o tema de estratégias de valorização de matéria-primas alimentares. O capítulo 6 finaliza este documento apresentando uma conclusão geral construída com o objetivo de sumarizar o significado dos principais achados obtidos durante a produção dessa tese de doutorado.

Figura 1 – Fluxograma da tese.



1.1 Objetivos

1.1.1 Objetivo geral

Avaliar estratégias de revalorização de matérias-primas para produzir ingredientes alimentícios derivados de frutas e vegetais e avaliá-los no que diz respeito as propriedades físico-químicas, funcionais e bioativas, bioacessibilidade, assim como a viabilidade ambiental.

1.1.2 Objetivos específicos

- Obtenção de extratos aquosos ricos em polifenois recuperados de resíduos agroindustriais de frutas (acerola, *Malpighia emerginata* e jambolão, *Syzygium cumini*) mediante aplicação de técnicas de extração convencional (extração sólido-líquido) e soluções emergentes (ultrassom);

- Avaliação da performance da extração de polifenois recuperados de resíduos agroindustriais de frutas por diferentes métodos através de modelagem matemática e comparação dos protocolos em relação a viabilidade ambiental avaliada através da ferramenta ACV (Avaliação do Ciclo de Vida);

- Produção de ingredientes alimentícios do tipo proteína-polifenol por *spray drying* usando diferentes estratégias: a) polifenois recuperados de resíduo do sabugueiro americano (*elderberry, Sambucus nigra canadensis*) complexados com isolado de proteína de soja e amido de tapioca e b) polifenois extraídos de folhas de alecrim (*Rosmarinus officinalis L)* ou recuperados do resíduo da uva muscadine (*Vitis rotundifolia)* usando a nova proteína de inseto (*insect protein*) ou a mistura de proteína de inseto e proteína de ervilha (*pea protein isolate*) como coadjuvantes de secagem.

- Caracterizar os ingredientes alimentícios do tipo proteína-polifenol produzidos em relação a suas propriedades físico-químicas, morfológicas e bioativas, bem como a performace em relação a digestão gastrointestinal simulada *in vitro* (bioacessibilidade dos polifenois).

CAPÍTULO 2
INTRODUÇÃO GERAL E REVISÃO BIBLIOGRÁFICA

Capítulo 2. Introdução geral

Nesse capítulo, as frutas acerola, jambolão, sabugueiro americano e uva muscadine estudadas nesta tese são apresentadas. Suas características botânicas, fitoquímicas, além de dados relevantes sobre os resíduos agroindustriais das mesmas são mostrados e discutidos. Além das frutas citadas, as características da planta medicinal alecrim e proteínas alternativas (proteína de inseto) também são abordadas. Técnicas de processamento e ferramenta de avaliação de impacto ambiental utilizadas neste trabalho finalizam a presente revisão bibliográfica.

2.1 Revisão bibliográfica

2.2 Matérias-primas vegetais: frutas e resíduos derivados

O Brasil possui grande biodiversidade natural, sendo o berço de espécies nativas, como culturas adaptadas ao clima tropical e semi-tropical (Gonçalves-Souza et al., 2021). Uma ampla variedade de matérias-primas vegetais são processadas pela indústria e o país é uma das nações líderes do agronegócio no mundo, sobretudo no que diz respeito a frutas tropicais (Ferreira, Arcanjo, & Peron, 2023).

No entanto, existem dificuldades em relação ao pleno aproveitamento desses recursos naturais, não só no Brasil, mas também no mundo. De fato, frutas, hortaliças, raízes e sementes oleaginosas representam 40 a 50% do volume de perdas ou desperdícios na produção mundial de alimentos (FAO, 2023a). A Organização da Nações Unidas para a Alimentação e a Agricultura (FAO) estima que 6% das perdas globais de alimentos ocorrem na América Latina e no Caribe, e a região perde e/ou desperdiça anualmente cerca de 15% de seu suprimento de alimentos. Perdas e desperdícios têm impacto na viabilidade dos sistemas alimentares, no fornecimento de alimentos local e globalmente, nos ganhos do produtor e no preço final apresentado ao consumidor. Além disso, prejudicam o ecossistema ao utilizar os recursos naturais de forma insustentável.

No que diz respeito especificamente a atividade agroindustrial, um volume considerável de resíduos são produzidos diariamente de Norte a Sul do país. Estimativas apontam que resíduos de frutas (constituídos principalmente por peles, sementes e polpa residual) podem representar até 50% em peso da fruta original (Calderón-Oliver & López-Hernández, 2022). Apesar de já ter sido amplamente demostratdo que os resíduos são fontes valiosas de compostos fitoquímicos residuais com potenciais aplicações na produção de alimentos (Alwazeer et al., 2023), ainda existem poucos produtos no mercado gerados a partir de subprodutos (Aschemann-Witzel et

al., 2023), especialmente em países em desenvolvimento como o Brasil. Segundo a FAO (2023b), a fome e a desnutrição podem ser combatidas através da inovação, a qual diz respeito não apenas ao surgimento de novas tecnologias, mas também ao uso racional de produtos, procedimentos ou conformações já conhecidos e novos.

2.3 Acerola (*Malpighia emerginata* D.C)

A acerola pertence à família *Malpighiaceae*, conhecida comumente por "Acerola cherry", “Jamaican cherry” ou "Barbados cherry" (Belwal et al., 2018; Silva et al., 2017). O cultivo da acerola foi iniciado por indígenas americanos por volta do período pré-colombiano, sendo disseminada pelas ilhas da América Central através de movimentos migratórios. Ganhou a denominação de “acerola” devido a comparação com a fruta “azarola” (cultivada na Europa). No Brasil, seu cultivo comercial pioneiro ocorreu na Zona da Mata pernambucana, mais precisamente no município de Pau d’alho – PE e depois difundiu-se para outras regiões do país (Souza et al., 2017). O interesse comercial por essa fruta (**Fig. 2**) foi motivado por sua alta concentração de vitamina C, que chega a ser 80 vezes ao encontrado em fontes tradicionais como laranja e limão. Além disso, essa fruta tropical possui elevados teores de vitamina B3, e outros compostos bioativos como flavonoides e antocianinas (Miskinis, Nascimento, & Colussi, 2023).

Figura 2 - Acerola (*Malpighia emerginata D.C.*)

Fonte: Puravida (2019).

No Brasil, a produção da acerola é expresssiva, com produção de 60.966 toneladas em 2017 (IBGE, 2023). Devido às suas propriedades físico-químicas, organolépticas e nutricionais, a produção de acerola tem aumentado nos últimos anos e ganhado maior importância no que diz respeito a seu uso industrial em setores como o alimentício, farmacêutico e cosmético (Gualberto et al., 2021). Benefícios a saúde derivados da alta concentração de vitamina C e os outros fitoquímicos como carotenoides e compostos fenólicos (Nascimento et al., 2018), tais

como ação anti-inflamatória, antioxidante, anticancerígena e anti-hiperglicêmica já foram reportadas na literatura (Belwal et al., 2018). Por todos esses motivos, a acerola tem ampla comercialização no mundo, tendo como principais consumidores Japão, União Europeia e Estados Unidos (Silva et al., 2017).

As frutas são principalmente utilizadas na indústria de polpas e sucos como também iogurte, licor, sorvete, cosméticos, suplemento alimentar, e na indústria farmacêutica para extração de vitamina C e fenóis (Farinelli et al., 2021; Silva et al., 2017). A acerola é comprovadamente uma das frutas de comercialização estabelecida, incluindo exportação para multiplos países (Silva et al., 2020). Em paralelo, significante volume de resíduos agroindustriais de acerola é produzido em resposta aos grandes volumes de utilização da fruta na indústria de alimentos brasileira. Por exemplo, para a processo de extração do suco ou da polpa, a quantidade de resíduo gerada na produção de sucos chega a 20-60% da matéria-prima (Miskinis et al., 2023). No entanto, grande parte desse resíduo é destinado ao descarte ou subutilizado, o que resulta em desperdício de energia e matéria-prima. Esses resíduos podem ser usados de forma mais eficaz tendo em vista seu rico teor em fitoquímicos (Silva, Duarte, & Barrozo, 2019), com teores que podem ser iguais ou maiores ao da sua polpa (Rezende, Nogueira & Narain, 2017). A necessidade de novos métodos para diminuir o impacto no meio ambiente e aumentar a eficiência dos recursos foi desencadeada pela mudança no ambiente global em direção ao desenvolvimento sustentável e à utilização racional de recursos (Miskinis et al., 2023).

Diferentes estudos abordaram o aproveitamento do resíduo de acerola. Por exemplo, seu uso foi testado para o desenvolvimento de biocompósitos de fécula de mandioca utilizando processos de extrusão e moldagem por injeção (Reinaldo et al., 2021). Além disso, nosso grupo de pesquisa e outros tem se dedicado a entender o potencial do resíduo de acerola para a produção de suplementos e ingredientes alimentares através de diferentes técnicas de secagem (Moraes et al., 2017; Monteiro et al., 2020; Nóbrega et al., 2015). Esse interesse é justificado por sua rica composição fitoquímica e nutricional, tais como compostos fenólicos, carotenoides, e ácido ascórbico, além da possibilidade de produzir produtos com atributos físico-químicos e organolépticos desejáveis, que atendem demandas das indústrias alimentícia, farmacêutica e química (Miskinis et al., 2023).

2.4 Jambolão (*Syzygium cumini* L.)

O jambolão é uma fruta da família Myrtaceae do gênero *Syzygieae* (Nascimento-Silva, Bastos & Silva, 2022). Essa fruta exótica é natural da Índia e sua produção foi difundida entre países

asiáticos, dentre eles Filipinas, Tailândia e Malásia, sendo também incorporada a países da África e em diversos países da América do Sul e América Central, onde se adequou a climas entre tropical a temperado (Sabino, Brito & Silva Júnior, 2018). O jambolão (**Fig. 3**) é também conhecido como "Black Jamun" e bastante utilizado com planta medicinal na Índia. Todas as partes da planta, incluindo folhas, cascas, frutos, raízes e sementes, têm usos medicinais reportados na literatura (Kumar et al., 2023). É usada na medicina tradicional como remédio digestivo, adstringente, antibacteriano e antidiarréico. Também possui efeitos antidiabéticos, anti-inflamatórios, antimicrobianos, antipiréticos, antiobesidade e antioxidante (Ahmad, Nawab & Kazmi, 2019; Negri et al., 2022).

Figura 3- Jambolão (*Syzygium cumini*).

Fonte: Frutíferas (2019).

Devido à sua curta vida útil e alta perecibilidade, a fruta é consumida *in natura* logo após a colheita ou, em outros países como a India, é usada para fazer produtos com valor agregado, como vinhos, sucos e geleias, em que são gerados resíduos que representam de 10-47% do fruto, principalmente compostos por sementes (Kumar et al., 2022).

Essa fruta tem sido pesquisada devido não apenas por suas propriedades organolépticas como cor e docura, além do alto teor de fitoquímicos, em especial antocianinas (Nascimento-Silva, Bastos & da Silva, 2022). No entanto, surpreendentemente, os frutos de jambolão são pouco explorados comercialmente no Brasil (Santiago et al., 2016), apesar de sua riqueza fitoquímica em compostos fenólicos, flanonoides, ácido ascórbico, capacidade antioxidante demonstrada em estudos do nosso grupo de pesquisa e outros (Borges et al., 2016; Correia et al., 2012; Farias et al., 2020). Ainda, seus atributos tecnológicos para uso em alimentos como corante natural e antioxidantes, e na área farmacêutica como medicamentos são reconhecidos na literatura científica, mas ainda pouco explorado pela indústria (Ayenampudi, Verma & Adeyeye, 2022; Koop et al., 2021).

O resíduo do jambolão composto por cascas e sementes é também uma rica fonte de macronutrientes, como carboidratos, proteínas e lipídios, também minerais e vitaminas (Correia et al., 2012; Kumar et al., 2022). Suas sementes são insumos de baixo custo e apreciado por seu potencial fitoquímico e biológico pela presença de ácidos fenólicos, flavonoides, taninos hidrolisáveis (Tak et al., 2022). Logo, devido a atributos nutricional e fitoquímico possuem potencial aplicação em alimentos funcionais ou composições em produtos terapêuticos (Borges et al., 2016; Kumar et al., 2022).

2.5 Fruto do sabugueiro - *Elderberry* (*Sambucus nigra* L.*)*

O sabugueiro (*elderberry*, em inglês) pertencente a família Adoxaceae é uma planta de fruto brilhante (**Fig. 4**), com formato de uva. O termo "*nigra*" refere-se à sua cor, ao passo que "*Sambucus*" expressa seu formato já que significa "pequena harpa" em latim. Essa pequena fruta do tipo *berry* cresce em praticamente todo o continente europeu, oeste da Ásia, norte da África e nos Estados Unidos da América. Os desafios na classificação taxonômica são provavelmente resultado de sua adaptação a várias regiões climáticas e consequente extensões geográficas. Por exemplo, há uma distribuição natural em grande parte da Europa Ocidental, no entanto, esta espécie de planta está também presente no Norte (Noruega e Suécia) e no extremo leste (Asgary & Pouramini, 2022; Corrado et al., 2023). De acordo com a taxonomia, a espécie européia foi nomeada *S. nigra var. nigra*, e a espécie norte-americana como *S. nigra var. canadensis*, além de outras espécies européias (*S. ebulus*) e norte-americanas (*S. cearulea*) (Coman et al., 2018). As espécies *canadensis* (norte-americana) e *nigra* (Europeia) possuem composição química distinta. A espécie norte-americana pode apresentar até 50% a mais de antocianinas aciladas comparada a européia. Esse fato constitui uma importante diferença do ponto de vista tecnológico, já que antocianinas aciladas são capazes de estabilizar mais eficientemente a cor dos produtos derivados (Osman et al., 2023).

Seus extratos tem ganhando popularidade para usos como ingredientes nutracêuticos, suplementos alimentares e como insumo para as indústrias farmacêuticas, além de seu uso na indústria de alimentos como corantes e aromatizantes (Ferreira, Silva & Nunes, 2022). Compostos polifenólicos como flavonois, ácidos fenólicos, proantocianidinas e antocianinas, que dão à fruta sua cor roxa intensa, estão presentes em concentrações significativas (Asgary & Pouramini, 2022), tanto na polpa quanto no resíduo (pele e semente) do *elderberry* (Coman et al., 2018).

Figura 4 - Sabugueiro americano (*Elderberry, Sambucus nigra L.*).

Fonte: Adobe Stock (2023).

O interesse comercial por *elderberry*, bem como o número de estudos científicos sobre essa fruta, ganhou grande impulso nos últimos anos, já que seus bem reconhecidos efeitos antivirais que promovem redução da duração e a gravidade dos sintomas do resfriado comum e da gripe em adultos, foi alvo de especial interesse durante a recente pandemia causada pela síndrome respiratória aguda grave (SARS-CoV-2) chamada abreviadamente de COVID-19 (Asgary & Pouramini, 2022; Harnett et al., 2020). Como resultado, ressurgiu o interesse pelo cultivo e horticultura do *elderberry* americano, levando ao desenvolvimento de vários novos produtos, muitos dos quais são suplementos alimentares (Thomas et al., 2020). Além disso, suas propriedades antioxidantes, anti-inflamatórias, imunoestimulantes, quimiopreventivas e ateroprotetoras já foram devidamente registradas em estudos científicos (Tundis et al., 2018).

Os subprodutos da indústria de suco de sabugueiro incluem fitoquímicos, como entre os polifenois a presença de antocianinas, também macromoléculas como carboidratos, sendo glicose e xilose os principais componentes (Nemetz, Schieber & Weber, 2021; Veloso et al., 2022). O resíduo agroindustrial da fruta contabiliza 25-40% do peso total do fruto, constituído principalmente por cascas e sementes, são muitas vezes destinados a ração animal ou adubação (Veloso et al., 2022). Além de carboidratos, o resíduo de sabugueiro possui proteínas, fibras, minerais e vitamina B6 (Costa et al., 2021). No entanto, esse material é atualmente subexplorado e passível de promover impactos econômico e ambiental (Veloso et al., 2022) que poderiam ser minimizados através do estabelecimento de novos protocolos de utilização e

producão de produtos amigos do ambiente (Domínguez et al., 2021).

2.6 Uva muscadine (*Vitis rotundifolia*)

A uva muscadine (**Fig. 5**) é principalmente cultivada no sudeste dos Estados Unidos. Nesta zona, as muscadines são apreciadas pelo seu sabor e aroma característicos e são cultivadas tanto para produção de suco, geleia e vinho como para consumo *in natura*. Possuem a peculiaridade de possuir casca mais espessa que a variedade de uvas comuns de mesa e possuem alta concentração de compostos polifenólicos com características antioxidantes antiplaquetárias, entre eles flavonois, ácido elágico e antocianinas. Quando comparadas as outras variedades de uvas comerciais, são menos conhecidas no mercado, e esforços para desenvolver novos cultivares com maior produtividade e apelo comercial além da diversificação de produtos derivados são importantes para impulsionar sua introdução comercial (Conner & Worthington, 2022; Hickey et al., 2019).

Entre as principais cultivares comerciais de muscadine estão a Carlos, Scuppernong, Nesbitt e a Noble. A *Vitis rotundifolia* apresenta benefícios a saúde humana como a atenuação de diabetes tipo 2, cardiopatias, síndrome metabólica e inflamações. Esses benefícios resultam da presença de polifenois como antocianinas, proantocianidinas e a capacidade antioxidante atribuída a essas moléculas ativas (Yuzuak & Xie, 2022).

Figura 5- Uva Muscadine (*Vitis rotundifolia*).

Fonte: Alves (2023)**.**

Os subprodutos da produção do suco de uva ou vinícola são compostos por cascas, sementes, polpa e sólidos residual. O rendimento desses resíduos consiste em 20% das uvas colhidas

(Wang et at., 2010) e podem apresentar maiores teores de fenólicos totais comparado ao suco da uva muscadine (Xu et al., 2017). Eles também são compostos por taninos hidrolisáveis, antocianinas e flavonoides, incluindo quercetina e miricetina (Sandhu & Gu, 2010). Os polifenois do resíduo possuem atributos biológicos como atividade antioxidante, antibacteriana e antibiofilme, e o extrato de casca demonstrou atividade anticancerígena, evidenciando assim a aptidão para aplicação em áreas como a farmacêutica e alimentícia (Hickey et al., 2019; Xu et al., 2014). O resíduo de uva tornou-se disponível para possível utilização como resultado do aumento da produção comercial de uva muscadine, apresentando propriedades aplicáveis em um ingrediente na formulação de alimentos (Wang et al., 2010).

2.7 Alecrim (*Rosmarinus officinalis* L.)

O alecrim (*Rosmarinus officinalis* L.*,* **Fig. 6**) é uma erva medicinal pertencente à família Lamiaceae*.* Originada na região do Mediterrâneo, pode ser encontrada em praticamente todos os países do mundo. O sul da Europa abriga variedades selvagens e domésticas de *R. officinalis*, subdivididas em três espécies: *R. officinalis subsp*. *officinalis*, *R. officinalis subsp*. *palaui malag* e *R. officinalis subsp. valentinus ferrer* (mais recente encontrada no sudeste da Espanha) (Aziz et al., 2022). É uma planta perene de aroma característico que pode crescer até dois metros de altura, com ramos arbustivos cobertos de folhas verdes (Oliveira, Camargo & Oliveira, 2019). O alecrim por ser uma especiaria singular, é comercializado para uso como antioxidante nos Estados Unidos e na Europa (Nieto, Ros & Castillo, 2018). No Brasil há diferentes variedades e cultivares dentro da espécie, algumas mais pesquisadas e comerciais são: *Rosmarinus officinalis* var. *albiflorum*, *R. officinalis* var. *angustissimu*, *R. officinalis* var. *genuina* f. *erectus*, *R. officinalis* var. *genuina* f. *humilis*, *R. officinalis* var. *genuina* f. *Albiflorus.* Eles são apreciados na culinária especialmente como tempero por agregar sabor e aroma aos alimentos (Oliveira & Veiga, 2019; Silva Junior & Osaida, 2007).

Devido as suas propriedades terapêuticas ficou conhecida a princípio na medicina popular e depois na indústria cosmética e farmacêutica. A sua capacidade antioxidante e anti-inflamatória é atribuída à presença dos ácidos carnósico e ursólico e do carnosol em seu extrato orgânico, o que justifica pesquisas direcionadas não apenas para doenças inflamatórias, mas também micoses, câncer de pele e cicatrização de feridas (Macedo et al., 2020). Seu óleo essencial tem sido utilizado para preservar alimentos, como antisséptico e para fins adstrigentes, que datam de anos antes do surgimento da refrigeração. Possui ainda efeitos antimicrobianos, aromaterapêuticos e anticarcinogênicos (Turasan, Sahin & Sumnu, 2015). Além disso, extratos

e óleo essencial de alecrim podem atuar na estabilização de óleos e gorduras em alimentos como a manteiga, evitando oxidação e ranço, e também em produtos cárneos fermentados (Sasikumar, 2012).

Figura 6 - Alecrim (*Rosmarinus officinalis* L.)

Fonte: Antunes Oliveira & Veiga (2019).

Estudos mostram um bom desempenho do alecrim como suplementação dietética em cultura de peixes (Naiel et al., 2020) e no desenvolvimento de fitoquímicos encapsulados para aplicação em produtos farmacêuticos, nutracêuticos e cosméticos (Bankole et al., 2020). Ja foi demonstratdo na literatura científica seus efeitos biológicos tais como atividade antitumoral, antimicrobiana, neuroprotetora, antidepressiva, antiobesidade, antireumáticas, carminativas, antiespasmódicas, hepatoprotetoras, antiangiogênica e potencial terapêutico para a doença de Alzheimer (Aziz et al., 2022; Nieto et al., 2018; Sasikumar, 2012). Extratos de alecrim tem um aroma e sabor únicos que proporcionam vantagens tecnológicas tendo em vista as suas diversas aplicações (Nieto et al., 2018).

3. Técnicas de processamento aplicados a frutas e derivados

Operações de processamento com o fim de preservar as características de alimentos são necessárias para aumentar a vida útil e o valor comercial dos mesmos. O processamento de alimentos proporciona valor agregado, maior variedade disponível e planejamento agrícola adequado. Pode ser classificado em térmicos (como branqueamento, resfriamento, congelamento, secagem, pasteurização) e não-térmicos (como alta pressão, campo elétrico pulsado, luz ultravioleta, ozônio e sonicação) (Aaliya et al., 2021).

Existe um esforço contínuo por parte da indústria de alimentos para aprimorar técnicas de

produção a fim de garantir qualidade e rentabilidade, além de atender a recente forte demanda por processos "verdes" e "amigos do ambiente" (Arshad et al., 2021; Calín-Sánchez et al., 2020; Correia et al., 2017). Essas técnicas procuram estabelecer respostas para atender algumas desvantagens reportadas para técnicas tradicionais de processamento de alimentos que acarretam em perdas de compostos nutricionais, baixa eficiência de produção, operações que consomem tempo, energia ou uso de grandes quantidades de água. O desafio é estabelecer protocolos que possam ser eficientemente conduzidos em menos tempo, usando quantidades menores de água e energia. Por exemplo, processamentos como micro-ondas, extração assistida por ultrassom, secagem por aspersão, processamento de fluido supercrítico ou processo de queda de pressão controlada são alguns exemplos (Chemat et al., 2017; Nirmal et al., 2023).

Questões nutricionais, financeiras e ambientais tem sido levantadas como resultado de perdas e desperdícios significativos nas indústrias de frutas. As operações de processamento de frutas geram volume substancial de subprodutos que representam um valor de até 50% de toda a categoria de *commodities*. De fato, resíduos agroindustriais são fontes de substâncias bioativas com elevado valor econômico e mercadológico (Freitas et al., 2021; Hoskin et al., 2022). Esses compostos incluem, entre outros, carotenoides, polifenois, fibras dietéticas, vitaminas, enzimas e óleos (Hoskin et al., 2019; Sagar et al., 2018). Assim produtos da indústria de frutas, bem como os subprodutos podem gerar produtos de valor agregado a partir de processos como a extração, sonicação e secagem por *spray dryer* usando tecnologias inovadoras (Hoskin et al., 2023; Hoskin, Xiong, & Lila, 2019).

3.1 Extração de compostos fenólicos a partir de resíduos de frutas

Resíduos de frutas são ricas fontes de compostos fenólicos e na literatura há muitos estudos a respeito de protocolos de extração (Sagar et al., 2018). Um dos métodos mais populares para extrair compostos fenólicos é a extração sólido-líquido. A recuperação de compostos fenólicos a partir de resíduos agroalimentares é documentada usando uma variedade de técnicas de extração sólido-líquido, a maioria das quais depende do uso de solventes orgânicos como metanol, etanol ou acetona, ou mesmo o uso de solvente eutético profundo (Panzella et al., 2020; Sharma et al., 2017).

A extração de polifenois em resíduos pode fazer uso de abordagens convecionais como a agitação e uso da temperatura, e não convencionais como o micro-ondas, ultrassom, fluido pressurizado, campos elétricos pulsados e alta pressão. Visando aprimorar a extração, como alternativa a técnicas convencionais tecnologias não convencionais são empregadas. Essas

técnicas podem ainda se destacar por produzir extrações "mais verde" ao ser comparada a técnica convencional devido a um menor uso da energia e de solventes tóxicos (Casazza et al., 2010; Putnik et al., 2017).

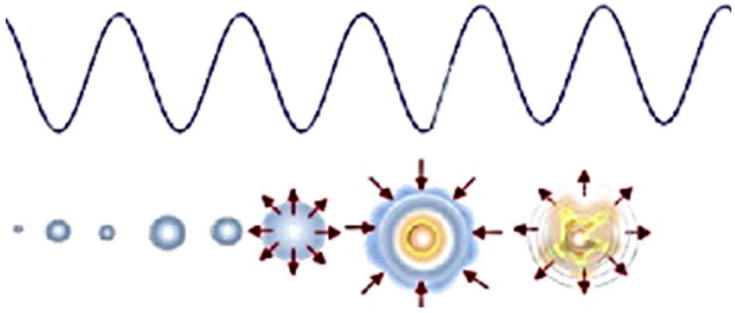
Devido a escassez de métodos de extração sustentável, resíduos de processamento de frutas podem ser vistos como tendo muito pouco valor em comparação a fruta processada. Tendo em vista que requisitos de tempo, energia e solvente para a extração convencional têm restrições, compostos fenólicos podem ser extraídos assistidos por ultrassom.

3.1.1 Extração por ultrassom

Sua ação ocorre em um período de tempo curto a baixa temperatura e com necessidade de menos energia ou solvente. Ultrassom é uma técnica de extração não térmica, de fácil uso, capaz de facilitar a recuperação de bioativos de forma eficiente em matrizes de plantas (Kumar, Srivastav, & Sharanagat, 2021; Nirmal et al., 2023). Ao contrário do ultrassom de baixa potência, as ondas ultrassônicas de alta intensidade (baixa frequência), são descritas como disruptivas e, portanto, têm um impacto significativo nos aspectos físicos, biológicos e mecânicos dos produtos alimentícios. Possuem frequências entre 20 e 100 kHz e intensidades entre 10 e 1000 W/cm^2. As aplicações incluem emulsificação, remoção de espuma, gerenciamento de microestrutura, devido a cavitação característica do ultrassom de alta intensidade (Bhargava et al., 2021; Yao, Pan, & Liu, 2020)

O uso do ultrassom na indústria de alimentos tem seu interesse fundamentado nos benefícios originados pela cavitação acústica (**Fig. 7**), que é a criação de microbolhas líquidas pela mudança de pressão interna da amostra, apresentando movimentos de expansão e compressão sucessivos até seu posterior colapso. Como consequência ocorrem rupturas na superfície provocando aumento do transporte de massa e velocidade da difusão (Araujo et al., 2021; Koshani & Jafari, 2019; Oliveira et al., 2022).

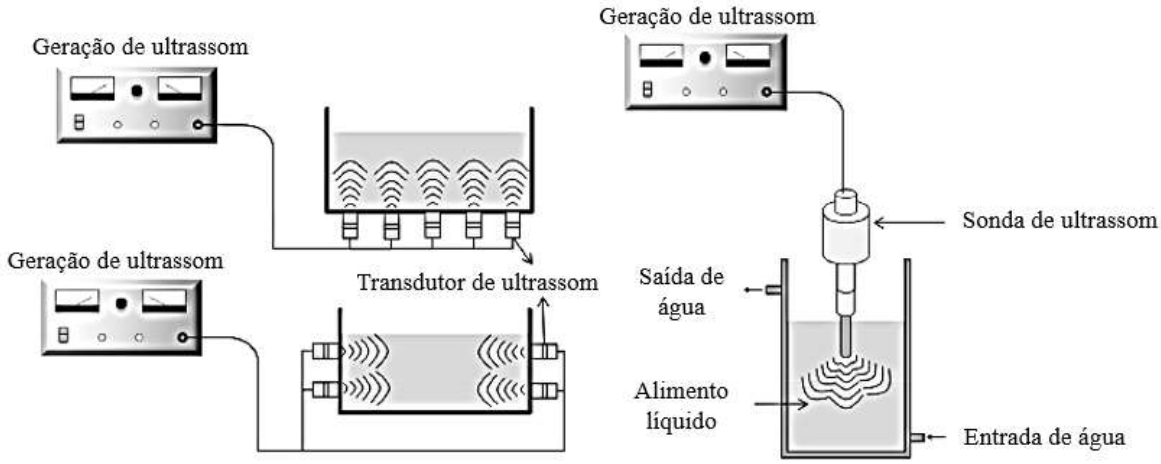
Figura 7 - Fenômeno de cavitação acústica associado ao processamento por ultrassom.



Fonte: Chemat *et al*. (2017).

A aplicação das ondas ultrassônicas ocorre através de equipamentos como banho e sonda. O banho ultrassônico (**Fig. 8a**), com sua aplicação de maneira indireta, pode propiciar a solubilidade pela redução do tamanho das partículas, contribuindo assim para difusão das partículas sólidas em solvente. A sonda ultrassônica (**Fig. 8b**) é empregada de forma direta ao ser imersa no recipiente com a solução usada para sonicação de volumes menores (Albero, Tadeo, & Pérez, 2019; Bernardi et al., 2021; Ojha et al., 2020).

Figura 8 – Modelos de aplicação de ondas ultrassônicas: a) Banho ultrassônico; b) Sonda ultrassônica.



a) b)

Fonte: Ojha, Tiwari e O'Donnell (2018).

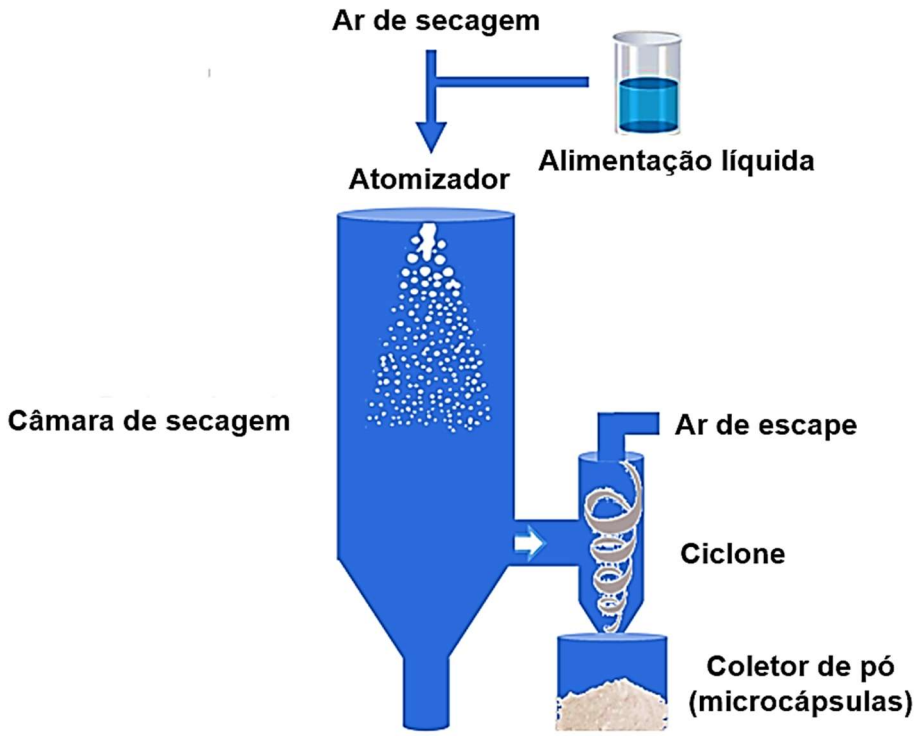
O processamento por ultrassom vem sendo considerado como eficiente, seja usado sozinho ou em conjunto com outras técnicas, e pode oferecer uma alternativa "verde" versátil para estabelecer protocolos de extração com maior eficiência energética. É considerada uma tecnologia inovadora e promissora capaz elevar o desempenho do processo e melhorar a qualidade final do alimento (Singla & Sit, 2021; Yao et al., 2020).

3.2 Microencapsulação por secagem em spray

O *spray drying* é uma técnica de secagem que usa a atomização de uma solução ou emulsão, normalmente preparada com auxílio de coadjuvante, através de uma corrente de ar em elevada temperatura, para obter produto desidratado através de um processo contínuo e controlável. Tem amplo uso industrial, compreendendo a produção de alimentos como laticínios, químicos, farmacêuticos, cerâmicos entre outros (Caglayan, Şöhret, & Caliskan, 2018; Choudhury, Meghwal, & Das, 2021; Ziaee et al., 2019). O secador *spray dryer* (**Fig. 9**) funciona através das seguintes etapas em sequência: 1) aumento de temperatura do ar de secagem; 2) formação de

partículas por atomização; 3) secagem das partículas na câmara; e 4) coleta de produto seco no ciclone (Arpagaus et al., 2018; Choudhury et al., 2021; Ziaee et al., 2019).

Figura 9 - Secador por atomização - Spray Dryer.



Fonte: Arenas-Jal, Suñé-Negre, & García-Montoya (2020).

Essa tecnologia de secagem é amplamente usada na indústria e oferece vantagens tais como controle da morfologia das partículas, rapidez, baixos custos de operação, eficiência energética, possibilidade de uso em amostras sensíveis ao calor e eficiência de encapsulamento elevado (Arenas-Jal et al., 2020; Arpagaus et al., 2018; Choudhury et al., 2021; Ziaee et al., 2019). Por isso, a tecnologia de encapsulamento tem sido amplamente utilizada no setor alimentício para produzir ingredientes com características primárias adequadas (textura, sabor e cor).

A qualidade dos pós (densidade, umidade e tamanho das partículas) depende da concentração da solução de alimentação, temperatura do ar de entrada e saída, como também da velocidade e tipo do atomizador, taxa de secagem e do ar do compressor (Marcillo-Parra et al., 2021). Quanto a dimensão, a microencapsulação varia de 3 a 800 μm, diferente da nanoencapsulação que é de 10 a 1000 nm. Os principais coadjuvantes são carboidratos (p.ex., maltodextrina), mas proteínas têm sido usadas com sucesso para esse tipo de aplicação (Marcillo-Parra et al., 2021; Rehman et al., 2019). O uso desses carreadores de alto peso molecular (polissacarídeos e proteínas) elevam a temperatura de transição vítrea do material, promovendo maior rendimento na secagem e maior estabilidade ao material seco (Moghbeli et al., 2019). Dentre as

características necessárias para a escolha do coadjuvante, estão a baixa higroscopicidade, baixa viscosidade, poder de emulsificação, proteção contra agentes como oxigênio, luz e pH, bom desenvolvimento do envoltório, como também baixo valor, ausências de odor e de sabor desagradável (Santos et al., 2019).

A microencapsulação por *spray drying* tem sido extensivamente testada para produzir ingredientes de frutas usando tanto suco como resíduos de frutas. Hoskin et al. (2019) desenvolveram e compararam as características de ingredientes funcionais obtidos através do *spray dryer* a partir do suco e extrato dos resíduos de mirtilo, amora e uva muscadine roxa. O estudo aponta diferenças importantes relacionadas a composição fitoquímica dos produtos finais, e ressalta a viabilidade desses ingredientes em formulações de alimentos funcionais. Vários outros estudos demonstraram, que o microencapsulamento de bioativos oriundos do resíduos de frutas por *spray dryer* pode gerar produtos funcionais encapsulados com relevantes efeitos para a saúde e podem, portanto, ser empregados como aditivos alimentares (Hoskin et al., 2022; Hoskin, Xiong, & Lila, 2019; Marcillo-Parra et al., 2021).

Além disso, tambem foi mostrada a produção de micropartículas obtidas por microencapsulação por *spray dryer* com maior estabilidade dos fitoquímicos durante o armazenamento. Isso acontece porque durante o processo de secagem, uma camada física externa é formada (podendo ser homogênea ou heterogênea), promovendo proteção para os compostos bioativos encapsulados. Esse comportamento foi observado em estudo sobre a microencapsulação de compostos fenólicos de folhas de alecrim com isolados de proteína de soja e do soro do leite, em que as partículas proteína-polifenol permaneceram estáveis em 20 semanas de armazenamento (Grace et al., 2021).

3.2.1 Produção de partículas do tipo proteína-polifenol por *spray drying*

Polifenois são produtos do metabolismo secundário das plantas. Além de atuarem como protetores das plantas contra parasitas, insetos e luz ultravioleta, são responsáveis também por sua pigmentação e adstringência. Essas substâncias podem ser encontradas em grande variedade de matrizes, incluindo, entre outras, frutas e vegetais (Albuquerque et al., 2021). Existe uma afinidade de ligação natural entre polifenois de média polaridade e proteínas, e isso pode ser usado racionalmente para criar partículas agregadas de proteína-polifenol com características melhoradas e/ou desenhadas para funções específicas (Hoskin, Xiong, & Lila, 2019; Lila et al., 2022).

Por exemplo, devido à sua atividade antioxidante, os polifenois proporcionam benefícios a saúde, incluindo tratamento de doenças cardiovasculares, obesidade e síndrome metabólica e por isso, existe um grande interesse por parte da indústria nesses compostos (Gasmi et al., 2022). No entanto, devido aos seus anéis benzenoides aromáticos apolares, muitos polifenois têm solubilidade limitada em água. Devido ao ambiente levemente alcalino do intestino delgado, eles também são suscetíveis à oxidação durante a digestão, o que reduz sua bioacessibilidade e biodisponibilidade. Assim, partículas proteína-polifenol podem ser desenhadas para exibir maior solubilidade em água, bem como reduzida oxidação durante a digestão (Langerijt, O'Mahony, & Crowley, 2023).

De fato, a bioacessibilidade/biodisponibilidade de polifenois durante o trânsito gastrointestinal foi reportado como superior quando comparado aos polifenois não-complexados (Grace et al., 2021; Lila et al., 2022). Também foram demonstradas as propriedades hipoglicêmicas e antimicrobianas dos complexos proteína-polifenol, além da redução de reações alérgicas e melhoria das bioassinaturas de metabólitos fenólicos ativos em circulação (Diaz, Foegeding, & Lila, 2021; Diaz et al., 2022) .

Assim, ingredientes funcionais a partir de partículas de proteína-polifenol podem ser criados para melhorar determinadas propriedades dos sistemas alimentares, preservando as propriedades dos polifenois (Correia et al., 2017; Grace et al., 2022). Estudos demonstrando as qualidades sensoriais desejáveis dessas partículas foram demonstradas, já que a complexação proteína-polifenol atua na mitigação do sabor amargo de alguns concentrados naturais fenólicos (Diaz et al., 2022). Além disso, maior estabilidade térmica, e capacidade espumante e emulsificante aprimoradas (Quan et al., 2019) foram demonstradas e encorajam o uso desses ingredientes em uma variedade de produtos alimentícios, como espumas, géis, emulsões e barras (Diaz et al., 2021).

4. Proteínas alternativas

Os sistemas agroalimentares globais vem passando por rápidas mudanças. Toda a produção agroalimentar e os sistemas da cadeia de abastecimento vem sofrendo mudanças significativas graças a avanços tecnológicos, independentemente da situação socioeconômica da área. Apesar disso, ainda há uma série de problemas persistentes de sustentabilidade global que precisam ser resolvidos (Bhat, 2022). O número de consumidores preocupados com questões ambientais e alimentos benéficos à saúde justifica o aumento crescente do interesse por alimentos orgânicos, funcionais, além de maior procura por fontes alternativas de proteína (Lavilla & Gayán, 2018).

Pesquisas futuras para compreender e encontrar oportunidades de estabelecer medidas eficientes para fortalecer e remodelar os mercados agroalimentares e promover a transição para processos sustentáveis são uma forte demanda mundial (Borsellino et al., 2020).

Um aumento significativo da produção e consumo de proteínas denominadas "alternativas" e derivadas de plantas e agora evidente em várias partes do mundo. Como exemplo, proteínas vegetais como leguminosas, soja, lentilhas e ervilhas, substitutos de carne processados (como Quorn e o *Impossible Burger*), carne cultivada e insetos são consideradas fontes alternativas a proteína animal (Ismail, Hwang, & Joo, 2020). No presente trabalho foram também avaliadas as proteínas de ervilhas com sua caracterização apresentada no Anexo 1. Compreender as percepções do consumidor em relação aos substitutos a proteína animal é essencial para promover a transição para um consumo alimentar mais sustentável. Os principais fatores que impulsionam esse setor de mercado são o aumento do interesse do consumidor em dietas vegetarianas e flexitarianas, aumento da renda individual nos países em desenvolvimento e aumento da consciência ambiental em escala global (Vatansever, Tulbek, & Riaz, 2020). De fato, previsões de empresas de consultoria apontam que ~10% do mercado global de carne será substituido por produtos feitos a partir de proteínas alternativas até 2029 (Siegrist & Hartmann, 2023).

Proteínas de leguminosas, além de saudáveis e ecológicas (demandam menos recursos ambientais para sua produção), são facilmente aceitas em países ocidentais, mas ainda existem desafios quanto ao sabor, digestão, e compatibilidade com a culinária tradicional (Siegrist & Hartmann, 2023). Exemplos desse tipo de proteína são ervilha (*Pisum sativum*), lentilha (*Lens culinaris*) e fava (*Vicia faba*). As proteínas de leguminosas são uma fonte proteica não transgênica com baixo teor de alérgenos, perfil aminoacidico comparável e taxas de digestibilidade semelhantes à proteína de soja. Além disso, propriedades tecnológicas tais como solubilidade, gelificação, ligação à água e texturização são apropriadas para a formulação de alimentos (Spaen & Silva, 2021; Vatansever et al., 2020). Aplicações como substitutos de carne sem glúten, com baixo teor de alérgenos e sem Organismo Geneticamente Modificado (OGM) são potenciais aplicações da proteínas de leguminosas no mercado internacional (Vatansever et al., 2020).

É necessária maior diversificação das proteínas alternativas disponíveis no mercado. Quinoa e sementes de cânhamo são fontes significativas de energia, proteínas de alta qualidade, fibras, vitaminas e minerais incluem fontes. Do ponto de vista nutricional, a mistura adequada de proteínas pode fornecer aminoácidos essenciais suficientes para atender às necessidades

humanas (Pihlanto et al., 2017)**.**

4.1 Proteína de inseto

O interesse pela entomofagia tem aumentado nos últimos dez anos. Atualmente, dois bilhões de pessoas em todo o mundo comem insetos e, recentemente, eles passaram a ser inseridos nos mercados americanos e europeu. Na literatura, mais de 2100 espécies de insetos foram listadas como comestíveis. Devido a preconceitos culturais, atualmente os insetos comestíveis nas nações ocidentais são mais usados como ração animal do que para alimentação humana. No entanto, várias nações na Ásia, Oceania, África e América Latina dependem fortemente de insetos como fonte de proteína (Kim et al., 2019). Além disso, o desenvolvimento de insetos comestíveis é incentivado pela Organização das Nações Unidas para Agricultura e Alimentação (FAO), sendo considerados como uma fonte saudável de proteína (Lucas et al., 2020; Zielińska, Karaś, & Baraniak, 2018).

A proteína de inseto (**Fig. 10**) pode ser usada para minimizar problemas com a cadeia de abastecimento alimentar convencional, sobretudo no que diz respeito a escassez mundial de energia, água e terra. Através do desenvolvimento de técnicas de processamento, pode ser possível aumentar as possibilidades de utilização desse tipo de proteína alternativa. No entanto, é muito importante que seja feita uma eficiente exposição dos benefícios para a saúde e ressaltar a necessidade de estabelecer menos dependência em relação a outras fontes de alimento, para que opiniões negativas sobre o consumo da proteína de insetos sejam minimizados (Kim et al., 2019).

Figura 10 – Proteína de inseto (grilo).

Fonte: Souza & G1 (2023).

A possibilidade de insetos comestíveis como um novo ingrediente em produtos de alto valor agregado tem sido pesquisada nos últimos anos para identificar substitutos para fontes de proteína convencionais, como a carne, usadas em excesso e prejudiciais ao meio ambiente. Todavia, o estudo da performance dessa proteína e o estudo de suas propriedades funcionais ao ser adicionado a matrizes alimentares ainda são incipientes (Gravel & Doyen, 2020). Pesquisas são necessárias em vários níveis para estabelecer maneiras de usá-los em diversas aplicações que contribuam para a desmistificação desse alimento. Por exemplo, sobre a relação estrutura-função das proteínas de insetos e como essas funcionalidades e processamento de insetos podem aumentar a aceitação do consumidor seriam benéficas para ampliar o mercado (Queiroz et al., 2023). Aliado a isso, uma das principais barreiras para plena aceitação desse alimento pelo consumidor esta relacionada com características organolépticas. Dessa forma, é crucial desenvolver estratégias visando melhorar características como apresentação, sabor e textura (Gravel & Doyen, 2020).

5. Ferramentas de avaliação de sustentabilidade – ACV

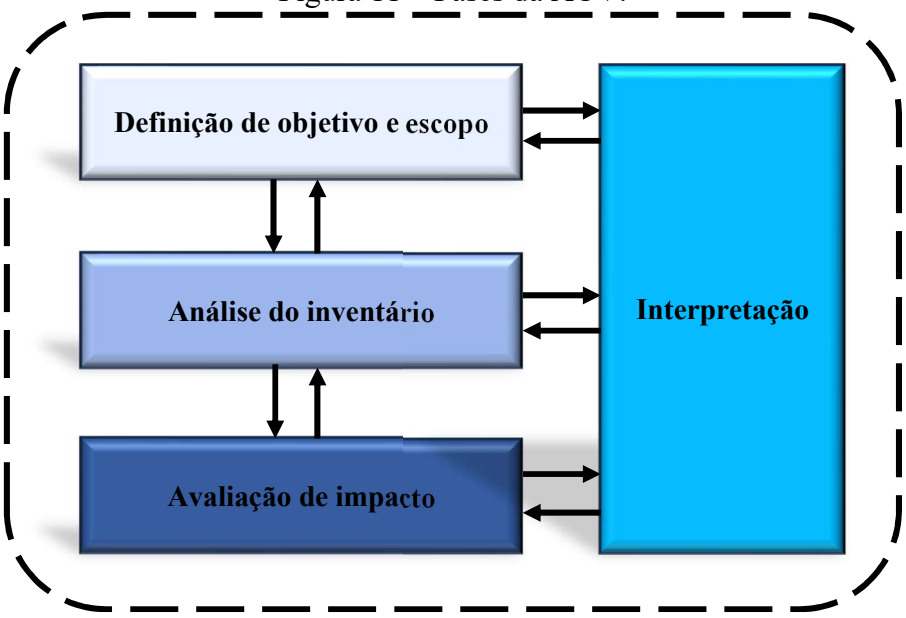
Na escala mundial, o setor industrial de alimentos é considerado um grande consumidor de energia e recursos naturais. Com uma contribuição acima de 25% das emissões de gases do efeito estufa (GEE), esse setor está entre os que mais usam e contaminam a água. No longo prazo, isso compromete a preservação de recursos para gerações futuras, por isso a necessidade de processos desenvolvidos de forma sustentável (Ahmad et al., 2019; Sanderson, Bamber, &

Pelletier, 2019).

Diante disso, avaliação do ciclo de vida (ACV) é uma ferramenta de potencial reconhecido para a avaliar os possíveis impactos ecológicos de indústrias ou produtos alimentícios considerando as fases do ciclo de vida. Visa avaliar a escolha de métodos, evidenciar pontos fracos e apresentar sugestões para minimizá-los (Nunes et al., 2018; Sanderson et al., 2019; Silva & Sanjuán, 2019). Também se aplica a desenvolvimento e melhoria do produto, marketing e planejamento estratégico (ABNT, 2014; Fraval et al., 2018).

Uma ACV pode ser conduzida considerando determinados segmentos do ciclo mas quando avaliada no formato *cradle to grave,* ela engloba todos os segmentos do ciclo de vida, indo desde a matéria-prima (*cradle*, berço) até o descarte (*grave*, túmulo). A ACV segue padrões constituídos pelo regulamento da *International Organization for Standardization* – ISO 14040/2006 e 14044/2006 (Filho, Junior, & Luedemann, 2016; Fraval et al., 2018; Garcia et al., 2018), e sua metodologia é dividida em quatro fases (**Fig. 11**): definição do objetivo e escopo, análise de inventário, avaliação de impacto e interpretação (ABNT, 2014; Rezende et al., 2022; Farjana, Huda, & Mahmud, 2019).

Figura 11 – Fases da ACV.



Fonte: ABNT (2014).

A primeira fase é qualitativa e inclui a identificação dos objetivos da ACV, seu escopo e limitações do sistema analisado em seu ciclo de vida, bem como seus limites temporais, dimensões de análise ambiental e da unidade funcional. A segunda fase é também quantitativa, formada pela construção do inventário do ciclo de vida (ICV). O consumo de recursos e as emissões pertinentes às categorias de impacto que estão sendo avaliadas são então

representados por um modelo de inventário de ciclo de vida para cada processo dentro dos limites do sistema. Para a terceira fase, a Avaliação de Impacto do Ciclo de Vida (AICV) ocorre relacionando o inventário a ponderação do impacto ambiental, para cada entrada/saída/resíduos do processo de produção. Na última fase, o processo interpretativo conecta dados do inventário e avaliação de impacto, propondo recomendações de melhoria de processo e indicadores de sustentabilidade para as principais vulnerabilidades ambientais do processo (ABNT, 2014; Filho et al., 2016; Fraval et al., 2018; Nunes et al., 2018).

As categorias de impacto ambiental consideradas em estudos de ACV estão relacionadas ao uso de recursos naturais, implicações sobre a saúde humana e consequências ecológicas (Detzel et al., 2022; Farjana et al., 2019). Por exemplo, em estudo prévio, o uso de energia na cadeia produtiva de alimentos foi indicado como a categoria de maior impacto (Wang et al., 2020). Quando a secagem da biomassa de algas marinhas foi avaliada, o processamento térmico (secagem) foi considerado como sendo a etapa de maior impacto (Oirschot et al., 2017). A ACV no setor alimentício tem sua maior concentração na produção de produtos primários, podendo avaliar fases do ciclo como do cultivo ao transporte (Silva & Sanjuán, 2019), como cerejas (Sanderson et al., 2019), mas tem se expandido por outros processos como consumo de frutas (Frankowska, Jeswani, & Azapagic, 2019), torrefação em casca de azeitona (Christoforou & Fokaides, 2016) e produção de passas (Elhami et al., 2019).

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CAPÍTULO 3

ARTIGO 1: ***Ultrasound-assisted polyphenol extraction of acerola and jambolan pomaces: comparison of extraction protocols, kinetic modeling, and life cycle assessment.***

Capítulo 3 - Apresentação do artigo 1

A pesquisa desenvolvida neste artigo foi realizada no Labolatório de Compostos Bioativos (LABTA) localizado no Laboratório de Engenharia de Alimentos-LEA (UFRN). Este artigo 1 foi publicado na revista Chemical Engineering and Processing – Process Intensification, que possui fator de impacto 4,30, e foi aceito em junho/2023. A versão do artigo 1 publicada está disponível no Anexo 2 deste documento.

Ultrasound-assisted polyphenol extraction of acerola and jambolan pomaces: comparison of extraction protocols, kinetic modeling, and life cycle assessment

Edilene Souza da Silva[a], Andréa Oliveira Nunes[a], Roberta Targino Hoskin[a,b]

[a] *Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Natal, RN. 59078-970, Brazil.*

[b] *Plants for Human Health Institute, North Carolina State University. Kannapolis, NC. 28081, USA.*

E-mail address: rtcorrei@ncsu.edu (R. T. Hoskin).

Abstract

In this study, tropical acerola and jambolan pomaces were submitted to four water-based polyphenol extraction methods: conventional solid-liquid extraction CSLE; heated conventional solid-liquid extraction HCSLE; static ultrasound-assisted extraction SUAE; and ultrasound-assisted extraction and mechanical stirring UAES. Our objective was to evaluate and compare the extraction protocols regarding their performance, extraction kinetics, mathematical modelling, and environmental viability using the life cycle assessment (LCA) tool. The highest total polyphenol content was obtained by UAES after 90 min (1,606.8 mg GAE/100g for acerola and 1,580.7 mg GAE/100g for jambolan). These results are significantly higher ($p \leq 0.05$) compared to CSLE (1,296.4 mg GAE/100g for acerola, 644.1 mg GAE/100g for jambolan). The Power Law model showed the best experimental fit compared to Peleg's and second-order models. Regarding the environmental viability, the LCA tool revealed that UAES had the lowest environmental impact among all extraction protocols, mainly due to its lower energy consumption. Overall, the combination of mechanical stirring and ultrasound improved water-based polyphenol extraction rates with reduced energy consumption. This

study shows UAES as an environmentally friendly strategy to achieve efficient extraction of naturally occurring polyphenols from tropical fruit pomaces.

Keywords: revalorization, fruit waste, environmentally friendly, bioactive compounds.

1. Introduction

Fruit pomaces are by-products of the fruit processing industry and consist of peels, residual pulp, and seeds. The pomace composition varies within fruits, but generally speaking, they are a complex mixture of macronutrients (carbohydrates, minerals, and vitamins) [1,2] with significant residual content of bioactive compounds such as phenolics and carotenoids that sometimes exceed the amount found in the fruit pulp [3]. For instance, acerola fruit (*Malpighia emarginata*) is one of the main natural sources of ascorbic acid in the world, and its residue retains significant amounts of bioactive compounds [4,5]. This "superfood" is cultivated in Central and South Americas, and used for the production of supplements and nutraceuticals [6,7]. Jambolan (*Syzygium cumini* (L.)) is another phytochemical-rich tropical fruit reported as a superfood due to its high content of phenolics, mainly anthocyanins, potent antioxidant activity [6], and health-relevant biological effects [8]. However, differently from acerola, its consumption and production are modest and not commercially relevant yet [9].

There is an increasing market for natural, clean label, environmental-friendly food products [10–12]. This trend has encouraged the investigation of new ways to manage industrial secondary streams and recover residual phytochemicals from fruit pomaces using extraction protocols that cause minimum impact to the environment, using less solvents and maximizing the use of natural resources [13]. Among upcycleable phytochemicals, the polyphenol extraction from agricultural pomaces consist of a major, increasingly important research field due to the well-established scientific evidence showing the health relevance of polyphenol compounds and their potential applications in the pharmaceutical and food industries [14,15]. In addition, finding ways to optimize the use of fruit pomaces is a rational strategy to add value to a widely abundant natural resource that would be, otherwise, discarded in the environment, incinerated or destined to less profitable end uses [16]. This is especially true for tropical fruit pomaces, that have their production concentrated in low-income countries that would benefit from increased income generation and stronger economic growth [17].

Extraction techniques have been referred as conventional (solid-liquid, liquid-liquid extractions using heat and/or organic solvents or water) and green technologies, such as ultrasound-assisted

extraction (UAE) [18]. Extraction processes can be intensified with the use of the ultrasound technique, a more efficient and ecologically correct method to extract phytochemicals from natural resources [20]. In addition to using less solvents, it requires shorter lead time, less energy, and can be combined with other extraction methods to enhance extraction efficiency [19]. This emerging extraction technology is based on an acoustic cavitation mechanism that uses compression and decompression cycles to cause fragmentation, erosion, and inter-particle shocks to rupture cellular structures, facilitating the release of entangled compounds such as phenolics, carotenoids and other phytomolecules [21]. It has proved to be particularly useful for fruit pomaces where rigid cell walls entrap compounds of interest and make it inefficient and/or not economically viable to use conventional solid-liquid extraction to recover phytomolecules [22]. For example, UAE has been successfully applied to several by-products such as jambolan bark [20], acerola pomace [23], grape pomace [24], peanut peels [25], green sweet lime peels [26], apple pomace [27], mango peels [28], lime [29] and passion fruit rinds [30].

Several studies have established the importance of the type of solvent for the recovery of bioactive compounds from natural plant materials using ultrasound-assisted extraction [27,31]. Water is a cheap, readily available food-grade solvent aligned with the current green chemistry principles valued by both consumers and industry [32]. Additionally, water can produce efficient polyphenol extraction yields, in comparison to organic solvents such as ethanol, acetone or methanol [33]. When ultrasound-assisted aqueous extraction of polyphenol was performed in kinetic study from brewer's spent grain it showed the best performance among pure solvents tested [34]. Mathematical modeling is an essential engineering technique used to simulate, describe, design and control a target process and it has been applied to study the kinetics extraction of multiple food materials such as olives [35], cocoa [36], basil leaves [37], soybeans [38] and yerba mate [39,40].

However, UAE can be conducted using multiple protocols and to determine their environmental friendliness, it is necessary to analyze the impact of UAE regarding several environmental factors. Life Cycle Assessment (LCA) is a powerful tool used by industries and production sectors to analyze and compare the potential environmental impacts during the life cycle of a product system. It helps to generate reliable data to assist in the decision-making process, design improvement strategies, and establish sustainability policies and ecological labeling [41,42].
In this research study, we investigated four polyphenol extraction protocols applied to acerola

and jambolan pomaces using only water as the extraction solvent: conventional solid-liquid extraction, heated conventional solid-liquid extraction, static ultrasound-assisted extraction and ultrasound-assisted extraction with mechanical stirring. Overall, the objective of this research study was to evaluate and compare these extraction strategies regarding their a) performance, b) extraction kinetics and mathematical modelling of experimental extraction data and, finally, c) environmental viability using the LCA tool. This research work evaluates ultrasound intensified polyphenol extraction to provide novel, meaningful scientific data regarding the recovery of bioactive compounds from underexplored fruit pomaces abundantly found in the tropical world and help unveil their potential as raw materials for multiple industries.

2. Materials and methods

2.1. Acerola (Malpighia emarginata) and jambolan (Syzygium cumini (L.)) pomaces

Acerola pomace resulting from industrial fruit pulp extraction was donated by a local fruit processing plant (Delícia da Fruta, Natal, RN, Brazil). Jambolan pomace resulted from processing jambolan fruits locally harvested (5° 24' 9.2880" S, 36° 57' 14.7780" W) in January 2019, using a fruit pulping machine (Juicer RI1858 650W, Philips Walita, Shanghai, China). Both pomaces consisted of skins, seeds, and residual pulp remaining after fruit pulp separation. They were kept frozen at -6 ^{o}C until further use and contained 78.0 % and 54.7 % of moisture, respectively.

2.2. Extraction protocols

Four different extraction protocols (**Figure 1**) were investigated using only water as the extraction solvent. A fruit pomace:water ratio of 1g:50 mL defined in preliminary experiments (data not shown) was used for all experiments.

2.2.1 *Conventional solid-liquid extraction (CSLE)*

The pomace:water solution was continuously stirred using a mechanical stirrer (TE-139 TECNAL, Piracicaba, Brazil) at room temperature (25 °C) and 150 rpm.

2.2.2 *Heated conventional solid-liquid extraction (HCSLE)*

The pomace:water solution was placed in a temperature-controlled water bath using a heating base (TE-0853 TECNAL, Piracicaba, Brazil) and continuously stirred (TE-139 TECNAL,

Piracicaba, Brazil) at 150 rpm. The temperature was monitored during extraction, and a gradual temperature increase was applied until 45 °C was reached (protocol established to match temperature increase observed for SUAE and UAES protocols described below).

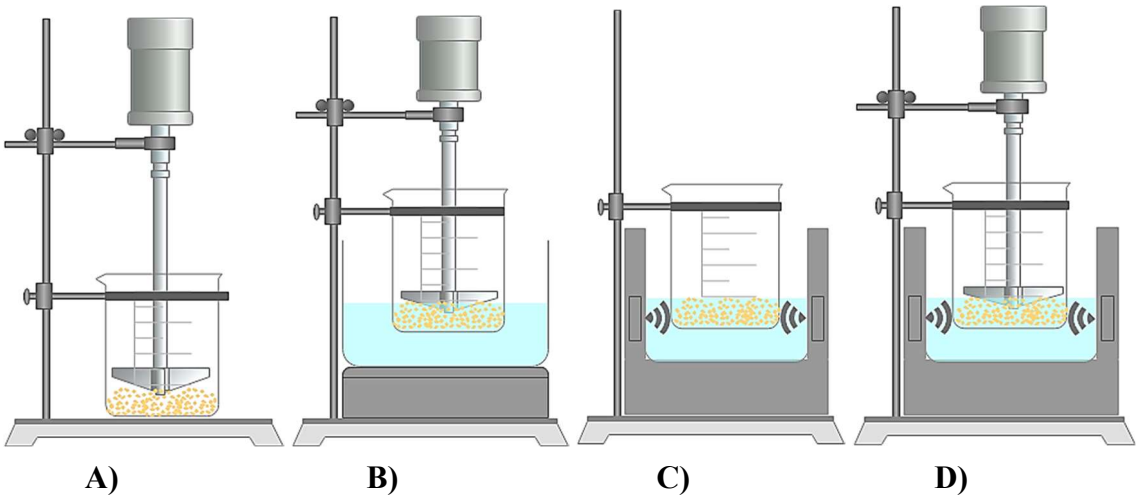


Figure 1. Representation of performed extractions: A) Conventional solid-liquid extraction (CSLE); B) Heated conventional solid-liquid extraction (HCSLE); C) Static ultrasound-assisted extraction (SUAE); and D) Ultrasound-assisted extraction and mechanical stirring (UAES).

2.2.3 *Static (no stirring) ultrasound-assisted extraction (SUAE)*

Indirect ultrasonication was applied to the pomace:water solution by placing the aqueous mixture in an ultrasonic bath (ALTSONIC Clean 3IA, 3L). The temperature of the solution increased progressively with time reaching a maximum of 45 ± 2°C within 100 min. The temperature increase resulted only from the UAE process and no external heat was applied. The ultrasound treatment was applied continuously using 40 kHz frequency and 100 W power.

2.2.4 *Ultrasound-assisted extraction and mechanical stirring (UAES)*

Indirect ultrasonication was applied to the pomace:water solution by placing the aqueous mixture in an ultrasonic bath (ALTSONIC Clean 3IA, 3L), using similar conditions described for SUAE treatment. However, the solution was mechanically stirred at 150 rpm. The ultrasound treatment was applied continuously using 40 kHz frequency and 100W power.

All extraction protocols were conducted for 100 min with samples collected every 10 min. The samples were vacuum filtered at 4 °C using qualitative filter paper and centrifuged for 10 min at 4000 rpm. The supernatants were collected, immediately frozen and kept protected from the light until further analysis.

2.3 Total polyphenol content (TPC)

Samples were analyzed for TPC using a previously published protocol with modifications [43]. Briefly, sample extracts (25 µL), distilled water (75 µl) and Folin-Ciocalteau reagent (1N, 25 µL) solutions were transferred to 96-well microplates and received 100 µL of Na_2CO_3 solution 7.5 %. Experiments were performed in triplicate. Results were calculated using a standard curve of gallic acid (0-500 mg/L, $R^2 = 0.99$) and expressed as mg of gallic acid equivalent per 100 g of sample (mg GAE/100 g).

2.4 Kinetic models

The kinetic modelling of all four polyphenol extraction protocols was evaluated using three different mathematical models (Peleg, Second-order and Power law models), commonly used for the modelling of solid-liquid extraction of plant-based natural products [44]:

a) **Peleg's model.** The Peleg's model uses a non-exponential model equation based on the extraction rate constant (k_1) and equilibrium concentration of total extracted TPC (C_{eq}) (Equation 1), where *t* (min) is the extraction time; *Ct* is the TPC (mg/100g) at a given time *t*; k_1 is the Peleg rate (100g/mg min) and k_2 is the Peleg capacity constant (100 g/mg); C_{eq} is the equilibrium concentration of total extracted TPC when $t \rightarrow \infty$ (C_{eq}, 100g/mg) defined as $C_{eq}=1/k_2$ [45].

$$C_t = \frac{t}{k_1+k_2.t} \quad \text{(Eq.1)}$$

b) **Second-order kinetic model**. It approaches the rate dynamics of the solid-liquid extraction process by considering parameters such as the initial extraction rate (H) and the content of polyphenols present when the extraction reaches equilibrium (Ce). A general second-order law [46] is described in Equation 2, where *t* (min) is the extraction time; *Ct* is the TPC (mg/100g) at a given time *t*; *h* is the initial extraction rate (mg/100g min) when t approaches 0, C_e is the TPC at equilibrium (mg/100g) and *k* is the second-order extraction constant (100g/mg min) described as $k = h/Ce^2$ [45].

$$C_t = \frac{t}{\frac{1}{h}+\frac{t}{C_e}} \quad \text{(Eq.2)}$$

c) **Power Law model**. The Power law model is typically used to assess the diffusion of target molecules and it is based on the extraction rate constant (B) and the diffusional exponent involved in transport mechanisms (n) [47] as defined by Equation 3, where *Ct* is the TPC

(mg/100g) at a given time *t* (min) *n* is the power law exponent (<1) and *B* is constant related to extraction rate (100 g / mg.min^{-1}).

$$C_t = B.t^n \quad \text{(Eq.3)}$$

2.4.1 Model evaluation

To determine the best fit to the experimental data, the concordance between experimental data and calculated values was established by means of correlation coefficient (R^2) and normalized root means squared deviation (*NRMSD*) criteria defined as (Equation 4):

$$NRMSD = \frac{RMSD}{Exp_{max}} = \frac{\sqrt{\left(\frac{1}{n}\right).\sum_{p=1}^{n}(Exp_p - Pred_p)^2}}{Exp_{max}} \quad \text{(Eq.4)}$$

where *n* is the number of kinetic experimental points, Exp_p is the experimental value at point *p*; $Pred_p$ is the predicted model value at point *p* and Exp_{max} is the maximum result within *n* experimental values.

2.5 Life cycle assessment (LCA)

The methodology conducted according to ISO 14040:2006 and 14044:2006 [48,49] consisted of four stages: a) Goal and scope definition; b) Life cycle inventory analysis (LCI); c) Life cycle impact assessment (LCIA) and d) Interpretation of results.

The LCA goal was to determine and compare the environmental impacts caused by the different polyphenol extraction protocols investigated in this study. All process inputs and outputs were based on a functional unit that standardized and guided all quantitative analysis. The functional unit (FU) was defined as 1400 mg of polyphenols, taking into account the average adult daily consumption in the United States of America [50]. The scope considered in this study was the cradle-to-gate approach which considers the extraction process and resources used in each one of the investigated protocols, such as energy and water [51].

The life cycle inventory analysis (LCI) consisted of primary and secondary data. The primary data consisted of the amount of water (L) used in the water bath and the energy consumption (kWh) of each extraction protocol measured by a standard digital AC wattmeter BR110 (220V). Secondary data such as electricity and water production were obtained from EcoInvent v.3.6 database.

Finally, the environmental impact assessment (LCIA) was evaluated using the *SimaPro*® 9.1 software using the impact assessment methodology implemented (CML-IA) baseline LCIA method V3.06/EU25, considering the following 12 impact categories: abiotic depletion, abiotic

depletion (fossil fuels), global warming (GWP100a), ozone layer depletion (ODP), human toxicity, freshwater aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity, photochemical oxidation, acidification and eutrophication.

2.6 Statistical analysis and model evaluation

All experiments were conducted in triplicate and results were expressed as mean ± standard deviation (SD). The analysis of variance (one-way ANOVA) and Tukey's test ($p \leq 0.05$), as well as the calculation of kinetic model parameters and correlation analysis were performed by *Statistica® v.10* software.

3. Results and discussion

3.1. Effect of the extraction protocol on polyphenol extraction

Results obtained by conventional solid-liquid extraction (CSLE and HCSLE) and ultrasound-assisted extraction protocols (SUAE and UAES) were evaluated and compared. For acerola pomace, the highest TPC concentration was observed for UAES at 90 min (1607 mg GAE/100g) and represents a TPC increase of 24 % compared to CSLE results obtained at similar time ($p \leq 0.05$; **Figure 2A**). For jambolan pomace (**Figure 2B**), a gradual TPC increase was observed between 0-90 min for all extraction methods, except CSLE. Once again, the highest TPC was found after 90 min for UAES extraction (1581 mg GAE/100g), which means an increase of 171 % compared to CSLE ($p \leq 0.05$) at the same extraction time. For jambolan, the TPC results increased in the following order UAES > SUAE > HCSLE > CSLE. For acerola, UAES also yielded the highest TPC, however HCSLE and SUAE came in second and third places. For both pomaces, CSLE yielded the lowest TPC among extraction protocols, while UAES was the most efficient method to extract polyphenols from both fruit pomaces (**Figure 2**).

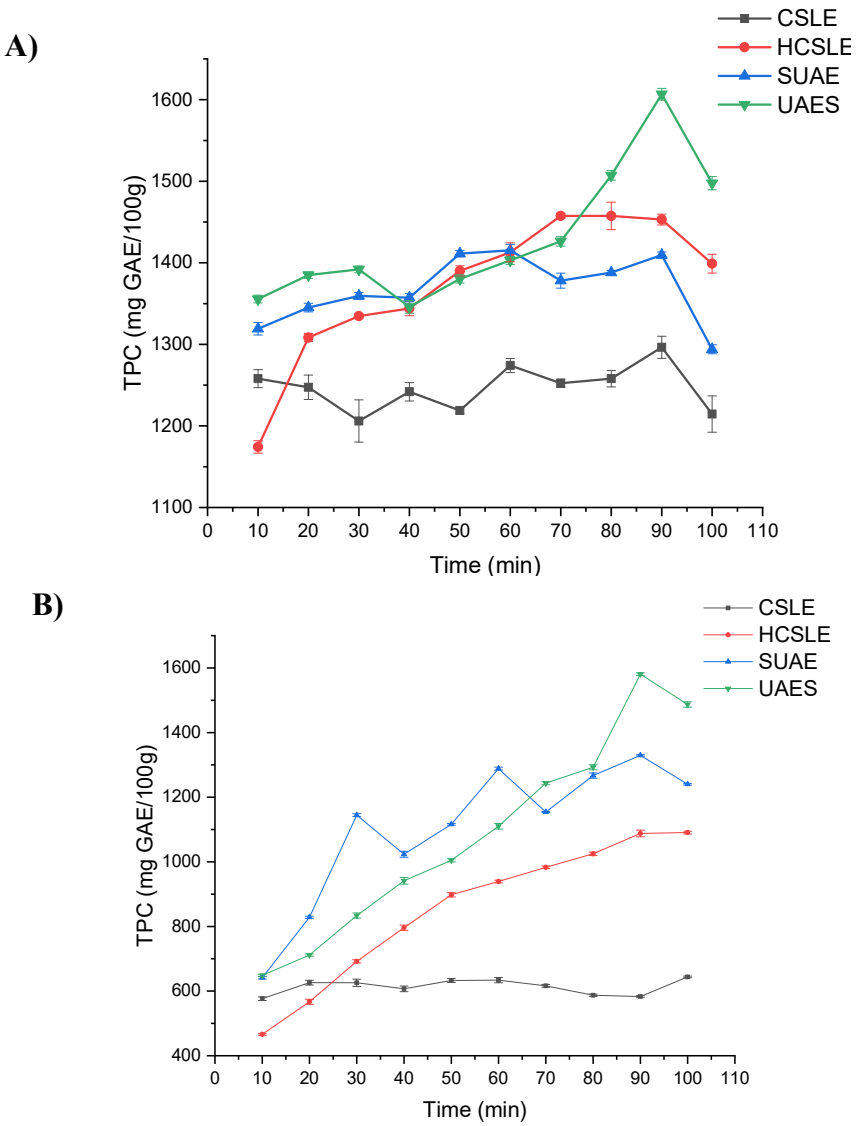


Figure 2. Polyphenol extraction of acerola pomace (A) and jambolan pomace (B) submitted to conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES) for 100 minutes. TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g). Bars represent standard deviation.

For acerola pomace (**Figure 2A**), TPC fluctuations were observed over time for all groups. Our hypothesis is that these variations occur because of an enhanced extraction/phenolic degradation cycle during acerola pomace extraction. Initially, the mechanical shearing resulting from both ultrasound or mechanical mixing ruptures cell walls (in the case of ultrasound), promotes the dissolution of active components in the media, and improves the polyphenol extraction rate. However, over time, degradation of acerola polyphenolic structures is likely to occur due to strong mechanical effects, reducing the TPC in solution. This cycle might occur over and over at different rates for each type of extraction, leading to the observed behavior.

Similar trend was observed for pitahaya peels [52] and pomegranate flowers [53].

Also, it was observed that TPC declined abruptly after 100 min of extraction of acerola pomace, mainly for SUAE and UAES. Indeed, ultrasonication time has been reported as a crucial factor influencing the extraction yield [54]. As the ultrasound-assisted extraction progresses, greater contact of the solvent with the sample (acerola pomace) is achieved, however, phenolic compounds can be oxidized and damaged, which reduces yield during longer times of extraction [19,21,55].

The extraction rate is controlled by mass transfer resistance and by intraparticle diffusion. Mechanical stirring decreases the mass transfer resistance by creating turbulence, but it has no effect on the intraparticle diffusion. Differently, ultrasonication intensifies both the mass transfer and the intraparticle diffusion [56], and enhances the mass transfer rate during the first extraction stage [57]. The ultrasound energy generates impact waves that cyclically compress/decompress the fibrous pomace structure, creating cavitation bubbles that induce fragmentation, pore formation and erosion and promote solubilization of the target compounds in the solvent, increased mass transfer rates in the solid:liquid interface, that ultimately leads to enhanced extraction yields [58,59]. Our hypothesis is that the synergistic combination of sonication and stirring (UAES treatment) provided greater cell wall disruption, facilitating mass transfer at both external and internal particle levels [26], which resulted in better polyphenol extraction. This configuration (stirring and sonication) has promising industrial use due to the intensifying synergistic effects on polyphenol extraction [60].

On the other hand, CSLE (stirring only, no heating or ultrasound) led to less efficient extraction, being necessary the combined use of ultrasound and stirring to enhance the extraction performance. Similar result was observed when phenolic compounds were extracted from dehydrated chicory by-products using agitation and sonication under various temperatures [61]. Green technologies like ultrasound-assisted technology has been referred as sustainable extraction strategies to achieve efficient, clean-label recovery of natural phytochemicals and pigments with reduced use or even elimination of harmful organic solvents and lower energy consumption yield [54,62].

For HCSLE (use of stirring and heating), a gradual temperature increase was applied to simulate what was observed with the use of ultrasound. HCSLE led to better polyphenol extraction results compared to CSLE, and we hypothesize that this is due the enhancement of polyphenol

diffusion, leading to higher polyphenol solubility in the solvent and more efficient extraction [37]. Heating facilitates solvent penetration and accelerates the mass transfer rate, leading to increased polyphenol extraction [23,65].

In this study, we used low frequency and high intensity (40 kHz, 100W) ultrasound treatments that have been reported as able to produce strong shear and mechanical forces to efficiently extract bioactive molecules from natural plant sources. In a study using aqueous ultrasonic-assisted extraction applied to grape pomace, González-Centeno et al. [64] reported that maximized phenolic content and antioxidant activity were observed when using similar sonication parameters shown here.

When conventional and ultrasound-assisted extraction (50 kHz) were applied to red araca peels, approximately 25 % higher TPC (589.49 mg GAE/100g) was observed for ultrasound-assisted extraction compared to conventional maceration method (477.53b ± 3.09 mg GAE/100g) after 90 min [67]. Our results for acerola pomace are higher than study by Rezende et al. [68], that used ultrasound-assisted extraction (50 kHz) applied to acerola pomace using ethanol/water 1:1 (1046 mg GAE/100) and just water (777.7 mg GAE/100g) as solvents. Similarly, our results after 90 min of extraction for both acerola and jambolan pomaces are superior than previous ultrasound-assisted extractions of grape pomace (Gonzáles-Centeno et al. [69], water-based, ultrasound probe, 55 kHz, 230 mg GAE/100g) and jambolan bark (Bhadange et al. [20], methanol-based, ultrasound probe, 20kHz, 13 mg GAE/g).

3.2 Extraction kinetics and mathematical modelling of polyphenol extraction

Because the extraction peak was observed at 90 min for both acerola and jambolan pomaces, the time interval 0-90 min was chosen for modelling the extraction kinetics. **Table 1** presents the kinetic parameters for each empirical model investigated in this study. The criteria adopted to determine the model that best represented the experimental values were higher values of R^2 and lower values of NRMSD.

Table 1 - Kinetic models constants and regression statistical parameters for acerola and jambolan pomaces submitted to different water-based polyphenol extraction protocols.

Acerola pomace		Model constant					Statistical parameters			
		CSLE	HCSLE	SUAE	UAES		CSLE	HCSLE	SUAE	UAES
Peleg model	K_1	-7.01E-05	1.79E-03	5.69E-04	7.77E-04	R^2	0.996	0.997	0.998	0.978
	K_2	8.03E-04	6.74E-04	7.13E-04	6.79E-04	NRMSD	0.020	0.016	0.013	0.042
	C_{eq}	1244.8	1483.1	1403.1	1492.9					
Second-order model	H	1480.6	529.7	1866.6	1253.4	R^2	0.996	0.997	0.998	0.978
	C_e	1253.6	1484.5	1408.0	1493.6	NRMSD	0.020	0.015	0.012	0.042
Power law model	B	1204.8	962.5	1233.5	1149.4	R^2	0.996	0.998	0.999	0.984
	n	0.01	0.09	0.03	0.06	NRMSD	0.019	0.012	0.011	0.036
Jambolan pomace		Model constant					Statistical parameters			
		CSLE	HCSLE	SUAE	UAES		CSLE	HCSLE	SUAE	UAES
Peleg model	K_1	6.36E-04	1.83E-02	9.02E-03	1.75E-02	R^2	0.994	0.993	0.984	0.962
	K_2	1.62E-03	7.54E-04	6.81E-04	5.47E-04	NRMSD	0.031	0.033	0.053	0.075
	C_{eq}	616.7	1325.7	1467.9	1829.0					
Second-order model	H	461.1	53.2	109.6	54.9	R^2	0.989	0.988	0.970	0.925
	C_e	617.9	1345.7	1476.3	1882.8	NRMSD	0.035	0.067	0.053	0.075
Power Law model	B	599.9	180.2	367.2	182.7	R^2	0.988	0.997	0.963	0.965
	n	0.004	0.400	0.287	0.455	NRMSD	0.033	0.017	0.059	0.051

Legend: Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES). R^2: Correlation coefficient; NRMSD: Normalized root means squared deviation. K_1 is the Peleg rate, K_2 is the Peleg capacity constant and C_{eq} is the equilibrium concentration of total extracted TPC; H is the initial extraction rate and C_e is the TPC at equilibrium; n is the power law exponent (<1) and B is constant related to extraction rate.

Generally speaking, all three models showed good fit to the experimental data ($R^2 > 0.9$, NRMSD < 0.1), however, when all extraction protocols were considered, the Power law model showed the best fit to the experimental TPC data for both acerola and jambolan pomaces **(Figures 3** and **4**, respectively). Indeed, it displayed the highest R^2 (0.984-0.999 for acerola pomace; 0.963-0.997 for jambolan pomace) associated to the lowest NRMSD (0.011-0.036 for acerola pomace; 0.017-0.059 for jambolan pomace). The NRMSD was the determining criterion for choosing the model, because low values suggest that the model properly describes the extraction progress with a good quality of fit and greater accuracy of the experimental data [47].

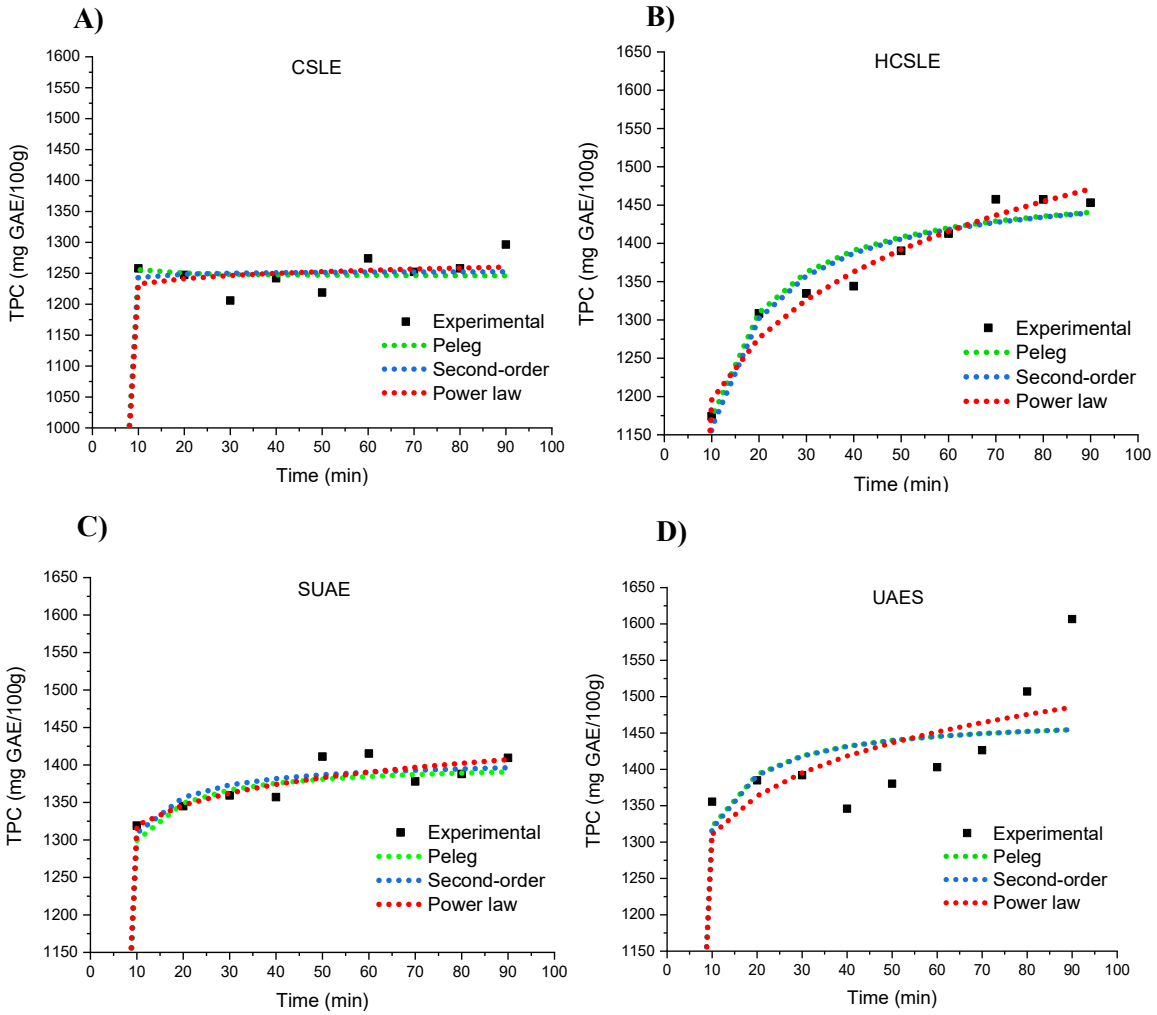


Figure 3. Kinetic modeling for acerola pomace submitted to conventional solid-liquid extraction (CSLE) (A), heated conventional solid-liquid extraction (HCSLE) (B), static ultrasound-assisted extraction (SUAE) (C) and ultrasound-assisted extraction and mechanical stirring (UAES) (D). TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g).

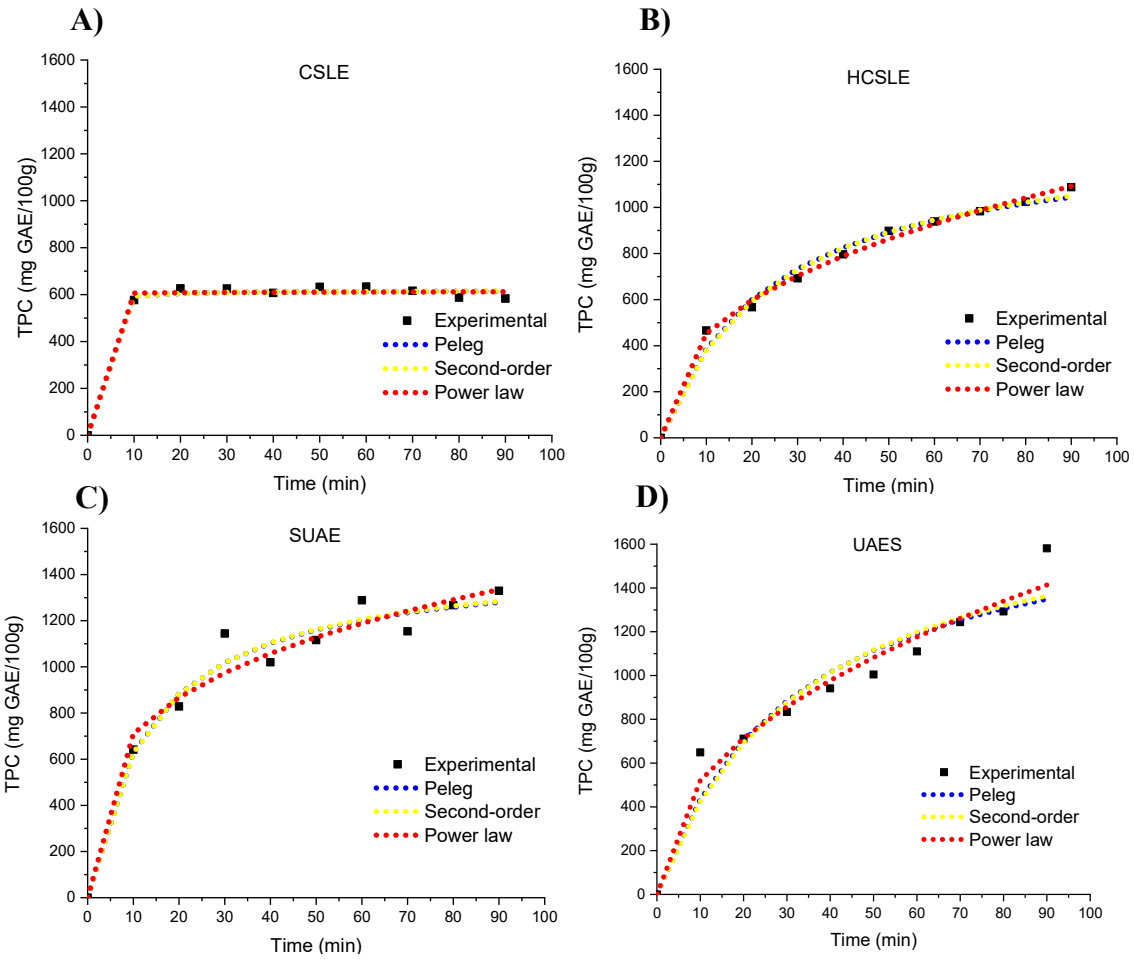


Figure 4. Kinetic modeling for jambolan pomace submitted to conventional solid-liquid extraction (CSLE) (A), heated conventional solid-liquid extraction (HCSLE) (B), static ultrasound-assisted extraction (SUAE) (C) and ultrasound-assisted extraction and mechanical stirring (UAES) (D). TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g).

Overall, acerola pomace extraction had higher constant B and lower time exponent n (also referred as diffusion coefficient), when compared to jambolan pomace. Higher B values were found for ultrasonication-assisted extraction protocols SUAE and UAES (Table 1), which indicates that the diffusion stage was positively affected when ultrasound-assisted extraction was performed. The time exponent n of Power Law model, also called the diffusion coefficient, showed low values for TPC extraction ($n \leq 0.6$), which indicates that the polyphenol extraction was controlled by Fickian diffusion [34]. A poor model fit was observed for experimental UAES data (**Fig. 3D**) of acerola pomace. We hypothesize that this might have happened due to the substantial TPC decrease at 40 min followed by increased TPC at 50 min. Apparently the models were not able to capture this abrupt change, which explains the lowest R^2 observed for UAES data (Table 1).

3.3 Environmental viability of extraction protocols using the LCA tool

In this study, acerola and jambolan pomaces were not submitted to drying before extraction, like in other extraction studies [68–70]. The polyphenol extraction (**Figure 5)** was evaluated under a perspective of cradle-to-gate approach, considering only the extraction protocols and processing inputs such as energy and water. The preparation of the extraction solution (water mixed with pomace) was not included in the inventory, since it is identical for all investigated extraction protocols, therefore it would add similar impact for all treatments.

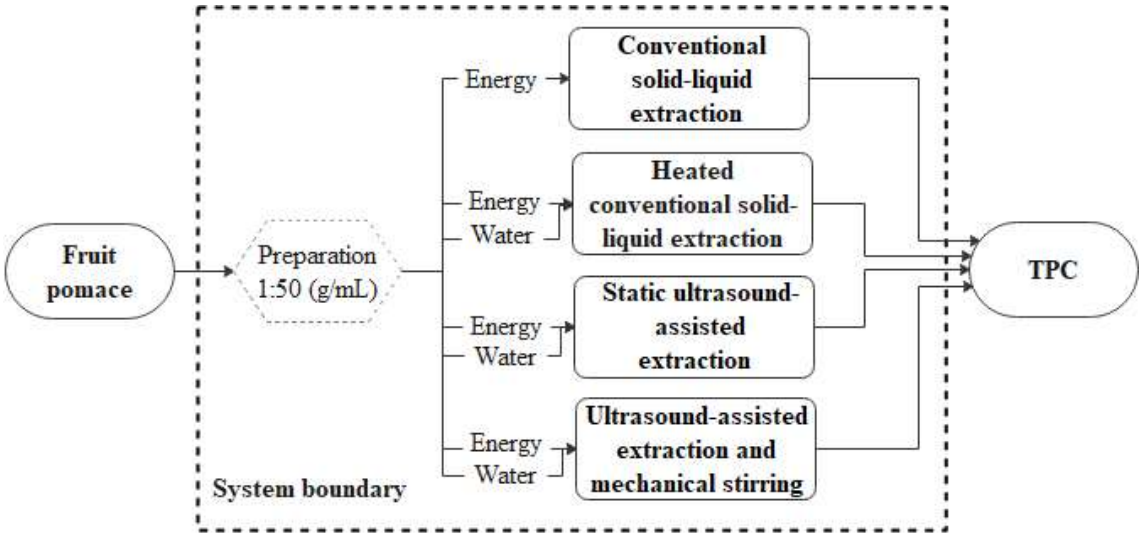


Figure 5. Flowchart and system boundaries used for the LCA evaluation of polyphenol extraction of acerola and jambolan pomaces. TPC: total polyphenolic content.

The best fit model (Power law model, **Equation 3**) was used to determine the time necessary to extract the functional unit (1400 mg of polyphenols**)** for each one of the four investigated extraction protocols. The experimental energy consumption data was plotted against time and linear equations were generated for each extraction protocol (**Fig. 6**). The time necessary to extract 1400 mg of polyphenols and the respective energy consumption are presented in the inventory table (Table 2). The lowest and highest energy consumption during the first 100 minutes of extraction were observed for CSLE and HCSLE, respectively. It was observed that the UAES protocol (mechanical stirring and sonication) exhibited similar energy consumption requirements compared to extraction using sonication only (SUAE) during this specific length of time (**Fig. 6**). It became evident that, despite the lower energy consumption during the first 100 minutes of extraction, the CSLE protocol required much longer extraction time to reach the functional unit (established as 1400 mg of polyphenols). Because of this, the estimated total energy consumption required to achieve the functional unit was the highest for CSLE among

all tested extraction protocols. On the other hand, because of the enhanced extraction capacity, UAES required the shortest time and lowest energy consumption to achieve the same functional unit for both fruit pomaces among all extraction protocols (Table 2).

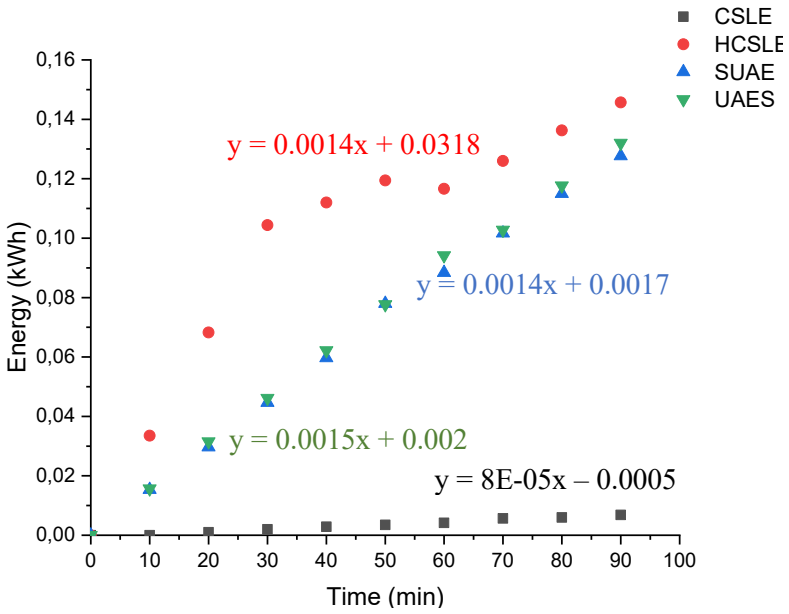


Figure 6. Graphical representation of time (min) x energy (kW/h) during the polyphenol extraction of acerola or jambolan pomaces submitted to conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES).

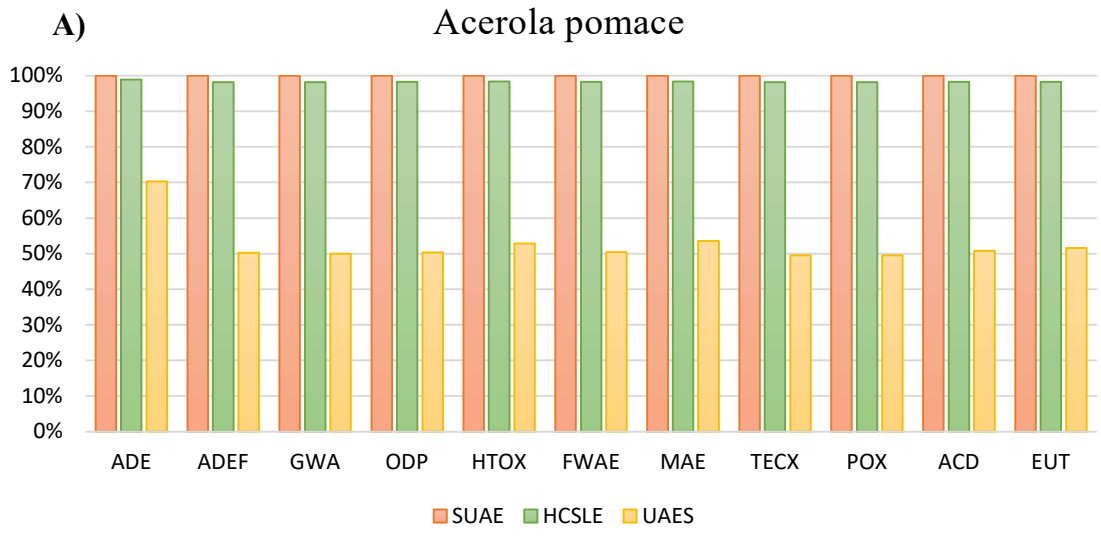
Table 2. Data inventory of polyphenol extraction protocols for acerola (AP) and jambolan (JP) pomaces submitted to different polyphenol extraction protocols.

	Input					**TPC output (mg)**
	Volume of water bath (liters)	**Time (min)**		**Electricity (kWh)**		
	Both AP and JP	AP	JP	AP	JP	
CSLE	0	3.59E+06	5.30E+82	287.099	4.24E+78	1400
HCSLE	3	53.18	167.49	0.106	0.266	
SUAE	3	75.61	106.15	0.108	0.15	
UAES	3	31.81	88.1	0.05	0.134	

Legend: Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES). kWh: kilowatt-hour; TPC: total phenolic content (expressed in mg); AP: acerola pomace; JP: jambolan pomace.

The time and energy consumption data were used to determine the environmental impact (LCIA). The CML-IA baseline LCIA method was applied to the data collected from each one

of the four different extraction protocols to evaluate the 12 impact categories (**Fig. 7A**, acerola pomace and **Fig. 7B**, jambolan pomace). Results were calculated as percentage (%) relative to the greatest impact (set as 100 %). The time needed to extract 1400 mg of polyphenols followed the order CSLE > SUAE > HCSLE > UAES for acerola pomace and CSLE > HCSLE > SUAE > UAES for jambolan pomace (Table 2). The CSLE results were not included here, due to the long time and high energy requirements (Table 2) needed to extract 1400 mg of polyphenols (functional unit used in this study) for both fruit pomaces. This result is corroborated by the highest K_2 (Peleg capacity constant) values obtained for CSLE, resulting in a low maximum extraction capacity. For acerola pomace, a similar high environmental impact measured by LCA was observed for both HCSLE and SUAE protocols (**Fig. 7A**). Indeed, both extraction protocols applied to acerola residue needed longer times compared to UAES to reach the FU, which leads to higher energy consumption (Table 2). The application of heat during HCSLE extraction protocol substantially increased the environmental impact in all assessed categories for jambolan pomace (**Fig. 7B**). The UAES protocol generated the lowest impact among all treatments for both pomaces and all 12 categories considered in this study. UAES applied to both pomaces had around 50% of the impact of HCSLE and SUAE for most of the categories, obtaining the highest value for the abiotic depletion category (70% for the acerola pomace and 63% for the jambolan pomace). The shortest extraction time required by UAES to reach the FU (1400 mg of phenolics) is a decisive factor that leads to lower energetic consumption and consequently, lower environmental impact compared to all other extraction protocols.



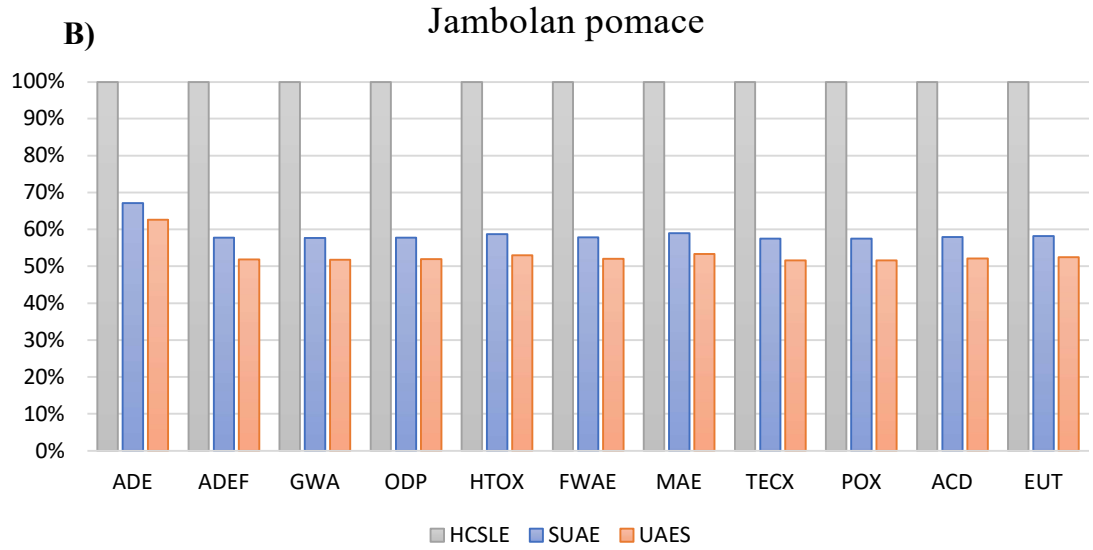


Figure 7. Impact evaluation of polyphenol extraction of acerola pomace (A) and jambolan pomace (B) using the CML-IA baseline LCA method assessed for 12 impact categories: Abiotic depletion (ADE); Abiotic depletion (fossil fuels) (ADEF); Global warming (GWP100a) (GWA); Ozone layer depletion (ODP); Human toxicity (HTOX); Fresh water aquatic ecotoxicity (FWAE); Marine aquatic ecotoxicity (MAE); Terrestrial ecotoxicity (TECX); Photochemical oxidation (POX); Acidification (ACD) and Eutrophication (EUT). Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES).

One of the main objectives of LCA is to compare the contributions of process parameters and conditions for the environmental impact and identify hotspots [71]. Results show that the UAES protocol had the best performance regarding time, water and electricity requirements for both acerola and jambolan pomaces. Based on these findings, the UAES protocol was further investigated to clarify the contributions of specific parameters to the impact evaluated by the LCA tool. The electrical energy was a major impact contributor for both pomaces, influencing the results in a more pronounced way when compared to water requirements (**Figures 8A** and **8B**). Overall, the water category had an impact below 30 % for AP and 20 % for JP, but the abiotic depletion (related to mineral resources and fossil fuels utilization) category presented a higher impact for both fruit pomaces - around 65 % for AP (**Fig. 8A**) and 40 % for JP (**Fig. 8B**).

Figure 8. Contribution of water and energy for the impact evaluation of ultrasound-assisted and mechanical stirring polyphenol extraction (UAES) of acerola pomace (A) and jambolan pomace (B) conducted by CML-IA baseline LCA method assessed for 12 impact categories: Abiotic depletion (ADE); Abiotic depletion (fossil fuels) (ADEFF); Global warming (GWP100a) (GWA); Ozone layer depletion (ODP); Human toxicity (HTOX); Fresh water aquatic ecotoxicity (FWAE); Marine aquatic ecotoxicity (MAE); Terrestrial ecotoxicity (TECX); Photochemical oxidation (POX); Acidification (ACD) and Eutrophication (EUT).

It is noteworthy that water was the only extraction solvent used in this study. Even though methanol, ethanol and other organic solvents can be successfully applied to polyphenol extraction, their use might incur in higher environmental impact and may require an additional solvent elimination step [71]. Besides being a food-grade, accessible and low-cost solvent, water was chosen for its good performance [72] and suitability to food processing operations [70,73].

Moreover, ultrasound-assisted extraction naturally leads to temperature increase in the system. Because of this, thermostatic baths or external heat application were not necessary and allowed for an eco-friendlier extraction protocol for both SUAE and UAES. In this regard, Vauchel et al. [71] showed that higher temperature (60 °C) and the use of ethanol as the solvent extraction led to a higher impact in all LCA categories when analyzing the antioxidant phenolics extraction from chicory waste using UAE.

Few research studies have used the LCA tool to compare extraction techniques protocols in the scientific literature [74]. The ones that did use it, were mainly focused on operating conditions such as time, temperature and solvent composition. The results obtained in this study strongly agree with previous results that showed ultrasound-assisted extraction protocols as effective strategies to reduce extraction time, energy consumption and environmental impact [71,74,75].

4. Conclusions

Indirect ultrasound coupled to mechanical stirring (UAES) proved to be an efficient polyphenol extraction protocol for both acerola and jambolan pomaces. The polyphenol content of acerola and jambolan polyphenol extracts obtained by UAES after 90 minutes were 1.2 and 3- fold compared to conventional solid-liquid extraction (CSLE), respectively. The Power law model showed the best fit to experimental kinetics extraction data for both acerola and jambolan pomaces and was used for the calculation of LCA. UAES extraction protocol demonstrated the lowest environmental impact among all extraction methods, mainly due to shorter extraction time and reduced energy consumption for both fruit pomaces. Our results highlight the importance of the use of green technologies for proper waste reutilization, biomolecules recovery and environmental protection. Overall, this study reveals that UAES is a rational and environmentally friendly strategy for obtaining polyphenol-rich extracts from fruit pomaces for multiple uses.

Declaration of Competing Interest

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

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CAPÍTULO 4

ARTIGO 2: ***Spray drying to produce novel phytochemical-rich ingredients from juice and pomace of American elderberry (Sambucus nigra canadensis).***

Capítulo 4 - Apresentação do artigo 2

A pesquisa que deu origem a este artigo foi realizada através de uma colaboração entre pesquisadores da Universidade Estadual da Carolina do Norte (NCSU) e da Universidade de Missouri (MU) nos Estados Unidos (EUA). Este estudo sobre o elderberry é parte de um projeto amplo financiado pelo Departamento de Agricultura dos Estados Unidos (USDA) que engloba diversos aspectos relacionados a valorização dos frutos de elderberry, incluindo aspectos botânicos relativos ao estudo de variedades do elderberry, bem como mecanização e impacto econômico de produção. Nossa contribuição neste estudo foi realizada na NCSU, com foco nas análises de quantificação de compostos bioativos, atividade antioxidante e bioacessibilidade do produto alimentício obtido pelo preparo de ingredientes a partir do resíduo do fruto do sabugueiro (elderberry) e sua secagem em spray dryer. O artigo 2 obteve recente publicação na revista Food Bioscience que possui fator de impacto 5,2, foi aceito em agosto/2023. A versão do artigo 2 publicada está disponível no Anexo 2 deste documento.

Spray drying to produce novel phytochemical-rich ingredients from juice and pomace of American elderberry (*Sambucus nigra canadensis*)

K. S. Ravichandran[1], E. S. Silva[3,6], M. Moncada[3], P. Perkins-Veazie[3], M. A. Lila[3], C. M. Greenlief[4], Andrew L. Thomas[5], R. T. Hoskin[3] and K. Krishnaswamy[1,2]

[1]Division of Food, Nutrition and Exercise Sciences, University of Missouri, Columbia, MO, USA
[2]Department of Chemical and Biomedical Engineering, University of Missouri, Columbia, MO, USA
[3]Food, Bioprocessing & Nutrition Sciences Department, Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, USA
[4]Charles W. Gehrke Proteomics Center, University of Missouri, Columbia, MO, USA
[5]Division of Plant Science and Technology, Southwest Research, Extension & Education Center, University of Missouri, Mt. Vernon, MO, USA
[6]Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Natal, RN. 59078-970, Brazil.

Author information

Corresponding Author: Kiruba Krishnaswamy − Division of Food, Nutrition and Exercise Science, University of Missouri, Columbia, Missouri 65211, United States; Department of Chemical, and Biomedical Engineering, University of Missouri, Columbia, Missouri 65211, United States; orcid.org/0000-0001- 7916-0311; Email: krishnaswamyk@umsystem.edu

Abstract

The cultivation and commercialization of American elderberries (*Sambucus nigra* subsp. *canadensis*), rich in acylated anthocyanins, is nascent. In this study, American elderberry juice and pomace extract were spray dried using soy protein isolate (SPI) or tapioca starch (TS) as carriers to develop functional food ingredients. Physicochemical, morphological, and bioactive properties were analyzed, and an *in vitro* gastrointestinal digestion model was used to study polyphenol bioaccessibility. An efficient spray drying process (solids recovery > 60 %) was established. Elderberry particles produced with SPI had higher solubility (60-64 %), lower porosity (69-70 %), and better flowability (22% Carr index, 1.29 Hausner ratio). Spray dried particles produced with tapioca starch showed significantly higher total polyphenol content (42-49 mg gallic acid equivalent/g sample), proanthocyanidin content (0.76-2.86 mg proanthocyanidin-B2/g sample), and anthocyanins (7.86-33.80 mg/g sample) for both elderberry juice and pomace extract, compared to SPI-derived ones. Particles of encapsulated elderberry juice or pomace extract with SPI had higher bioaccessibility compared to non-encapsulated elderberry juice or TS-derived particles. Overall, spray drying American elderberry juice and pomace extract is an effective and sustainable strategy to create novel ingredients for multiple food applications. These findings offer an industry-friendly technological solution to develop value-added ingredients for the emerging American elderberry market.

Keywords; *Sambucus*, dietary supplement, repurposing, powder ingredients, bioaccessibility, phytochemicals.

1. Introduction

Elderberry (*Sambucus* spp.), a deciduous shrub belonging to the family *Viburnaceae*, produces a small dark-purple fruit that is well-known in Europe, North Africa, Asia, and the USA. The fruit and flowers of elderberry are used in a variety of dietary supplement products, in part due to their flavonoids, phenolic acids, terpenoids, lipids, and alkaloids. Elderberry has been used in traditional medicine to treat various diseases due to its antioxidant, anti-bacterial, anti-carcinogenic, anti-allergic, immune-stimulating, and anti-viral properties (Domínguez et al., 2020; Liu et al., 2022; Thomas et al., 2020). Moreover, during the respiratory syndrome SARS-CoV-2 (COVID-19) pandemic, the interest in elderberry supplements skyrocketed to over $320 million (American Botanical Council report) (Osman et al., 2023), as the populations

across the world looked for alternatives and complementary therapies to support the prevention and/or treatment of upper respiratory symptoms, complications, and adverse events caused by this severe illness (Wieland et al., 2021).

The cultivation and commercial utilization of American elderberry (*Sambucus nigra* subsp. *canadensis*) are emerging, in contrast to the well-established European elderberry (*Sambucus nigra* subsp. *nigra*). The chemistry of the two species is different, as American elderberries contain more than 50 % acylated anthocyanins (known for imparting color stability) while European elderberries have little or no acylated anthocyanins (Osman et al., 2023).

European elderberries have been used to prepare several products (Terzić et al., 2023), but the technological potential of American elderberries has not been fully explored yet, as they are traditionally used to prepare a very limited number of products such jams, jellies, syrups, juice, and wine (Thomas et al., 2015a). Further, the by-product of elderberry juice processing (pomace consisting of seeds and skin, and residual pulp) is a good source of fatty acids, dietary fibers, and polyphenols that can be used to produce value-added ingredients to formulate functional foods and nutraceuticals (Costa et al., 2021). Indeed, the rational use of fruit pomaces can enhance the nutritional value of food products and reduce food waste which aligns with one of the important targets of sustainable development goals laid down by the FAO (Food and Agriculture Organization) for the 2030 agenda (*FAO Publications Catalogue*, 2021).

Berries are highly susceptible to spoilage due to their high moisture content and delicate structure, making them more prone to microbiological contamination and enzymatic degradation. Spray drying, one of the most used drying techniques in the food and pharmaceutical industries, has multiple advantages such as high energy efficiency, scalability, and short drying time (Kandasamy & Naveen, 2022). Spray drying encapsulation is a one-step drying technique that enables the production of food particles with reduced moisture, extended shelf-life, preserved and concentrated phytochemical content, all in a convenient particulate format (Correia et al., 2017). Spray drying operates with a short residence time of the product inside the drying chamber that allows for sufficient removal of moisture content while protecting the degradation of heat-sensitive compounds (Jafari et al., 2023; Bassani et al., 2022; Ravichandran & Krishnaswamy, 2021). However, spray drying of sugar-rich materials, such as fruit juices, is challenging because of the low glass transition temperature and consequent stickiness associated with the presence of low molecular weight sugars and organic acids in the solution (Sobulska & Zbicinski, 2021).

Carriers, also referred to as drying aids and/or wall materials, are used to minimize

excessive stickiness and consequent loss of product and to provide better retention of bioactive compounds (Grace et al., 2021). Polysaccharides and proteins are the two major carriers used for the encapsulation of food-bioactive compounds. Protein-based carriers exhibit superior film forming, surface activity, and emulsifying capabilities and provide a good source of energy with high nutritional value. In particular, plant-based proteins are more affordable options with better hydrophobic properties, lower toxicity, and less allergenicity compared to animal proteins (Akbarbaglu et al., 2021). On the other hand, polysaccharide-based carriers have lower cost, neutral flavors, and low viscosity at higher concentrations (Shishir & Chen, 2017).

Our research team has demonstrated that the spray drying microencapsulation of berry juice and pomace extracts is an efficient method to produce dried fruit ingredients with preserved phytochemical content, enhanced functional attributes, and extended storage while maintaining desirable organoleptic and biofunctional properties (Correia et al., 2017; Hoskin et al., 2019; Hoskin et al., 2022). In this study, we use spray drying encapsulation of American elderberry juice and pomace extract to obtain novel and convenient elderberry powdered ingredients. We investigated and compared the spray drying performance and quality attributes of spray dried particles (physicochemical content, bioactive properties, and bioaccessibility) produced with both elderberry juice and pomace extract using two carriers – polysaccharide-based (tapioca starch) and protein-based (soy protein isolate). Our results provide technical insights to create novel, value-added ingredients for the incipient market of American elderberry products, as an effort to diversify and increase the market opportunities of this underexploited, rapidly emerging specialty crop.

2. Materials and methods

2.1 Materials

American elderberry fruits were harvested (August 2022) from research orchards located at the University of Missouri's Southwest Research, Extension, and Education Center, near Mt. Vernon, MO. Ripe fruits were sealed in zippered plastic storage bags, immediately cooled, then promptly frozen (-20 °C). Berries from the cultivar 'Bob Gordon' were pressed to obtain elderberry juice, whereas the cultivars 'Bob Gordon', 'Kelly 7-14', 'Ozark', 'Pocahontas', 'Rogersville', and 'Wyldewood' were used to produce a concentrated liquid extract from pomace. Soy protein isolate (SPI, 90 % protein) was obtained from Bulk Supplements (Henderson, NV, USA) and organic tapioca starch (TS) from PuroRaw (Brooklyn, NY, USA) and Anthony's Goods (CA, USA). Gallic acid (G7384), L-ascorbic acid (A7506), 2,2-diphenyl-

1-picrylhydrazyl (DPPH, D9132), and 4-dimethylamino cinnamaldehyde (DMAC, D4506) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for chromatographic analyses were HPLC grade and all other reagents were of analytical grade.

2.2 Production of elderberry juice and concentrated pomace extract

Frozen elderberries were thawed at room temperature and rinsed with water to remove impurities or foreign materials before being pressed. The elderberry juice (EJ) was produced using a pulper (C80 Automatic Sieve, Ridgeland, MS, USA). The resulting pomace and juice were then stored at -20 °C until further use. The elderberry pomace (EP) was used to prepare a concentrated pomace extract according to an adapted extraction protocol (Hoskin et al., 2019). First, the freeze-dried elderberry pomace was mixed with a 50 % ethanol solution (1:3 extraction ratio w/v) and kept in a water bath at 80 °C for 2 h. Next, the mixture was filtered through double layer cheesecloth under vacuum, then centrifuged for 20 min at 24,400*g. Finally, the ethanol was removed using a rotary evaporator (Buchi Labortechnik AG, Switzerland), and the final concentrated extract was kept at -20 °C until further use.

2.3 Production of spray dried particles using carbohydrate and protein carriers

The frozen elderberry juice was thawed at room temperature and centrifuged at 15,552*g (10 minutes, 20 °C; Sorvall LYNX 6000 centrifuge, Waltham, MA, USA). The supernatant was collected, mixed with either soy protein isolate, or tapioca starch at a concentration of 8% (w/v) then homogenized at 3,200*g for 10 minutes (IKA, Ultra Turrax T25 homogenizer, Wilmington, NC, USA). Similarly, the concentrated liquid elderberry pomace extract was mixed with either soy protein isolate or tapioca starch (8 % w/v) and homogenized for 2 h using a magnetic stirrer to allow complete hydration.

The drying procedure was performed using a B-290 spray dryer (Buchi Labortechnik AG, Switzerland), with a concurrent flow and a nozzle diameter of 0.7 mm. The feed flow was controlled at 10 mL/min by a peristaltic pump and the drying air had an inlet temperature of 120 °C and an outlet temperature ranging from 67-77 °C. The elderberry juice or pomace extract mixed with tapioca starch or soy protein isolate was kept at constant magnetic stirring at 30 °C during the drying process. The resulting spray dried elderberry particles were collected, weighed, sealed, and kept frozen at -20 °C until use. As a result, four experimental groups of aggregate particles were produced and tested: EJ-TS (elderberry juice with tapioca starch), EJ-SPI (elderberry juice with soy protein isolate), EP-TS (elderberry pomace extract with tapioca

starch), and EP-SPI (elderberry pomace extract with soy protein isolate).

2.4 Determination of total soluble solids, pH, and berry size

The pH of elderberry juice and pomace extract was determined at room temperature (25 °C) using a digital pH meter (Seven Compact S220, Mettler Toledo). Similarly, the total soluble solids (TSS) content was measured at room temperature using a digital refractometer (HI96800, Hanna Instruments) and recorded as °Brix. Berry size was measured in length (diameter, mm) using ImageJ software (National Institutes of Health).

2.5 Solids recovery

Solids recovery was calculated as the relationship between the solids content of the spray dried powder recovered after spray drying to the total solids in the feed solution according to Equation 1 (Grace et al., 2021).

$$Yield\ \% = \frac{mass\ of\ powder}{total\ solids\ in\ feed\ solution} * 100 \quad (1)$$

2.6 Physicochemical properties of spray dried particles

2.6.1 Moisture content and water activity

The moisture content was determined using a halogen moisture analyzer (HE53, Mettler Toledo, Columbus, OH, USA) at 105°C. The water activity (Aw) was determined using a water activity meter (Cx-2, Decagon Devices, Inc., Pullman, WA, USA) at room temperature.

2.6.2 Color parameters

The color measurements were performed using a Colorimeter (Konika Minolta CR-410, Ramsey, NJ, USA) with a 0° viewing angle and a pulsed xenon lamp as the light source. The instrument displays readings in terms of color coordinates (L*: whiteness to darkness, a*: redness to greenness, and b*: yellowness to blueness). Instrument calibration was performed using the standard white tile, and samples were placed on a Petri dish during each measurement. The total color change (ΔE), hue angle (H°), and chroma (C) were calculated by Equations 2-4 (Correia et al., 2017):

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (2)$$

where L_0, a_0, b_0 denote the color coordinates of elderberry juice, used as a standard reference to compare the color change of the powder.

$$H° = 360 + \ tan^{-1}\left(\frac{b^*}{a^*}\right),\ when\ \ b^* < 0 \quad (3)$$

$$C = \sqrt{(a^*)^2 + \ (b^*)^2} \quad (4)$$

2.6.3 Density and porosity

Approximately 2 g of each sample was transferred into a 10 ml graduated cylinder and the volume occupied by the powder was used to calculate the bulk density (ρ_b, ratio of mass to bulk volume, Equation 5). A gas pycnometer (Quantachrome ultra pycnometer 1000 Anton Paar, Graz, Austria) was used to measure the true density. A sample cell containing approximately 1 g of powder was filled with a known volume of compressed helium gas. The volume measured by the pycnometer was used to calculate the true density (ρ_T) (Nani & Krishnaswamy, 2023). The porosity (ε) values were obtained based on the relationship between bulk and true densities (Equation 6).

$$Bulk\ density\ \left(\frac{g}{cm^3}\right) = \frac{Sample\ \text{mass}}{\text{bulk volume}} \quad (5)$$

$$Porosity\ (\%) = \frac{True\ density - \ bulk\ density}{True\ density} * 100 \quad (6)$$

2.6.4 Particle size distribution and zeta potential

The particle size and zeta potential of spray dried elderberry particles were analyzed using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern, MA, USA and DelsaNano C, Beckman Coulter, CA, USA respectively). For this, 0.5 g of the sample was dispersed in water and diluted to a final concentration of 0.005 % (w/v) according to Nani & Krishnaswamy (2023) with slight modifications.

2.6.5 Glass transition temperature (T_g)

The glass transition temperature (T_g) was determined using a differential scanning calorimeter (Q200 DSC, TA Instrument, Schaumburg, IL, USA). Each sample, weighing approximately 7-8 mg, was placed in a sealed aluminum pan. The samples were cooled to 20 °C and then heated to 180 °C at a rate of 10 °C per minute in a nitrogen-purged environment with a flow rate of 50 mL/min. A reference aluminum pan was used for comparison. The midpoint values of Tg were determined using Universal V4 5A TA Instruments analysis

software from Schaumburg, IL, USA (Singh et al., 2022).

2.6.6 Morphology

The powder morphology was examined by scanning electron microscopy (FEI Quanta 600F ESEM). The spray-dried elderberry particles were mounted using a double-sided carbon adhesive and coated with a 25 nm platinum layer in a vacuum. The examination was conducted at 5 kV with an 8 mm working distance, 30 μm objective aperture, and 3.5 spot size, under a magnification of 1000x.

2.6.7 Flowability

The Hausner ratio (HR, Equation 7) and Carr index (CI, Equation 8) were used to estimate the cohesiveness and flow characteristics of spray dried particles, respectively (Correia et al., 2017). Flowability of spray dried particles is classified based on a previous report (Supplementary Table 1, Goyal et al., 2015).

$$Hausner\ ratio\ (HR) = \frac{tapped\ density}{bulk\ density} \quad (7)$$

$$\text{Carr's compressibility index (CI)} = \frac{tapped\ density - bulk\ density}{tapped\ density} * 100 \quad (8)$$

2.6.8 Hygroscopicity

The hygroscopicity of spray dried elderberry particles was determined as described by Nani & Krishnaswamy (2023). Approximately 0.5 g of sample was placed in a desiccator with a NaCl saturated solution (75 % relative humidity at 30 °C) for 7 days. The hygroscopicity was calculated as a percentage based on the ratio of the mass of absorbed moisture to the initial mass of powder (Equation 9).

$$Hygroscopicity\ \% = \frac{mass\ of\ powder\ after\ 7\ days - initial\ mass\ of\ powder}{initial\ mass\ of\ powder} * 100 \quad (9)$$

2.6.9 Solubility

Solubility was determined based on Correia et al. (2017) with slight modifications. Approximately 0.5 g of powder was mixed with 10 ml of water and placed in a circulatory water bath at 37 °C for 30 min. Next, the mixture was centrifuged at 3074*g for 10 min, and the supernatant was poured on a pre-weighed petri dish, then oven dried at 105 °C until a constant weight was obtained (equation 10).

$$Solubility\ \% = \frac{weight\ of\ dried\ supernantant}{weight\ of\ powder} * 100 \quad (10)$$

2.7 Phytochemical analyses

2.7.1 Sample preparation

Briefly, 20 mg of spray dried elderberry particles was suspended in 1 mL of 1 % acetic acid in 80% methanol in water, followed by 5 min of sonication at 55 °C and 10 min centrifugation at 21,130*g. Next, the filtered supernatant was collected, and the process was repeated. Finally, the eluates were pooled together and used for phytochemical analysis.

2.7.2 Total polyphenol content (TPC) and proanthocyanidin content (PAC)

An adapted Folin-Ciocalteau method was used to measure the total polyphenol content spectrophotometrically in microplates (Spectramax Plus 384, Molecular Devices, Sunnyvale, CA). Samples were read at 765 nm against a gallic acid standard curve, and results were expressed as gallic acid equivalent (mg GAE/mL or mg GAE/g sample) (Hoskin et al., 2019).

The total proanthocyanidin (PAC) content was determined using an adaptation of the DMAC (4-dimethylamino cinnamaldehyde) assay with a calibration curve of PAC-B2 (3.125-100 µg/mL) and results were expressed as mg PAC-B2/100 g sample (Prior et al., 2010).

2.7.3 Ascorbic acid content

Ascorbic acid was determined following a previous protocol (Jiang et al., 2017). Filtered samples (20 µL) were injected into a Hitachi Elite LaChrom high-performance liquid chromatograph (Hitachi Ltd., San Jose, CA) equipped with a UV-Vis diode array detector, controlled temperature autosampler (4°C), and column compartment (30°C). Ascorbic acid detection and quantification were performed using a C18 column (Synergi 4µ Hydro-RP 80Å, 6 × 250 µm; Phenomenex Inc., Torrance, CA). The mobile phase consisted of 0.0065N H_2SO_4 with a flow rate of 1 mL/min. Total ascorbic acid content was calculated from standard curves generated by injecting 20 µL of L-ascorbic acid and reported as mg/100g fresh weight.

2.7.4 Anthocyanin profile

The anthocyanin profiles of elderberry juice, pomace extract, and spray dried elderberry particles were determined by double extraction. For this, 0.2 g of elderberry juice or pomace extract and 0.02 g of spray dried elderberry particles were mixed with 1.5 mL of acidified

methanol following a previous protocol (Perkins-Veazie et al., 2016). Filtered samples (20 µL) were injected into an ultra-high performance liquid chromatograph (Waters Acquity, Milford, MA), equipped with a diode array detector, controlled temperature autosampler (4 °C), and column compartment (45 °C). Anthocyanin and phenolic separation were performed using a C18 column (Waters Acquity UPLC BEH 1.7 µm, 2.1 x 50 mm). The mobile phase consisted of 5% formic acid in water (A), and 100% methanol (B) with a flow rate of 0.3 mL/min using a step gradient of 0 min, 10 % B; 5 min, 15 % B; 15 min, 20 % B; 20 min, 25 % B; 25 min, 30 % B; 45 min, 60 % B; 47 min, 10 % B; 60 min, 10 % B. Compound concentrations were estimated using standard curves generated by injecting 20 µL of 0.0625–0.5 mg/mL preparations of cyanidin 3-O-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside-5-glucoside, and cyanidin 3-sambioside as external standards. Compound identification was performed based on retention time compared to authentic standards and those reported by Lee & Finn (2007). Samples were reported as mg/g dry weight (DW) and sums of anthocyanins were calculated to obtain total anthocyanin and phenolic contents.

2.7.5 Antioxidant activity - 2.2-diphenyl-1-pricrylhydrazil (DPPH) assay

The radical scavenging activity measured by DPPH assay was conducted according to our previous protocol (Correia et al., 2017). Briefly, 20 µL of eluted samples were added to 180 µL of DPPH solution (150 µmol/L) in methanol-water 80 % (v/v) using 96-well microplates. After 40 min in the dark at room temperature, the absorbance was measured at 515 nm. Results were calculated using a standard curve built with different concentrations (100–500 µM) of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as µM Trolox equivalents/g.

2.8 *In vitro* simulated gastrointestinal digestion.

A modified standardized static *in vitro* gastrointestinal digestion (GID) method was adapted from Minekus et al. (2014). Simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) electrolyte stock solutions were added to elderberry-derived samples with previously standardized total phenolic content (7.8-9.2 mg TP). The mixtures were suspended in 0.5 mL water and thoroughly mixed with 0.35 mL of SSF, followed by sequential addition of 50 µL of 1500 U/mL porcine pancreas α-amylase solution, 25 µL of 0.03 M $CaCl_2$ and 75 µL of water (pH 7.0). For the gastric phase, the resulting oral bolus (1 mL) was mixed with 0.64 mL of SGF electrolyte stock solutions, 160 µL porcine pepsin stock solution

of 25,000 U/mL, 5 μL of 0.03 M $CaCl_2$, 20 μL of 1 M HCl, and 175 μL of water (pH 3.0). The reaction vessel was placed into an incubator at 37 ℃ for 2 h under agitation. For the intestinal phase, 2 mL of gastric chyme was mixed with 1.1 mL of SIF electrolyte stock solution, 0.5 mL of pancreatin solution 800 U/mL, 0.25 mL fresh bile (160 mM in fresh bile), 40 μL of 0.3 M $CaCl_2$, 15 μL of 1 M NaOH and 95 μL of water (pH 7.0) and shaken once again for 2 h at 37 ℃. After the intestinal digestion, samples were centrifuged to obtain the soluble fraction (supernatant) and the residual fraction, then frozen and freeze-dried. The bioaccessibility index (BI) was calculated as:

BI (%) = (TP post-digestion of supernatant/TP pre-digestion) × 100

where TP post-digestion is the total phenolic (μg/mg sample) quantified in the intestinal supernatant after the complete digestion process, and TP pre-digestion is the total phenolic (μg/mg sample) quantified before the *in vitro* digestion (Grace et al., 2021; Xiong et al., 2020).

2.9 Statistical analyses

Two-way analysis of variance of mean values was carried out using JMP 14.0 statistical software (SAS Institute Inc, Cary, NC, USA). *In vitro* simulated gastrointestinal results were analyzed by Prism 8.0 (GraphPad Software, San Diego, CA, USA) to perform ordinary one-way ANOVA analysis. Means were compared by Tukey test with statistical significance $p < 0.05$ at a 95 % confidence interval unless stated. Principal component analysis (PCA) was conducted and cross-verified using both Origin Pro, 2021, and Jmp 14.0, and the PCA graphs from Origin Pro, 2021 were used. Results are presented as the mean ± SD. All physical and chemical analyses were performed in triplicate unless noted.

3. Results and Discussion

3.1 Spray drying process evaluation.

The solids recovery, also referred to as spray drying yield, is an important indicator of the feasibility and efficiency of the spray drying process (Hoskin et al., 2019). The solids recovery of EJ-SPI, EJ-TS, EP-SPI and EP-TS treatments were similar ($p > 0.05$) and averaged 68.80±3.24, 62.18±2.47, 68.8±5.60, 71.7±2.90 % respectively. Our results are within the successful range for lab-scale spray drying (solids recovery > 50%; Tontul & Topuz (2017)) and are considerably higher than those obtained for the spray drying of blueberry with SPI (50.1 %), jussara (*Euterpe edulis*) pulp with SPI (39.8 %) and chokeberry (*Aronia* spp.) with tapioca starch (35.3 %) (Correia et al., 2017; Santana et al., 2016; Gawałek & Domian 2020). One of

the primary reasons for reduced product recovery in fruits is the stickiness caused by the presence of low molecular weight sugars and organic acids (Leyva-Porras et al., 2019). Indeed, elderberries contain high sugar concentrations (fructose, glucose, sucrose) and a variety of organic acids (citric, malic, shikimic, and fumaric acids) (Thomas et al., 2015b; Veberic et al., 2009) which might jeopardize the spray drying operation when no carriers are used (Verma & Singh, 2015). The elderberry juice and pomace extract had a pH of 4.61 and 3.72 respectively and total soluble solids of 8.33 and 5.9 °Brix, respectively. Hence, the findings of this study suggest that the addition of high molecular weight carriers, such as SPI or tapioca starch at a relatively low concentration (8%) enabled the efficient spray drying of elderberry juice and pomace extract, making it an efficient strategy to obtain powdered elderberry ingredients.

3.2 Physicochemical characterization of spray dried elderberry particles

3.2.1 Morphology

Figure 1 shows the morphology of spray dried elderberry juice and pomace extract particles. Those produced with SPI (EJ-SPI, EP-SPI) have a predominantly spherical shape with varying sizes, with rough or wrinkled surfaces. The observed wrinkled surface on SPI-derived particles can be attributed to uneven shrinkage during drying or cooling, a common characteristic of powders produced using proteins as a carrier. The increased flexibility of the protein film formed on the droplet surface leads to particle shrinkage without ruptures, resulting in particles with more wrinkles and rough surfaces (Muzaffar & Kumar, 2016). On the other hand, elderberry particles produced with tapioca starch (EJ-TS, EP-TS) had a smoother surface with oval-shaped particles and fewer wrinkles, or dents. Particle morphology can also directly influence the stability of the microencapsulated core, as well as the flowability, and bulk density of powders. For instance, particles with more wrinkles or rough surfaces could be more susceptible to oxidation due to increased surface area, and the reduced surface contact results in a more free-flowing powder (Correia et al., 2017; Zhang et al., 2020).

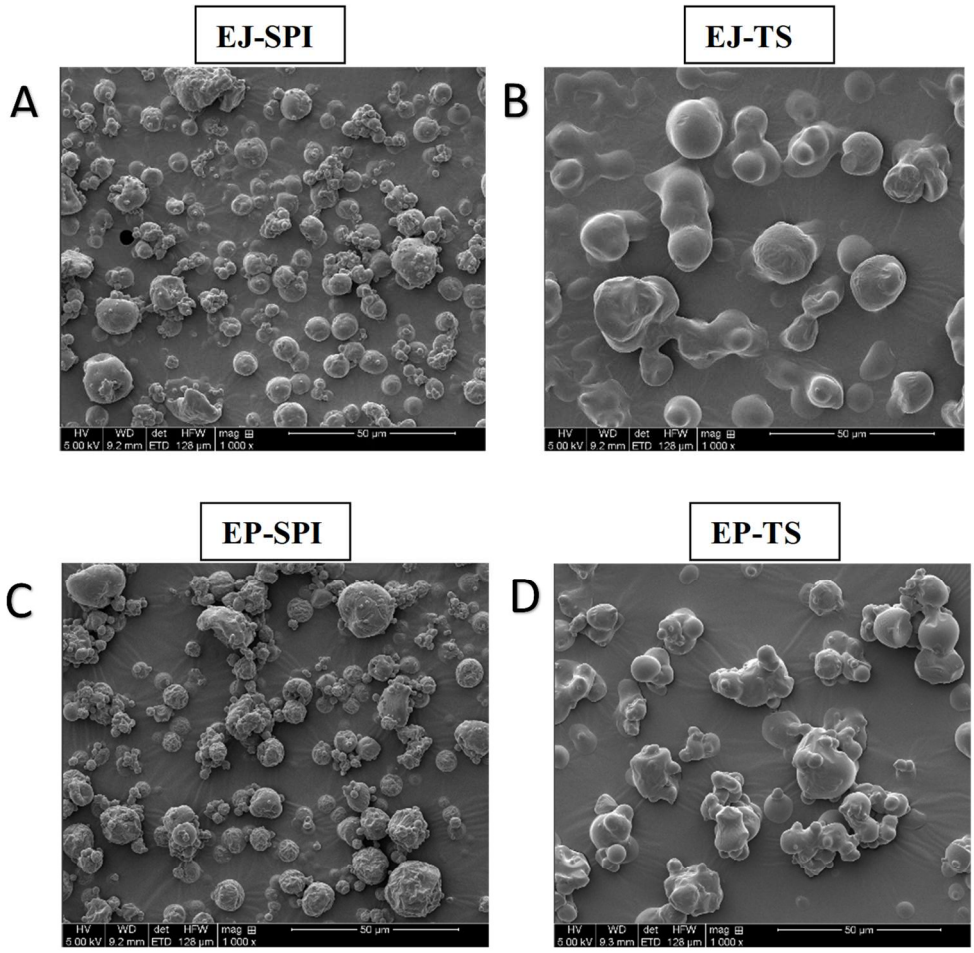


Figure 1. Scanning electron micrographs (1000x magnification) of spray dried American elderberry juice and elderberry pomace extract particles. A) EJ-SPI: elderberry juice with soy protein isolate; B) EJ-TS: elderberry juice with tapioca starch; C) EP-SPI: elderberry pomace extract with soy protein isolate; D) EP-TS: elderberry pomace extract with tapioca starch.

3.2.2 Moisture content and water activity

Both the moisture and water activity of spray dried particles play a crucial role in determining the flowability, stickiness, and storage stability of powdered food products due to their influence on the glass transition temperature and crystallization properties of food materials (Righi da Rosa et al., 2021). The moisture content of spray dried elderberry samples was between 2.68 % and 4.31 % (Table 1), consistent with previous reports for spray dried black mulberry juice and chokeberry extract (Fazaeli et al., 2012; Vidović et al., 2019). Reducing the moisture level can minimize the agglomeration and caking in spray dried particles, which are undesirable phenomena that can jeopardize the proper storage of the final product. Therefore, it is crucial to maintain a moisture content of less than 6% for a food powder intended for long-term storage (Bajac et al., 2022). Water activity (A_w) measures the availability

of free water responsible for deteriorative reaction and provides an important index to estimate the shelf life of particulate products. Powders with a water activity of less than 0.3 are considered as microbiologically and chemically stable (Shishir & Chen, 2017). The water activity of spray dried elderberry particles was between 0.16-0.21 (Table 1), typical values for spray dried products (Santhalakshmy et al., 2015).

Table 1. Physical properties of spray dried American elderberry juice and elderberry pomace extract particles.

Parameters	EJ-SPI	EJ-TS	EP-SPI	EP-TS
Moisture content (%)	4.31 ± 0.21^{a}	2.68 ± 0.05^{b}	3.06 ± 0.13^{b}	3.04 ± 0.15^{b}
Water activity	0.218 ± 0.005^{a}	0.169 ± 0.003^{c}	0.174 ± 0.001^{c}	0.176 ± 0.005^{b}
Bulk density (kg/m^3)	416.67 ± 11.22^{a}	373.49 ± 15.37^{b}	404.29 ± 17.19^{a}	415.41 ± 17.29^{a}
True density (kg/m^3)	1362.17 ± 10.55^{b}	1415.18 ± 7.27^{b}	1364.20 ± 4.40^{b}	1471.53 ± 17.56^{a}
Porosity %	69.36 ± 0.75^{b}	73.18 ± 0.76^{a}	70.73 ± 1.13^{b}	72.48 ± 1.21^{a}
Carr Index (%)	22.62 ± 1.52^{b}	31.18 ± 3.88^{a}	22.33 ± 2.99^{b}	34.79 ± 2.54^{a}
Hausner ratio	1.29 ± 0.03^{b}	1.46 ± 0.08^{a}	1.29 ± 0.05^{b}	1.54 ± 0.06^{a}
Particle diameter (µm)	1.14 ± 0.95	8.13 ± 6.72	0.51 ± 0.33	0.64 ± 0.48
Polydispersity index (PDI)	0.697	0.684	0.416	0.568
Zeta potential	-18.57 ± 2.26^{a}	-15.96 ± 0.91^{a}	-15.85 ± 2.45^{a}	-15.04 ± 3.27^{a}
Glass transition temperature (Tg, °C)	73.66 ± 3.72^{b}	62.32 ± 0.95^{c}	62.25 ± 1.46^{c}	120.93 ± 3.17^{a}
Hygroscopicity %	22.72 ± 0.65^{a}	21.26 ± 1.25^{a}	21.22 ± 3.77^{a}	18.77 ± 0.72^{a}
Solubility %	60.60 ± 2.16^{a}	51.16 ± 1.70^{b}	64.38 ± 0.41^{a}	49.40 ± 1.93^{b}

Results are shown as average ± SD. Different letters in the same row indicate statistical difference by Tukey's test (2-way ANOVA, $p < 0.05$). EJ-SPI: elderberry juice with soy protein

isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

3.2.3 Density and porosity

The density of food powders influences a product's processing, packaging, storage, and shipping. Higher bulk density is desired during transport as it requires less storage and transportation space and contains fewer cavities in the powder for oxygen penetration, thereby increasing storage stability (Bajac et al., 2022). Spray dried elderberry juice and pomace extract particles had a bulk density between 290 and 416 kg/m^3, where EJ-TS treatment had a significantly lower value ($p < 0.05$) compared to other treatments. Similar values were observed for spray dried blueberry juice and chokeberry extract (Darniadi et al., 2018; Tzatsi & Goula, 2021). True densities of spray dried elderberry juice and pomace extract particles were 1362 and 1471 kg/m^3, respectively. EP-TS treatment resulted in significantly higher true density compared to other treatments. Encapsulation of blackberry juice using maltodextrin and gum Arabic gave similar results of true densities (1487-1512 kg/m^3) (Ferrari et al., 2012) as those reported in our work.

High porosity implies a large interparticle void space containing oxygen available for degradation reactions (Bajac et al., 2022). Additionally, Lu et al., (2021) reported that the major drawback of carbohydrate-based wall materials is their high porosity, which can lead to decreased storage stability. The carrier type dictated porosity values, and tapioca starch treatments showed higher values ($p < 0.05$) compared to SPI. The porosity of elderberry spray dried particles was similar to those reported for blackberry powder using maltodextrin and gum Arabic (70–73 %) (Ferrari et al., 2012).

3.2.4 Flowability

Low-cost physical analyses such as bulk and tapped densities are used to estimate the Hausner ratio (HR) and Carr index (CI), parameters that enable the prediction of the flow behavior of powders (Goyal et al., 2015). The CI and HR values ranged from 22-34 % and 1.29-1.54, respectively (Table 1). Similar results were obtained when blueberry pomace extract was spray dried with SPI (Correia et al., 2017) and chokeberry juice spray dried with tapioca starch (Gawałek & Domian, 2020). Overall, the type of carrier had a significant influence on the flowability of powders, as elderberry particles produced with SPI (EJ-SPI, EP-SPI) showed significantly lower ($p < 0.05$) CI and HR, indicative of better flowability. According to Muzaffar & Kumar, (2016), powder flowability depends on moisture content, particle size, and

shape. Higher moisture content leads to increased cohesiveness because of the plasticizing effect of water, which makes the particle surface more viscous and reduces flowability. Nonetheless, the presence of significantly higher moisture content in EJ-SPI did not negatively affect its flow properties (Table 1). In addition, smaller particle sizes can increase cohesion and reduce flowability by providing more contact points for interparticle bonding. However, roughness on the surface can obstruct the particles from approaching each other resulting in better flowability. Our study revealed that particles produced with SPI (EJ-SPI, EP-SPI) were smaller than those produced with tapioca starch (EJ-TS, EP-TS), but the rough or wrinkled surface of the former might play an important role in the observed better flowability. Figure 1 revealed that EJ-SPI and EP-SPI particles had a greater degree of sphericity compared to EJ-TS and EP-TS. Generally, particles with a higher degree of sphericity tend to exhibit better flow properties (Gagneten et al., 2019) as observed for SPI treatments. Similar behavior was observed with spray dried raspberry, blackcurrant, and elderberry extract powders. Raspberry powders had a smoother surface with less shrinkage and showed less flowability compared to others (Gagneten et al., 2019).

3.2.5 Particle size and zeta potential

The particle size of food powders influences their handling, stability, transportation, and storage requirements. The wall material was the main factor that affected the size of spray dried elderberry particles, as SPI treatments (EJ-SPI, EP-SPI) showed smaller particle size compared to tapioca starch treatments (EJ-TS, EP-TS; Table 1). This finding could be related to the shrinkage of spherical particles during drying. In this regard, ESEM images (Figure 1) show more shrinkage for SPI treatments compared to tapioca starch treatments, which might play a role in the observed smaller particle size. Further, the PDI values (Table 1) indicate that the elderberry particles have a polydisperse (PDI > 0.1) particle size distribution (Raval et al., 2019). Comparable behavior was observed when raspberry and elderberry extracts were spray dried with maltodextrin. Elderberry particles showed a smaller particle size (6 μm) which was related to their shrinkage, while raspberry powders showed less shrinkage and increased particle size (8.5 μm) which decreased their flowability (Gagneten et al., 2019).

Zeta potential is linked to the electrostatic repulsion between particles and it was measured to determine the stability of spray dried elderberry particles in an emulsion. Bhattacharjee (2016) classifies particles with zeta potential values of ±0-10 mV, ±10-20 mV, ±20-30 mV, and > ±30 mV as highly unstable, relatively stable, moderately stable, and highly stable,

respectively. The obtained microparticles had negative zeta potential values ranging from -15 to -18 mV (Table 1) which indicates that the powders would be relatively stable when dispersed in an aqueous solution. However, our results indicate lesser stability in comparison to spray dried chokeberry particles produced with maltodextrin and skimmed milk (-35 to -39 mV) (Ćujić-Nikolić et al., 2019).

3.2.6 Glass transition temperature (T_g)

The glass transition temperature (T_g) is the temperature at which amorphous materials transform from a high-viscosity, glass-like state to a lower-viscosity, rubber-like state due to the increased molecule mobility. Powders stored below their T_g have an increased viscosity, hindering molecular mobility, whereas storage above T_g results in decreased viscosity causing structural changes such as stickiness and product collapse (Daza et al., 2016; Santhalakshmy et al., 2015). Table 1 shows T_g values for spray dried elderberry juice and pomace extract particles ranging from 62-120 °C, similar to what was previously observed for blackberry (51-60 °C) and black mulberry (40-76 °C) powder using maltodextrin or gum Arabic as carriers (Fazaeli et al., 2012; Ferrari et al., 2013). Interestingly, EP-TS treatment showed significantly higher T_g values compared to other treatments. In this study, the T_g values of wall materials SPI and tapioca starch were 39.07 ± 2.31 and 55.54 ± 10.67, respectively. Also, the expected lower sugar level of elderberry pomace extract, compared to elderberry juice, may explain the significantly higher T_g values of the EP-TS particles.

3.2.7 Color parameters

Color parameters are the direct indicators of quality and deeply influence the consumers' desire to purchase a certain food product. Both factors (carrier type and polyphenol source) and their interactions significantly influenced ($p < 0.05$) L*, a*, and b* values (Table 2) (Figure 2). EJ-TS particles had a significantly lower ($p < 0.05$) lightness (L*), indicating that this treatment looks darker than the others. According to Murugesan & Orsat, (2011), higher L* does not necessarily mean the quality deterioration of a product; rather, it could be the dilution effect of wall materials. The products of Maillard reactions (furfural and hydroxymethylfurfural) condense with anthocyanin to form brown pigments which could lead to lower lightness (Ferrari et al., 2013). Parameter b* shows that elderberry pomace extract particles are more towards the yellow coordinate while the juice particles are closer to the blue coordinate. The coordinate a* is the most sensitive color measurement as it directly correlates to the anthocyanin content. The

a* and chroma values (Table 2) are significantly higher ($p < 0.05$) for EJ-TS and EJ-SP suggesting higher anthocyanin content in these treatments compared to EP-TS and EP-SPI. Ferrari et al., (2012) spray dried blackberries with gum Arabic and maltodextrin and found that increased a* and chroma directly correlated with the anthocyanins and antioxidant capacity, which agrees with the present study.

Figure 2. Spray dried elderberry particles from juice (A, B) and pomace extract (C, D). Legend: (A) EJ-SPI: elderberry juice with soy protein isolate; (B) EJ-TS: elderberry juice with tapioca starch; (C) EP-SPI: elderberry pomace extract with soy protein isolate; (D) EP-TS: elderberry pomace extract with tapioca starch.

The hue angle reflects the characteristic color of the powder. A hue angle of 0°, 90°, 180°, and 270° represents red, yellow, green, and blue colors, respectively (Santhalakshmy et al., 2015). All elderberry particles showed very distinct hue angles ($p < 0.05$), as elderberry juice-derived particles were in the range of 330-350° (purple-red coordinate) and elderberry pomace

extract particles presented values between 14 and 18° (red coordinate). Previously, a much lower hue angle (270°), closer to the blue coordinate, was observed for European elderberry spray dried with SPI (Murugesan & Orsat 2011). These significant differences could be attributed to the different cultivars and sub-species, mainly regarding the diverse anthocyanin profile of subsp. *nigra* (rich in non-acylated compounds) that can influence the color stability and hue of the product (Jokioja et al., 2021). The total color change (ΔE) is a significant color parameter to analyze the color variation between fresh and processed products. Significant differences ($p < 0.05$) in ΔE values (Table 2) were observed for both factors (elderberry source, carrier type) and their interactive effects. In general, elderberry particles produced with SPI (EJ-SPI, EP-SPI) had a higher ΔE compared to particles produced with tapioca (EJ-TS, EP-TS), which could be due to the inherent color of SPI (Murugesan & Orsat, 2011).

Table 2. Color analysis of spray dried American elderberry juice and elderberry pomace extract particles.

Parameters	EJ-SPI	EJ-TS	EP-SPI	EP-TS
L*	36.76 ± 0.21^{c}	32.27 ± 2.14^{d}	53.48 ± 0.31^{a}	45.99 ± 0.02^{b}
a*	33.05 ± 0.11^{b}	34.67 ± 0.91^{a}	11.35 ± 0.15^{d}	17.60 ± 0.15^{c}
b*	-14.66 ± 0.04^{d}	-6.69 ± 0.25^{c}	3.86 ± 0.06^{b}	4.59 ± 0.07^{a}
Chroma (C)	36.15 ± 0.11^{a}	35.70 ± 0.56^{a}	11.99 ± 0.16^{c}	18.19 ± 0.16^{b}
Hue angle (°)	336.09 ± 0.06^{b}	349.19 ± 0.34^{a}	18.78 ± 0.04^{c}	14.62 ± 0.09^{d}
Total color change (ΔE)	28.74 ± 0.12^{c}	21.12 ± 0.5^{d}	40.88 ± 0.40^{a}	32.30 ± 0.17^{b}

Results are shown as average ± SD. Different letters in the same row indicate statistical difference by Tukey's test (2-way ANOVA, $p < 0.05$). EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

3.2.8 Hygroscopicity

Hygroscopicity refers to a product's ability to absorb moisture from the environment. The hygroscopicity values of spray dried elderberry juice and pomace extract were similar ($p < 0.05$) and between 18.77 and 22.72 %. However, according to GEA Niro, (2023) (15.1% to 20.0% - hygroscopic; 20.1% to 25% - very hygroscopic) they receive different classifications, with EP-

TS being classified as hygroscopic and all other treatments classified as very hygroscopic. In general, high hygroscopicity causes particles to clump together, which can alter the nutritional value and flow characteristics of the powder and impair handling and storage (Bajac et al., 2022). Despite EP-TS particles showing lower hygroscopicity compared to the other treatments, they exhibited the least flowability among them, as indicated in Table 1. This implies that while EP-TS particles had a lower tendency to absorb moisture from the surroundings, they had a poorer ability to flow smoothly. The results in the present study are comparable to those obtained for spray dried beetroot powder with whey protein isolate (20.15-23.18 %) but lower than spray dried tamarind pulp with SPI (20-34 %) and lulo (*Solanum quitoense*) pulp with maltodextrin or gum Arabic (54%) (Igual et al., 2014; Muzaffar & Kumar, 2015).

3.2.9 Solubility

The practical application of particulate products in the food industry is also influenced by the extent of their solubility in water. Poorly soluble powders may cause difficulties in processing, formulation, and incorporation, causing economic losses (Bajac et al., 2022). Ideally, food powders would wet quickly and sink rather than float (Santhalakshmy et al., 2015). Table 1 shows that the solubility of spray dried treatments ranged from 49.40-77.84 %. Elderberry particles with SPI (EJ-SPI, EP-SPI) showed statistically higher solubility ($p < 0.05$) than particles produced with tapioca starch (EJ-TS, EP-TS). The decreased solubility of EJ-TS and EP-TS treatments can be explained by the structure and functional properties of tapioca starch. Tapioca starch has a ratio of approximately 1:5 between amylose and amylopectin, with highly branched amylopectin forming clusters held together by hydrogen bonds, creating a tightly packed granular structure. This hinders water penetration and starch dissolution (Mukerjea et al., 2007; Stephen & Phillips, 2006). Additionally, a study by Babic et al., (2006) showed that the solubility of tapioca starch in water was around 7 % at 65 ºC and it increased with temperature. In the present study, tapioca starch was homogenized with elderberry juice or pomace extract at room temperature, which may explain its lower solubility. Relative to other studies, the solubility of both SPI and tapioca starch-derived treatments in this study was superior to spray dried blueberry pomace extract with SPI and similar to tamarind pulp with SPI (Correia et al., 2017; Muzaffar & Kumar, 2015). In contrast, the solubility of elderberry extract spray dried with beta-glucan, maltodextrin, and gum Arabic (Sobieralska & Kurek, 2019) was higher (89.14-90.18 %) than the spray particles in this study (49.40-64.38%).

3.3 Phytochemical content of spray dried elderberry particles

Processing of foods, particularly thermal processing, may result in decreased nutritional value and bioactive content. Therefore, finding processing methods that provide end products with undegraded phytochemical molecules is a primary challenge for the food industry (Zhang et al., 2020). Elderberries are a rich source of natural polyphenols and anthocyanins with relevant biological activities, potent coloring attributes, and antioxidant capacities. The elderberry juice has TPC (7623 mg GAE/L), with an expressive part of this being anthocyanins (8418 mg/L) and proanthocyanidin (238 mg/L). The elderberry pomace extract has a TPC of 9216 mg/L, and anthocyanins of 1012 mg/L, with a remarkable concentration of proanthocyanidins (562 mg/L). Both the elderberry juice and pomace extract showed high antioxidant activity (2945 and 5413 μM Trolox equiv/L) measured by the DPPH method. The elderberry pomace extract has a lower concentration of anthocyanins compared to the juice but is extremely high in proanthocyanidins with greater antioxidant activity than the juice (Hoskin et al., 2019).

The phenolic compounds present in spray dried elderberry particles come primarily from the elderberry juice or pomace extract since the TPC of both carriers (SPI and tapioca starch) was negligible (< 2 mg/g). Overall, particles produced with tapioca (EJ-TS, EP-TS) showed significantly ($p < 0.05$) higher TPC (42-49 mg GAE/g sample) compared to those produced with SPI (EJ-SPI, EP-SPI) (32-39 mg GAE/g sample) (Figure 3A). Moreover, the elderberry pomace extract particles had higher TPC than the juice particles ($p < 0.05$), indicating that the pomace, composed of skins, seeds, and pulp, is a rich source of phytochemicals and may have the potential to produce functional ingredients. TPC of spray dried elderberry juice with SPI and tapioca (EJ-SPI, EJ-TS) in this study was between 32-42 mg GAE/g, which is comparable to spray dried elderberry juice powders (subsp. *nigra*) obtained with different wall materials in a 1:1 ratio of total solids to wall material (Murugesan & Orsat., 2011). These wall materials include soy milk powder (46 mg GAE/g), soy protein powder (36 mg GAE/g), isolated soy protein (44 mg GAE/g), gum acacia (48 mg GAE/g), and maltodextrin (40 mg GAE/g), as reported by Murugesan & Orsat. (2011). Furthermore, the TPC content of spray dried elderberry juice particles shown here is higher than previously shown for freeze dried elderberry juice powder (12.42 mg GAE/g, Casati et al., 2019).

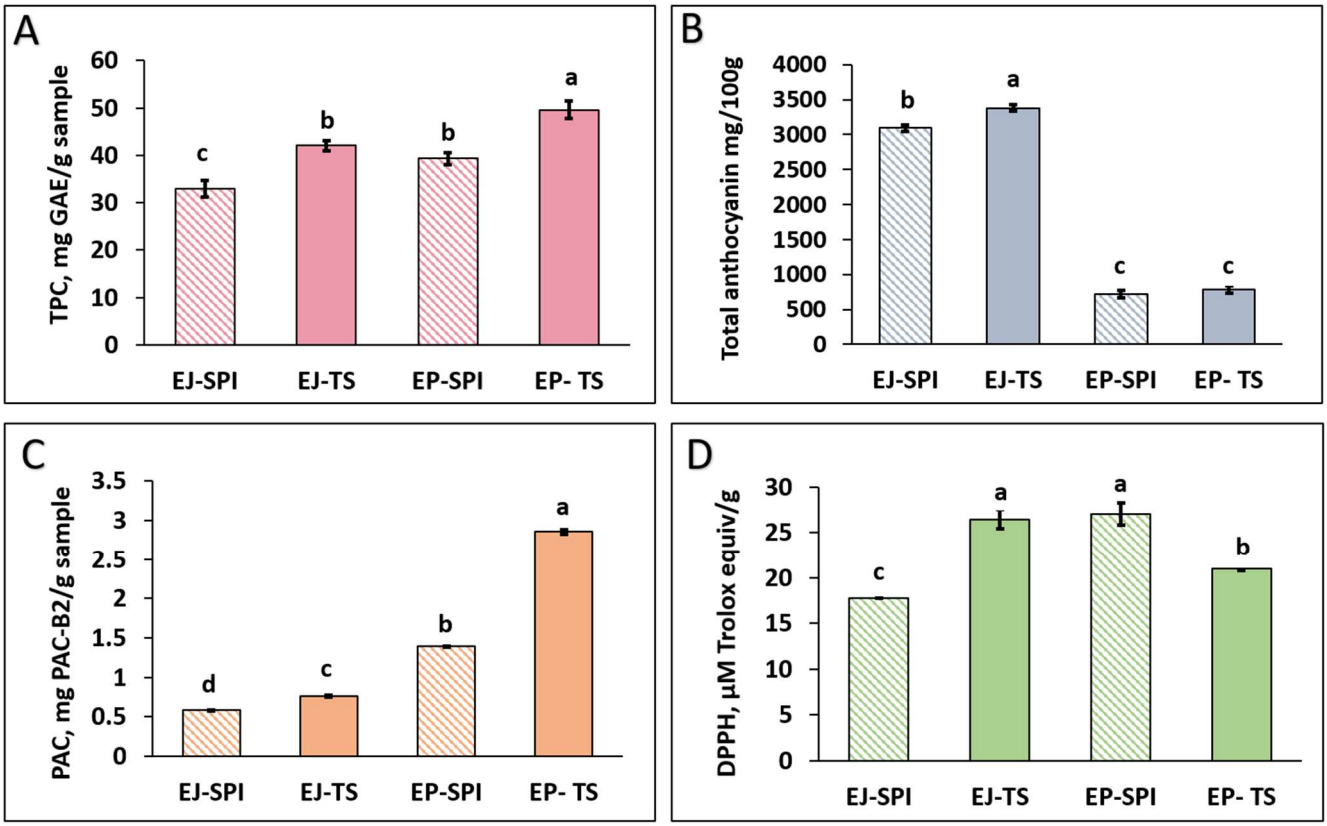


Figure 3. (A) Total polyphenol content, (B) Total anthocyanin content, (C) Proanthocyanidin content (PAC), and (D) Antioxidant capacity for spray dried particles. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch. Bars with different letters are significantly different by Tukey's test (2-way ANOVA) test, $p < 0.05$.

Table 3. Concentration of anthocyanins identified by UPLC (Ultra Performance liquid chromatograph) analysis of American elderberry juice, pomace extract and their spray dried particles [a].

Anthocyanin	Elderberry juice mg/100ml	Elderberry pomace extract mg/100ml	EJ-SPI mg/100 g	EJ-TS mg/100 g	EP-SPI mg/100 g	EP-TS mg/100 g
cyanidin 3-sambubioside-5-glucoside	144.77±40.40	16.12±0.88	734.26±13.64	685.92±28.73	115.62±18.37	112.77±13.64
cyanidin 3-glucoside	ND	ND	6.07±0.60	6.58±0.56	ND	ND
cyanidin 3-sambubioside	0.87±0.09	ND	17.41±0.77	16.61±0.81	ND	ND
cyanidin-based anthocyanin	3.11±0.39	ND	6.70±0.03	7.12±0.28	ND	ND
delphinidin 3-rutinoside	11.09±3.50	ND	51.69±0.78	56.07±2.71	10.52±0.46	9.49±1.09
cyanidin 3-(Z)-p-coumaroyl-sambubioside-5-glucoside	21.07±1.91	3.42±0.09	74.20±0.72	79.65±1.11	22.06±2.04	24.29±1.93
cyanidin 3-p-coumaroyl-glucoside	6.00±1.00	0.60±0.16	19.77±0.19	21.23±0.12	5.98±0.32	6.40±0.25
cyanidin 3-(E)-p-coumaroyl-sambubioside-5-glucoside	654.09±33.10	81.08±4.89	2181.78±3.61	2500.99±9.35	564.66±37.46	633.14±33.47
cyanidin 3-p-coumaroyl-sambubioside	ND	ND	ND	6.05±0.58	ND	0.76±1.32
Total anthocyanins	841.81±80.29	101.22±4.37	3091.88±43.07	3380.22±41.75	718.84±57.97	786.86±50.70

[a] Results are shown as mean ± SD. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

Overall, the anthocyanin profiles of spray dried juice and pomace extract particles mirrored the anthocyanins originally present in the elderberry juice and pomace extract. For instance, cyanidin 3-(E)-p-coumaroyl-sambubioside-5-glucoside was the major anthocyanin in juice and pomace extract, and a similar trend was observed in the resulting spray dried particles (Table 3). Both factors (elderberry source and carrier type) and their interaction significantly affected the total anthocyanin content of spray dried particles (Figure 3B). Notably, spray dried juice particles (EJ-SPI, EJ-TS) had almost four times the concentration of anthocyanins found in the counterpart pomace extract particles (EP-SPI, EP-TS), which indicates that the elderberry fruit pulp is a major source of anthocyanins. Notably, the total anthocyanins of spray dried American elderberry juice with carriers (3091-3380 mg/100g) in this study is significantly higher than the total monomeric anthocyanins in freeze dried European elderberry pulp using different carriers such as maltodextrin (1059 mg/100g), corn-based soluble fiber (1200 mg/100g), waxy maize modified starch (1251 mg/100g), k-carrageenan (1363 mg/100g) (Baeza et al., 2021). Therefore, spray drying is a viable alternative to deliver elderberry phytochemicals in a stable format with increased shelf life (Ferrari et al., 2013).

The proanthocyanidin content (PAC) of spray dried particles derived from elderberry pomace extract (EP-SPI, EP-TS) is significantly ($P < 0.05$) higher than juice particles (EJ-SPI, EJ-TS) (Figure 3C). Similar to what was observed for TPC and anthocyanins, tapioca starch-derived particles showed higher PAC compared to SPI. Lower levels of PAC were observed for elderberries compared to blueberries, cranberries, chokeberries, and blackcurrant (Hoskin et al., 2019; Sidor & Gramza-Michałowska, 2015). Proanthocyanidins are characterized by their degree of polymerization, which has only been reported in the range of dimeric to hexameric forms in elderberries (Wu et al., 2004). Further, as the degree of polymerization increases, astringent yellow to brown compounds are formed (Krenn et al., 2007). Because of the negligible detection of oligomers and polymers of proanthocyanidin in elderberries, they have less astringent taste than chokeberries (Wu et al., 2004).

A serving size of ½ cup (73 g) of American elderberries contains approximately 434 mg of TPC and 183 mg of total anthocyanins (Perkins-Veazie et al., 2015). Thus, small amounts of spray dried juice samples (10-14 g and 5-6 g) would yield equivalent amounts of TPC and anthocyanins respectively. It is noteworthy that the elderberry pomace constituted 32 % of the original weight of elderberries used in this study. Although fruit pomaces are often discarded as processing waste, the elderberry pomace proved to be a valuable source of phytochemicals as 8-11 g and 22-26 g of spray dried pomace extract samples provide the same amounts of TPC

and anthocyanins as in ½ cup serving size of elderberry fruits, respectively.

The small size of elderberries (2.3 mm diameter; 50 mm^3 volume) and their delicate structure make it challenging to handle the fruits without causing damage. A study by Johnson et al. (2015) showed that frozen storage of American elderberry juice over 3, 6, and 9 months resulted in a significant decrease in total phenolics and anthocyanins. While fruit juice concentrates are natural FDA-approved food colorants, the limited stability and color fading of juice anthocyanins restrict their use. Nevertheless, the American elderberry juice particles obtained in this study are rich in acylated anthocyanins (Table 3), and therefore, enhanced stability against pH, light, and heat might be expected (Osman et al., 2023).

3.4 Antioxidant capacity

The radical scavenging activity of spray dried elderberry juice and pomace extract is shown in Figure 3D. The antioxidant capacity refers to substances that delay or mitigate oxidative reactions by free radicals that cause structural or functional damage to cell structures (Lobo et al., 2010). The DPPH method is a popular and effective assay for evaluating the antioxidant properties of samples due to its simplicity, low cost, and efficacy in hydrophilic environments. This method measures the sample's ability to reduce a stable DPPH radical in the presence of an antiradical compound (Chedea & Pop, 2019).

Greater antioxidant capacity was observed for EJ-TS and EP-SPI treatments (Figure 3D). Pearson's correlation coefficient was employed to examine the relationship between antioxidant capacity and TPC, anthocyanin, and PAC values. For spray dried elderberry juice particles, a strong and significant positive correlation was observed between DPPH and TPC, PAC, and anthocyanin content ($r = 0.974$, $p < 0.05$; $r = 0.995$, $p < 0.05$; $r = 0.986$, $p < 0.05$, respectively), which confirm that these bioactive compounds contributed to the observed free radical scavenging activity. Likewise, for spray dried elderberry pomace extract particles, DPPH showed a positive correlation with TPC and PAC ($r = 0.995$ $p < 0.05$; $r = 0.98$, $p < 0.05$ respectively, while no such correlation was observed for anthocyanin ($r = 0.73$, $p > 0.05$). This demonstrates that both TPC and PAC play an important role in the antioxidant activity exerted by particles produced with elderberry pomace extract. However, compared to the elderberry juice, the elderberry pomace extract produced in this study is not a relevant source of anthocyanins (Figure 3B, Table 3) and therefore, this class of phenolic compounds did not contribute to the observed DPPH results of EP-SPI or EP-TS.

3.5 Ascorbic acid content

No ascorbic acid was detected in juice, pomace extract, or spray dried elderberry particles in this study. Sobieralska & Kurek. (2019) reported ascorbic acid in both elderberries (subsp. *nigra*) extract (179 ± 3.257 mg/100g) and the spray dried elderberry powder produced with beta-glucan (9.4–21.37 mg/100g). According to Młynarczyk et al. (2018), the presence of ascorbic acid is inconsistent across studies. Depending on the cultivar and location, the ascorbic acid in subsp. *nigra* ranged from 6 to 25 mg/100g (Kaack & Austed, 1998). In that study, when European elderberry juice was purged with oxygen, the levels of selected anthocyanin and quercetin increased as the ascorbic acid concentration decreased. This suggests that oxygen can promote the interaction of ascorbic acid with flavonoids which would lead to ascorbic acid depletion in the final product. Hence, a possible reason for the negligible detection of ascorbic acid in the elderberry juice or pomace extract could be their oxidation and consequent decrease during pulping.

3.6 *In vitro* bioaccessibility assay

To exert pharmacological activity, bioactive substances must be effectively absorbed from the intestinal system into the bloodstream, and transported to the appropriate location in the body (Gullon et al., 2015). *In vitro* digestion models are popular research strategies to simulate and predict the *in vivo* digestion of target phytochemicals. Compared to *in vivo* models, they are more affordable, simpler, and efficient and do not require ethical committee approvals (Grace et al., 2021).

In this study, the bioaccessibility of spray dried particles was evaluated based on the percentual bioaccessibility index using the total polyphenol content as the marker phytochemical group. Bioaccessibility ranged from 12.26 % (elderberry juice, liquid, non-encapsulated) to 26.86 % (spray dried EJ-SPI) and important differences among elderberry groups were observed. Similarly low BI was observed for non-encapsulated elderberry juice and pomace extract ($p > 0.05$, Figure 4). For both elderberry juice and pomace extract, the bioaccessibility was enhanced when elderberry polyphenols were complexed and encapsulated with SPI ($p < 0.05$), resulting in more than a 2-fold increase in the bioaccessibility of EJ-SPI, compared to liquid, non-encapsulated elderberry juice. Previous studies on the *in vitro* GID of elderberry (*S. nigra* subsp. *nigra* and *S. lanceolata*) polyphenols *reported significant loss or degradation post-digestion process* (Olejnik et al., 2016; Pinto et al., 2017).

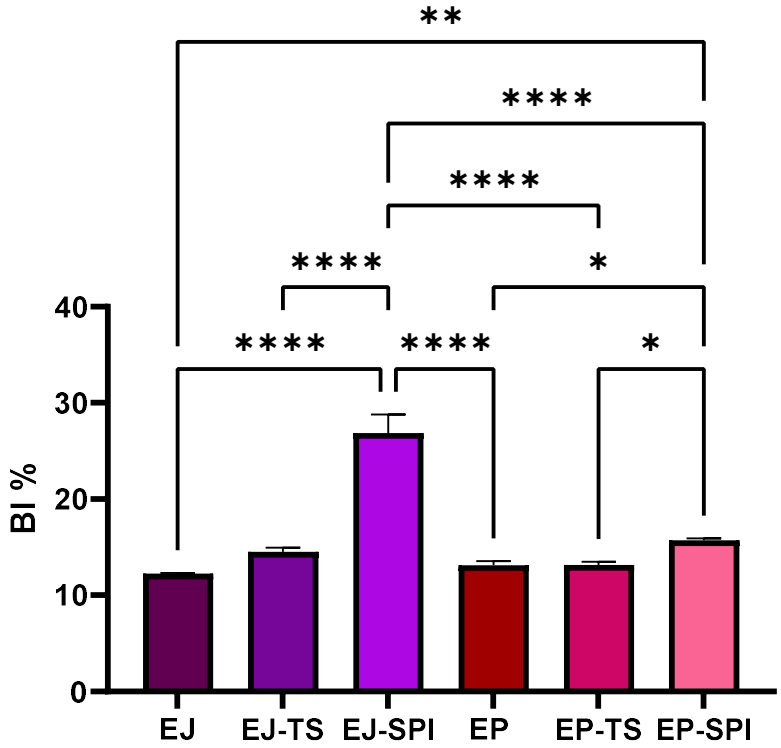


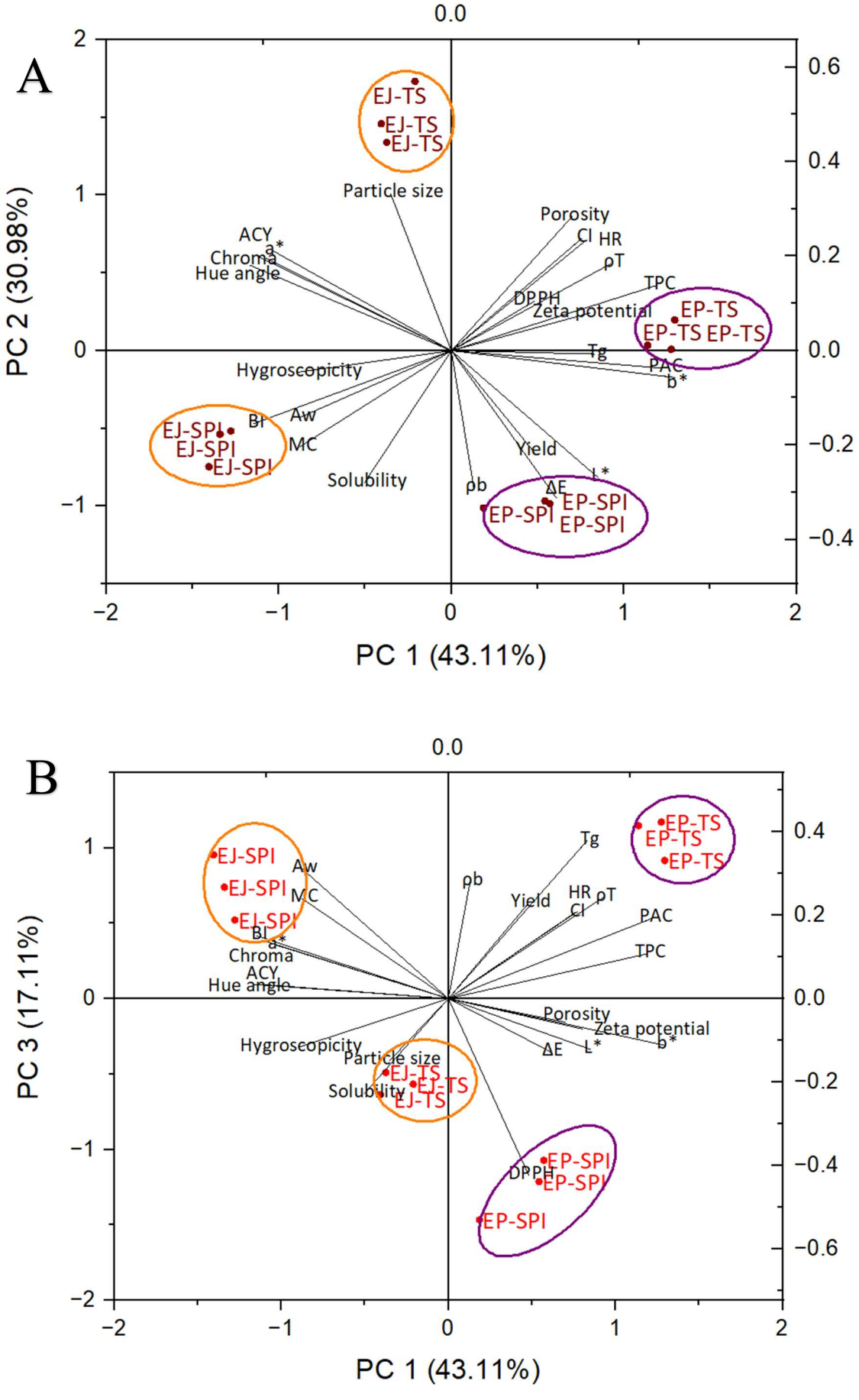
Figure 4**.** Bioaccessibility index (%) for spray dried American elderberry particles after simulated in vitro gastrointestinal digestion. EJ: elderberry juice (non-encapsulated); EJ-TS: elderberry juice with tapioca starch; EJ-SPI: elderberry with soy protein isolate; EP: elderberry concentrated pomace extract (non-encapsulated); EP-TS: elderberry pomace extract with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate. Asterisks denote statistical differences among mean values according to Turkey's test: *p < 0.05; **p<0.005; *** p<0.001; **** p < 0.0001.

Phenolic compounds during GID are unstable due to dietary components, pH changes, and digestive enzymes, leading to conversion, and degradation (Pinto et al., 2017). The potential of using protein-polyphenol complexation to enhance the bioavailability of dietary bioactives was demonstrated by Diaz et al., (2020). In a study by Grace et al. (2021), the bioaccessibility of polyphenols from rosemary (*Rosmarinus officinalis*) extract increased significantly after spray drying with whey protein and soy protein isolates as wall materials, compared to the uncomplexed rosemary extract. The bioaccessibility increased from 20.2% in the rosemary extract without protein carrier to 56.7% and 53.8% in rosemary-protein particles produced with whey and soy protein isolates, respectively. Protein-bound polyphenols exhibit greater bioavailability than unbound polyphenols, primarily due to the protein-polyphenol interactions occurring via hydrogen bridge binding, van der Waals, and hydrophobic interactions Lila et al. (2022). Hydrogen and hydrophobic interactions were predominant in soybean protein-polyphenol binding; SPI is mainly composed of glycinin, β-conglycinin, and lipophilic proteins

(Ao et al., 2021). The hydroxyl group of flavan-3-ol polyphenol can form hydrogen bonds with protein polar groups, while non-polar aromatic rings of polyphenols bind to the non-polar surface of soy proteins through hydrophobic interactions (Lila et al., 2022).

3.7 Principal component analysis (PCA)

To better understand the trends and relationships among the different variables and factors studied, principal component analysis (PCA) was performed. In the PCA biplot (Figure 5), PC1, PC2, and PC3 accounted for 43.11%, 30.98%, and 17.11% of the variance, respectively. The cumulative PC (91.20%) is high enough to explain the total variance in the data set. PC1 is mainly positively contributed by TPC, PAC, b*, and true density. On the other hand, PC1 was negatively contributed by bioaccessibility index, anthocyanins, a*, chroma, hue angle, and hygroscopicity. For PC2, particle size, porosity, Carr index, and Hausner ratio showed positive contributions while L*, bulk density, solubility, and ΔE had negative contributions. PC3 was mainly positively contributed by T_g, moisture, A_w, and yield, while it was mainly contributed negatively by DPPH. The biplot shows that particles of different treatments are clearly separated based on the PCA scores, revealing that the processing method and the addition of wall material influenced the properties of the spray dried particles. On PC1, the negative side displays spray dried juice particles, whereas the positive side shows pomace extract particles. Conversely, on PC2, particles produced with tapioca starch are located on the positive side, while those obtained with SPI are on the negative side (Figure 5A).


A
0.0
EJ-TS
EJ-TS
EJ-TS
Particle size
Porosity
Cl
HR
ρT
ACY
a*
Chroma
Hue angle
TPC
DPPH
Zeta potential
EP-TS
EP-TS EP-TS
Tg
PAC
b*
Hygroscopicity
Bl
Aw
EJ-SPI
EJ-SPI
EJ-SPI
MC
Yield
L*
Solubility
ρb
ΔE
EP-SPI
EP-SPI
EP-SPI
PC 2 (30.98%)
PC 1 (43.11%)
B
0.0
EP-TS
EP-TS
EP-TS
Tg
EJ-SPI
Aw
EJ-SPI
MC
ρb
Yield
HR
ρT
Cl
EJ-SPI
Bl
PAC
a*
Chroma
TPC
ACY
Hue angle
Porosity
Zeta potential
Hygroscopicity
ΔE
L*
b*
Particle size
EJ-TS
EJ-TS
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EP-SPI
DPPH
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EP-SPI
PC 3 (17.11%)
PC 1 (43.11%)

4.

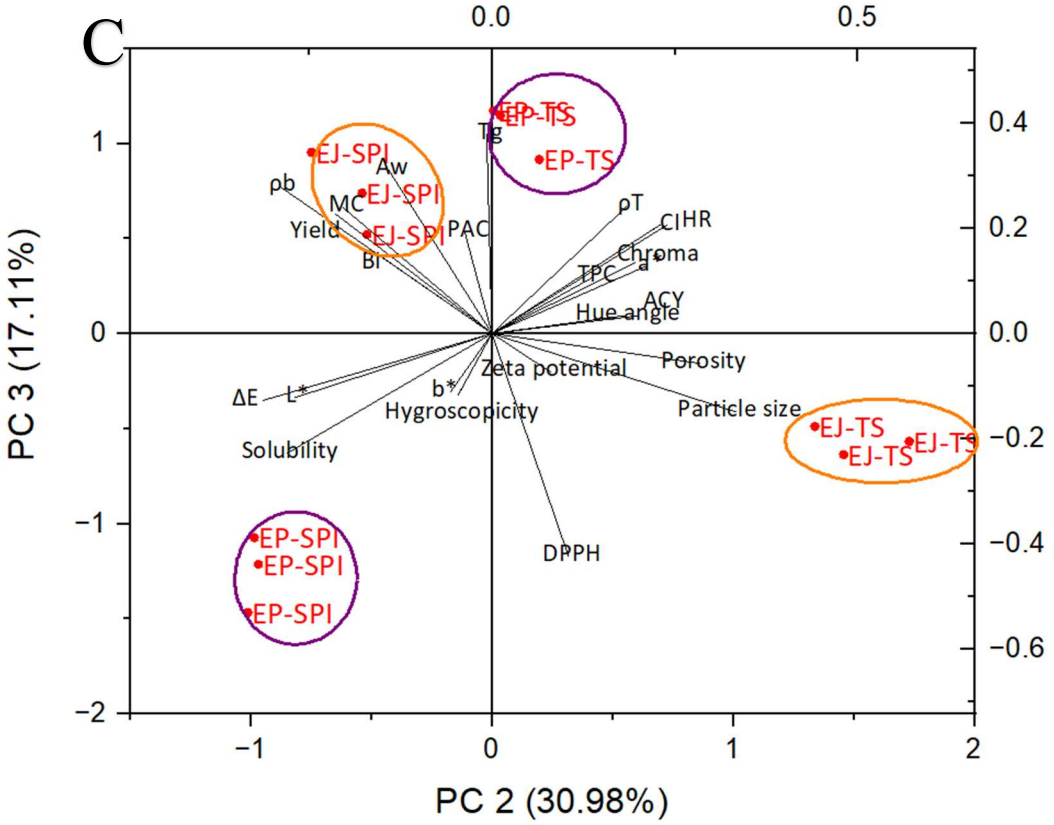

Figure 5A-C. Two-dimensional biplot ordination on PCA of (A) PC 1 and 2; (B) PC 1 and 3; (C) PC 2 and 3 of spray dried American elderberry juice or elderberry pomace extract particles displaying the relationship among treatments and variables. Aw: water activity, MC: moisture content, Tg: glass transition temperature, b*: yellowness/blueness, a*: redness/greenness, L*: lightness/darkness, ACY: anthocyanin, BI: bioaccessibility index, CI: Carr index, HR: Hausner ratio, ΔE: total color change, DPPH: antioxidant capacity, ACY: total anthocyanins, TPC: total polyphenol content, PAC: proanthocyanidins, ρ_b: bulk density, ρ_T: true density. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

Conclusion

Elderberry juice and pomace extract were spray dried with tapioca starch and soy protein isolate. The process proved to be efficient as satisfactory solids recovery was obtained for all treatments and phytochemical-rich and stable elderberry particles were produced. Furthermore, higher bioaccessibility was observed for spray dried elderberry particles produced with both juice and pomace extract compared to non-encapsulated elderberry sources. Therefore, in this study, we show that spray dried elderberry particles are a convenient and versatile format to deliver polyphenols from underexplored American elderberries to be used in multiple food applications. These phytochemical-rich fruit ingredients can be efficiently prepared both from

elderberry juice or pomace, creating sustainable, diversified, and profitable avenues for fruit growers and processors. The spray drying encapsulation of polyphenol-rich juice and pomace extract obtained from American elderberries is a commercially sound and industrially friendly strategy to maximize the marketability and overall value of this underutilized American crop.

Author contributions

KSR: formal analysis, investigation, methodology, data curation, writing–original draft, writing–review and editing, and visualization. ESS: investigation and formal analysis. MM: writing–review and editing. PP: methodology, and writing–review and editing. MAL: writing–review and editing. MG: writing–review and editing. AT: funding acquisition, resources, and writing–review and editing. RH: conceptualization, methodology, writing–review and editing. KK: conceptualization, methodology, writing–review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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CAPÍTULO 5

ARTIGO 3: ***Spray dried insect protein-polyphenol particles deliver health-relevant value-added food ingredients.***

Capítulo 5 - Apresentação do artigo 3

O estudo para composição deste artigo foi realizado na Universidade Estadual da Carolina do Norte (NCSU) nos Estados Unidos da América (EUA). O artigo 3 foi submetido na revista Future Foods, que possui fator de impacto: 4,0, em julho/2023 para publicação em edição especial sobre proteínas de inseto.

Spray dried insect protein-polyphenol particles deliver health-relevant value-added food ingredients.

Edilene Souza da Silva[a,b], Jia Xiong[b] , Medeiros, Fábio Gonçalves Macêdo[b], Mary Grace[b], Marvin Moncada[b], Mary Ann Lila[b], Roberta Targino Hoskin[a,b, *]

[a] Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Campus Central, s/n, Natal, RN 59078-970, Brazil.
[b] Plants for Human Health Institute, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081, United States.
*Corresponding author: rtcorrei@ncsu.edu

ABSTRACT

A novel strategy to create value-added insect protein-derived ingredients is presented in this study. Spray dried protein-polyphenol particles were produced using aqueous polyphenol extracts of rosemary (RM) or muscadine grape pomace (MG) complexed with insect protein (IP) alone or blended with pea protein 50:50 (IPP). The spray drying process was evaluated (solids recovery SR and polyphenol retention PR) and the four experimental protein-polyphenol treatments IP–RM, IP-MG, IPP–RM and IPP–MG were characterized regarding their physicochemical, bioactivity, functional, bioaccessibility and thermal stability properties. Higher SR (53.7-53.3%) and PR (53.1-62.5%) was observed for IPP-derived particles ($p<0.05$). Particles had water activity in the microbiologically stable range (0.24-0.32) and high protein content (29.5%-38.3%). All particles had low hygroscopicity (<15%) and solubility between 44-52.83%. Remarkably high phenolic content (>68.5 mg GAE/g) was shown for MG-derived particles. Good emulsifying activity (1.85 to 16.46 m^2/g) and emulsifying stability (> 60%), besides foaming capacity (4-57%) and foaming stability (2-37.3%) were observed for insect protein-polyphenol particles. Differently from MG-derived particles, RM-derived treatments

showed higher polyphenol bioaccessibility than non-complexed polyphenols ($p<0.05$). Overall, our study demonstrates that spray drying microencapsulation is an efficient strategy to produce attractively colored, value-added functional protein-polyphenol ingredients using insect protein.

Keywords: alternative protein, cricket, food ingredients, revalorization.

1. Introduction

Food systems would be greatly benefited by increased production, consumption, and consumer acceptance of edible insects. Although conventional animal-based proteins are of good nutritional quality, their production is resource-intensive and often involves unsustainable practices (Bryant, 2022). Edible insects, on the contrary, are a novel food source with high nutritional value produced through less intensive and more sustainable practices. Besides converting food into energy more efficiently, their production emits less greenhouse gases (GHGEs) than traditional animal husbandry, while using considerably less water and land (Queiroz et al., 2023). In addition, edible insects have a balanced nutritional profile, providing lipids and proteins, fiber, vitamins, minerals, and bioactive compounds. Although variations among species are expected, in general, insect protein fulfills the required amino acid composition for human composition, as established by the World Health Organization (WHO) (Lucas et al., 2020). Indeed, insect protein has been considered one of the most promising alternative protein sources to mitigate the global problem of protein production and affordability and constitutes a new business opportunity for the food industry to capitalize on the ever-increasing consumer demand for alternative, healthier and environmentally friendly diets (Gravel & Doyen, 2020; Lucas et al., 2020).

However, the incorporation of edible insects in our Western diet is challenging. Entomophagy (consumption of insects as a whole) is largely refused by consumers based on disgust and/or neophobia (rejection of food perceived as non-familiar) reasons (La Barbera et al., 2018; White et al., 2023). Insect protein concentrates have been considered as a possible approach to eliminate insect appearance and improve consumption (Queiroz et al., 2023), but it still has questionable taste and appearance, which might impair consumer acceptance. Therefore, the development of novel ways to deliver the benefits of high-quality insect protein to the consumer is needed. Convenient insect protein-derived products constitute a new opportunity to increase marketability by enabling easier incorporation of unknown insect protein into more familiar

food products (Meshulam-Pascoviche et al., 2022).

Developing insect protein-derived food ingredients with enhanced appearance, functionality and desirable physicochemical attributes and stability was the main motivation of this work. Our research group has demonstrated that the complexation between polyphenol sources and protein is a versatile tactic to produce value-added food ingredients. Better polyphenol bioavailability, good sensory properties and desirable functionality have been demonstrated for protein-polyphenol particles, which encourages their use as healthy functional ingredients (Hoskin et al., 2022; Lila et al., 2022). Proteins with different characteristics, derived from both animal or plants, and a wide array of polyphenol sources can be used for the manufacturing of protein-polyphenol ingredients, including greens, herbs, fruit juices or pomaces, an abundant side stream of the food industry (Grace et al., 2021, 2022; Hoskin, Xiong, & Lila, 2019). For example, muscadine grape (*Vitis rotundifolia*) pomace (constituted mainly by seeds, skins, and residual pulp) is an abundant phytochemical-rich resource from United States southeastern region. This natural resource is rich in polyphenols, with antioxidant and antimicrobial properties (Xu et al., 2017), including nutraceutical molecules such as anthocyanins and proanthocyanidins, with health-relevant properties (Collard et al., 2020; Yuzuak & Xie, 2022). Likewise, rosemary (*Rosmarinus officinalis*) leaves are a valuable source of antibacterial, neuroprotective, anti-inflammatory, and anti-obesity molecules (Khatoon et al., 2021).

In this study we describe a protocol to produce insect protein-polyphenol particles by spray drying insect protein alone or insect protein bound to pea protein with polyphenols from rosemary leaves or recovered from muscadine grape pomace. The spray drying process was evaluated and the resulting protein-polyphenol particles were characterized regarding their composition, physicochemical attributes, phytochemical content, functionality, thermal stability, and bioavailability. To the best of our knowledge, this is the first study reporting the spray drying encapsulation of polyphenols with insect protein to produce phytochemical-rich particulate food ingredients. The results of this study unveil a sensible strategy to produce value-added, versatile and convenient products and broaden the use of insect protein in food applications.

2. Materials and methods

2.1. Materials

Rosemary leaves (*Rosmarinus officinalis*) were obtained from John Weddington Greenhouse

(Salisbury, North Carolina, USA) as fresh rosemary shoots. The leaves were carefully removed from the stalks and kept in frozen storage (-80 °C) under vacuum before the extraction process. The grape muscadine pomace (*Vitis rotundifolia*) from Noble cultivar (also known as purple or black muscadine grape) were obtained from Muscadine Products Corporation (Wray, Georgia, USA) as the by-product of the juicing process and was comprised of residual pulp, peels and seeds. The dried muscadine grape pomace was preserved at -20 °C until use. The protein sources used in this study were insect protein (IP) obtained from crickets (*Acheta domesticus;* Entomo Farms; 70 % protein) and pea protein (PP) (Nutralys S85 Plus N, 84% protein). A protein blend consisting of insect protein and pea protein (IPP) 50:50 (w/w) was prepared. Before processing, the insect protein was ground for 1 min (high-speed multifunction Grinder HC-2000, Cgoldenwall, Glendale, CA, USA) and sieved (80 mesh).

2.2. Production of polyphenol extracts from rosemary leaves and muscadine grape pomace

The rosemary leaves were extracted using a 3-step sequential process (Grace et al., 2021). Firstly, batches of 200 g of rosemary leaves were blended with 1 L of 100 % ethanol (1:5 w/v extraction ratio) for 5 minutes under vacuum (Vita-Mix Corp, Cleveland, OH, USA). The ethanolic extract was then sonicated at 50 °C for 10 min and centrifuged at 4 °C for 20 min. The supernatant was collected and set aside. The precipitate obtained at this first stage was resuspended in 500 mL of 80 % aqueous ethanol and was then sonicated at 50 °C for 10 min and centrifuged at 4 °C for 20 min. The precipitate resulting from this second extraction step was dispersed in 400 mL of 50 % aqueous ethanolic solution, and once again, it was sonicated and centrifuged using the same conditions described before. The supernatants of all three stages were pooled together and vacuum evaporated (45 °C) to eliminate the ethanol to prepare the final concentrate rosemary extract.

For muscadine grape pomace, a previously reported optimized protocol was used to produce concentrated muscadine extract (Hoskin et al., 2019). Briefly, a 50 % ethanol solution was vigorously blended (Ninja Professional Plus System, Needham, MA, EUA) with freeze-dried muscadine grape pomace (1:4 w/v extraction ratio) and then transferred to a water bath at 80 °C for 2h. The mixture was vacuum filtered, centrifuged at 4000 rpm for 20 minutes, and the ethanol was evaporated in a rotary evaporator at 45 °C under constant rotation to obtain the final muscadine grape pomace concentrated extract. Multiple batches of rosemary or muscadine pomace extract were combined to form a single batch of each polyphenol source that was used for all subsequent experimental steps.

2.3. Production of spray dried insect protein-polyphenol particles

2.3.1. Experimental groups

Before spray drying, 8 % (w/v) of protein (insect protein IP alone or the insect-pea protein blend IPP, 50:50 w/w) was added to the concentrated liquid extracts produced with rosemary leaves or muscadine pomace extract. These four experimental protein-polyphenol experimental groups were prepared and investigated: IP-RM (insect protein with concentrated rosemary extract); IP-MG (insect protein with concentrated muscadine grape pomace extract); IPP-RM (blend of insect protein and pea protein 50:50 w/w with concentrated rosemary extract); and IPP-MG (blend of insect protein and pea protein 50:50 w/w with concentrated muscadine grape pomace extract). Each experimental group was prepared by mixing the protein and polyphenol sources for 2 h, until complete hydration, using a magnetic stirring plate.

2.3.2. Spray drying

Immediately before spray drying, the protein-polyphenol suspension was processed by high-speed homogenization (PRO Scientific PRO250, Cole Parmer, Vernon Hills, IL, USA) for 4 min to eliminate any possible clusters or agglomeration that would cause clogging during spray during. The spray drying process (B-290, Buchi Labortechnik AG, Flawil, Switzerland) used air in co-current flow under the following conditions: 0.7 mm nozzle, 10 mL/min of feed flow (controlled by peristaltic pump) kept under constant magnetic stirring at 30 °C, drying air inlet temperature of 130 °C and aspirator rate of 100 %. The resulting protein-polyphenol spray dried (SD) particles were collected from the collection chamber, weighed, then sealed in plastic bags and stored at -20 °C until further use.

2.3.3. Solids recovery and polyphenol retention

The percentage of solids recovery of spray dried protein-polyphenol samples was determined using the equation [total solids content of resulting particles (protein-rosemary or protein-muscadine particles)/total solids content in the feeding solution (before spray drying)] x 100 (Grace et al., 2021). Additionally, the percentage of polyphenol retention was calculated using Equation 1 (Zhang et al., 2020):

$$Polyphenol\ retention(\%) = \frac{TPC\ of\ SD\ powder\ \left(\frac{mg}{g}\right)}{Total\ solids\ of\ SD\ powder\ (g)} \div \frac{TPC\ of\ feed\ solution\ \left(\frac{mg}{g}\right)}{Total\ solids\ of\ feed\ solution\ (g)} x100 \quad (1)$$

2.4. Phytochemical evaluation of spray dried insect protein-polyphenol particles

2.4.1. Elution of phytochemicals

The protein-polyphenol matrices were extracted according to Grace et al. (2013). For each spray dried experimental group, 1 mL of 1 % acetic acid in 80 % methanol in water was added to 20 mg of sample, followed by sonication (5 min at 50 °C) and centrifugation (10 min, 4000 rpm). The procedure was carried out twice in 2 mL centrifuge tubes, and the eluates were used to assess the total phenolic (TPC), proanthocyanidin (PAC) and anthocyanin (ANC) contents, as well as the antioxidant activity.

2.4.2. Total phenolic content (TPC)

TPC was assessed spectrophotometrically by a modified Folin-Ciocalteu procedure using a microplate-adapted technique (Xiong et al., 2020). Results were calculated as gallic acid equivalents (mg GAE/ g sample) based on the absorbance readings at 765 nm using a standard curve for gallic acid (Sigma-Aldrich, St. Louis, MO, USA).

2.4.3. Proanthocyanidins (PAC)

PAC was measured calorimetrically in a 96-well plate using the previously published DMAC method (Grace et al., 2013; Prior et al., 2010). A procyanidin B2 standard curve was used to determine the total PAC concentration, which was then expressed as mg of proanthocyanidin B2 equivalent (mg PAC-B2/g sample).

2.4.4. Anthocyanins (ANC)

Samples were initially filtered through 0.2 μm PTFE syringe filter (Fisher Scientific, Fair Lawn, NJ, USA), and submitted to HPLC analysis using an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) connected with a photodiode array detector (Hoskin et al., 2022). Separation was performed with using a RP Supelcosil- LC-18 column, 250 mm × 4.6 mm × 5 μm (Supelco, Bellefonte, PA, USA). Five anthocyanidin compounds were detected and identified based on a previous study (Xiong et al., 2020). Concentrations were based on peak area measurements against a standard curve constructed with cyanidin-3-O-glucoside. The concentration of each detected anthocyanin was used to determine the total anthocyanin content expressed as mg cyanidin-glucoside equivalent/g.

2.5. Radical scavenging activity by 2.2-diphenyl-1-pricrylhydrazil (DPPH)

Radical scavenging activity was measured in 96-well microplates using 20 µL of eluted samples and 180 µL of DPPH solution (150 µmol/L) in methanol-water (80:20, v/v). The absorbance was determined at 515 nm following 40 min of darkness and room temperature. A calibration curve was constructed using solutions of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at various concentrations (100-500 µM), and the results were expressed as µmol Trolox equivalents (TE) (µmol TE/ g sample).

2.6. Water activity, pH, and proximate composition

An Aqualab water activity meter (Decagon, Pullman, WA, USA) was used to measure the water activity (Aw) and a moisture analyzer was used to assess the moisture content (Mettler Toledo HE53, Columbus, OH, USA) of spray-dried protein-polyphenol particles. The pH (XL50 dual-channel pH meter, Fisher Accumet, Hampton, NH, EUA), fat content (Rapid NMR Fat Analyzer, Oracle, Austin, TX, EUA), ash (method no. 942.05, AOAC 2005) and protein (method no. 992.15, AOAC 2012) were determined. Total carbohydrates were calculated by difference.

2.7. Physicochemical characterization

2.7.1. Bulk and tapped density

The volume occupied by samples (1-2 g) in a 10 mL graduated cylinder was recorded and used to determine the bulk density (ρB) (weight per volume). After gently tapping the graduated cylinder carrying the sample 120 times against a rubber pad, the volume was measured to determine the tapped density (ρT).

2.7.2. Flowability

The Hausner ratio (HR) and Carr's compressibility index (CI) were used to measure the flowability, which is defined as the capacity of samples to flow freely in a constant and predictable manner, according to Equations (2) and (3) (Saifullah et al., 2016):

$$HR = \frac{\rho T}{\rho B} \quad (2)$$

$$CI = \left(\frac{\rho T - \rho B}{\rho B}\right) * 100 \quad (3)$$

Samples were classified considering the following scales (Bhusari et al., 2014): Hausner ratio:

1.0 < HR < 1.1, free flowing powder; 1.1 < HR < 1.25, medium flowing powder; 1.25 < HR < 1.4, difficult flowing powder; and HR > 1.4, very difficult flowing powder. Carr's index: 5 < CI < 15, excellent flowability; 15 < CI < 24 passable to fair; CI > 25, poor flowability.

2.7.3. Hygroscopicity

Hygroscopicity was assessed by placing 0.5 g of sample in a desiccator with a saturated NaCl solution (RH 75.3%) and measuring the mass of absorbed water after 7 days of storage. Results were expressed as g H_2O/100 g sample (Correia et al., 2017).

2.7.4. Solubility

50 mL of distilled water was added to samples (0.5 g) of protein-polyphenol particles or protein sources (non-complexed) that were thoroughly homogenized at high velocity for 5 min and centrifuged at 4000 rpm for 5 min. An aliquot of the supernatant (25 mL) was placed onto aluminum dishes and vacuum dried (Isotemp 285A vacuum oven, Fisher Scientific, Hampton, NH, EUA) until constant weight in a 105 °C. The weight of the supernatant divided by the weight of the sample was used to calculate solubility, represented as a percentage (%).

2.8. Color

Color parameters lightness (L*), greenness (-a*) or redness (+a*), and blueness (-b*) or yellowness (+b*), were measured using a reflectance spectrophotometer (CR-400, Konica Minolta, Japan) that had been previously calibrated with white and black standards. The browning index was calculated using Equations (4) and (5).

$$Browning\ Index = \frac{[100(x - 0.31)]}{0.17} \quad (4)$$

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (5)$$

2.9. Functional attributes

2.9.1. Emulsifying properties: emulsifying activity index (EAI) and emulsion stability index (ESI)

Dispersions prepared with 1% (w/v) of protein-polyphenol particles or protein only (non-complexed) were prepared in water. The dispersions had the pH adjusted to 2, 4, 7 or 10 using

1 M HCl or 1 M NaOH solutions. After that, 15 mL of the solutions were transferred to round bottom centrifuge tubes and 5 mL of corn oil (Mazola, Jeddah, SA) was added. A high-speed homogenizer (PRO Scientific PRO250, Cole Parmer, Vernon Hills, IL, USA) was used to emulsify the mixture for 2 minutes. After this, 50 µL of the recently prepared emulsion was transferred to a 5 mL Eppendorf tube containing 4.9 mL of 0.1 % sodium dodecyl sulfate (SDS) in water. The emulsions absorbance was measured at the beginning (A0) and after 10 minutes (A10) at 500 nm (Spectramax Plus 384, Molecular Devices, Sunnyvale, CA, USA). The emulsifying properties EAI and ESI were calculated as follows EAI: (m^2/g) = [(2 x 2.303 x A0 x DF)/ c x l x Θ x 10000] and ESI (%) = [A0 x 10/ A0-A10] x 100, where DF is the dilution factor (100), c is the sample concentration (0.01 g/mL), l is the route length (1 cm), Θ is the oil volumetric fraction (0.25) and A0 and A10 are the absorbance values after 0 and 10 minutes, respectively (Deng et al., 2019).

2.9.2. Foaming properties: foaming capacity (FC) and foam stability (FS).

Water-based dispersions of 50 mL were prepared with 1% (w/v) of protein-polyphenol particles or protein only (non-complexed). The dispersions had the pH adjusted to 2, 4, 7 and 10 using 1 M HCl or 1 M NaOH solutions and whipped at 16,000 rpm for 2 min using a high-speed homogenizer (PRO Scientific PRO250, Cole Parmer, Vernon Hills, IL, USA). At 0 and 30 minutes, the foam volume was measured. The results were calculated as FC (%) = [(volume after whipping at 0 min – volume before whipping)/ (volume before whipping)] x 100 and FS (%) = [(volume after standing for 30 min- volume before whipping)/volume before whipping] x 100, respectively (Hoskin et al., 2022).

2.10. Thermal stability

Samples (0.35 g) of protein-polyphenol particles were evenly spread on Petri dishes (55 mm diameter) and submitted to 30 min drying in a lab oven dryer (Isotemp 285A Vacuum Oven, Fisher Scientific, Hampton, NH, EUA) at 65°C, 100°C or 135°C, based on previous protocol (Medeiros et al., 2019). The obtained material was stored at −20°C until subsequent TPC analysis. Extracts from heat-treated samples had their polyphenol content evaluated and results were expressed as percentage of TPC retention (%) in comparison to non-treated samples.

2.11. Bioaccessibility

An adapted static in vitro gastrointestinal digestion method was used in this study. Simulated

salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) electrolyte stock solutions were prepared with the corresponding electrolytes based on the parameters of Minekus et al. (2014). For the oral phase, protein-polyphenol particles (42-72 mg, normalized to about 10 mg TP) were suspended in 0.5 mL of water and mixed with 0.35 mL of SSF, followed by sequential addition of 50 μL of 1,500 U/mL porcine pancreas α-amylase solution, 25 μL of 0.03 M $CaCl_2$, and 75 μL of water (pH 7.0), thoroughly mixed for 2 min. For gastric phase, the 1 mL oral bolus was mixed with 0.64 mL of SGF solution, 160 μL porcine pepsin solution of 25,000 U/mL, 5 μL of 0.03 M $CaCl_2$, 20 μL of 1 M HCl, and 175 μL of water (pH 3.0). The reaction vessel was placed under agitation at 37 °C for 2 h (150 rpm). For the intestinal phase, 2 mL of gastric chyme was mixed with 1.1 mL of SIF solution, 0.5 mL of a pancreatin solution 800 U/mL, 250 μL fresh bile (160 mM in fresh bile), 40 μL of 0.03 M $CaCl_2$, 15 μL of NaOH, and 95 μL of water (pH 7.0), and shaken once again for 2 h at 37°C. After the intestinal digestion, samples were centrifuged to obtain the soluble fraction (supernatant) and the residual fraction, then immediately frozen and freeze-dried. The recovery index (RI) (Eq. 6) and bioaccessibility index (BI) (Eq. 7) for TPC, after the simulated gastrointestinal digestion, were calculated as (Grace et al., 2021):

$$RI\ (\%) = (TPC\ post\ digestion/TPC\ pre\ digestion) \times 100 \quad (6)$$

$$BI\ (\%) = (TPC\ post\ digestion\ of\ supernatant/TPC\ pre\ digestion) \times 100 \quad (7)$$

Where TPC post-digestion and TPC pre-digestion correspond to the TPC of supernatant samples (μg) after the in vitro digestion (after oral, gastric, and intestinal) and before the in vitro digestion.

2.12. Statistical analysis

Prism 9.0 (GraphPad Software, San Diego, CA, USA) was used to perform the Ordinary one-way ANOVA analysis and Tukey multiple comparison tests, with statistical significance determined at $p < 0.05$, unless indicated otherwise. Results are presented as the mean ± SD.

3. Results and discussion

3.1 Solids recovery and phenolic retention

The spray dried protein-polyphenol particles derived from muscadine grape pomace were a reddish color, distinctive of muscadine grapes of the cultivar Noble, while the particles derived from rosemary had the typical green color of rosemary leaves. The groups produced with insect

protein as the only protein source (IP-RM, IP-MG) acquired a darker color, because of the natural brownish color of IP (**Figure 1**).

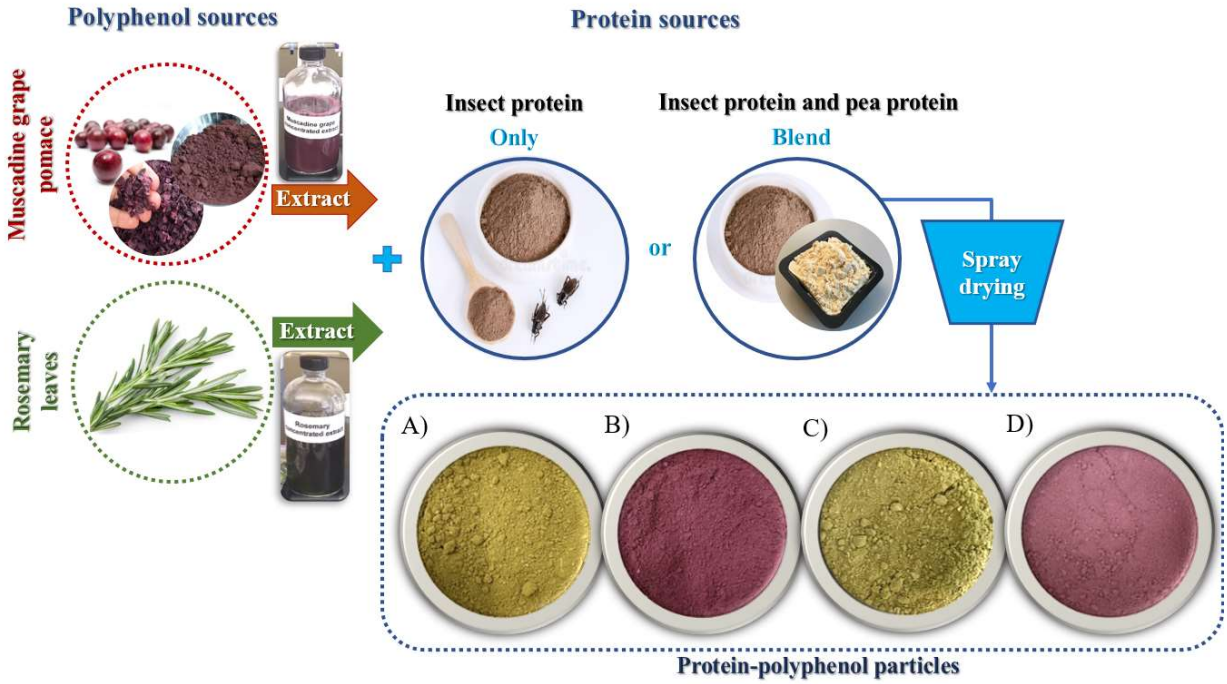


Figure 1. Spray dried insect protein-polyphenol particles. Treatments: A) IP-RM (insect protein with concentrated rosemary extract); B) IP-MG (insect protein with concentrated muscadine grape pomace extract); C) IPP-RM (blend of insect protein and pea protein 50:50 with concentrated rosemary extract); and D) IPP-MG (blend of insect protein and pea protein 50:50 with concentrated muscadine grape pomace extract).

Spray drying yield, measured as the percentage of solids recovered after spray drying, is an important index to evaluate the feasibility and potential scalability of the spray drying process (Samborska et al., 2022). Proteins are biopolymers widely used as carriers for the reduction of shear stress, heat stress and structural alterations during spray drying, in addition to acting as buffering and bulking agents (Akbarbaglu et al., 2021). Proteins improve the spray drying performance by migrating to the air/water interface, as a consequence of their surface-active properties, which mitigates excessive stickiness of feed solutions to the dryer walls (Fang & Bhandari, 2012). In fact, polysaccharide-based carriers and blends composed of protein-polysaccharides are commonly tested (Benito-Román et al., 2020; Gong et al., 2018), while protein blends have comparable spray drying efficiency, but fewer studies have focused on them (Hoskin et al., 2022, 2019).

The experimental groups IP-RM (45.91 %) and IP-MG (43.82 %) achieved solids recovery below 50 % while the groups IPP-RM (53.67 %) and IPP-MG (53.29 %) reached levels above 50 %, with an increase of approximately 10 %, when pea protein was used as part of the protein blend (**Figure 2**). Previous work using protein blends (pea and buckwheat) with muscadine grape pomace obtained similar results (55.9 %) using 10 % (w/v) addition rate of protein (Hoskin et al., 2019). Solids recovery of 50 % or higher has been accepted as a desirable level when dealing with lab-scale spray drying protocols (Fang & Bhandari, 2012). Likewise, higher polyphenol retentions were observed for IPP-derived groups (**Figure 2**), and the best results among all experimental groups were observed for IPP-RM (92.5%; $p < 0.05$).

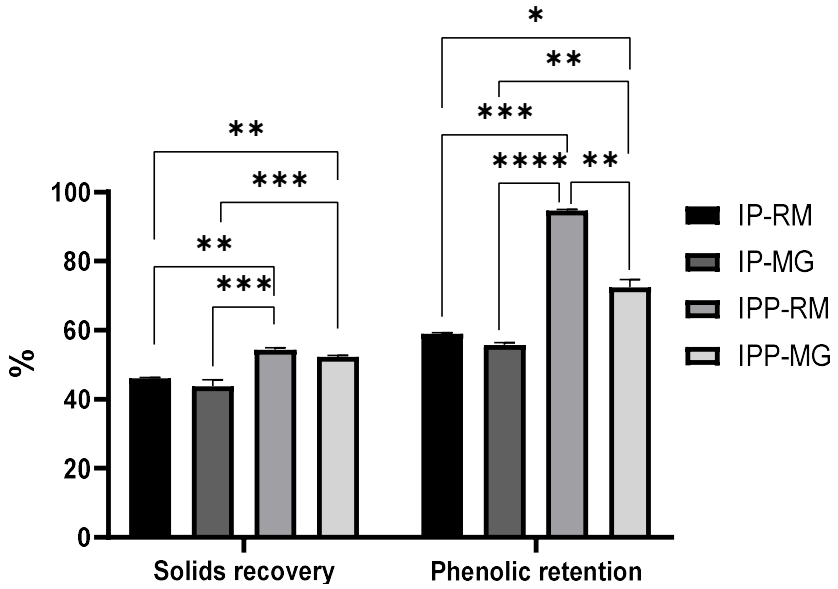


Figure 2. Solids recovery and phenolic retention (%) of spray dried insect protein-polyphenol particles produced with rosemary leaves or muscadine pomace extract. Legend: IP-RM: insect protein with concentrated rosemary extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 with concentrated rosemary extract, IPP-MG: blend of insect protein and pea protein 50:50 with concentrated muscadine grape pomace extract. Bars indicate standard deviation. Samples marked with an asterisk are significantly different: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ **** $p < 0.0001$.

Overall, it is evident that protein-polyphenol particles produced with a blend of insect protein and pea protein provided both higher solids recovery and polyphenol retention (**Figure 2**). This finding shows the influence of the type of carrier in the performance of the spray drying process. The protein content of insect-pea protein blend (approx. 80 %) is higher than the insect protein alone (70 %). The pea protein used in this study is more soluble (37.5 %) than insect protein, which explains the higher solubility of the IPP blend (27.75%) compared to IP alone (21.6 %; **Table 1**). We hypothesize that both the higher protein content and solubility of IPP blend enhances the performance of the spray drying process (Grace et al., 2021). Therefore, the IPP

protein blend has better film-forming characteristics, creating a more efficient physical barrier to protect rosemary and muscadine polyphenol molecules, which results in higher polyphenol protection (and consequent retention) and enhanced solids recovery (Hoskin et al., 2019; Muzaffar & Kumar, 2015).

3.2. Phytochemical content (total polyphenol content, proanthocyanidins and anthocyanin) and antioxidant activity of polyphenol extracts and protein-polyphenol particles

Polyphenols are compounds of interest for both food and pharmaceutical industries (Alwazeer et al., 2023; Bitwell et al., 2023). The polyphenol source determined the final bioactivity of the protein-polyphenol particles since the polyphenol content of protein sources (insect protein, pea protein) used in this study was negligible (< 5 mg/g). Both concentrated plant extracts proved to be phenolic-rich sources with 6,460 mg GAE/L (total solids 3.92 g/100g) for rosemary extract and 9,840 mg GAE/ L (total solids 4.61 g/100g) for muscadine grape extract. Our results are higher than rosemary extract prepared by pulsed ultrasound extraction (100 Hz, pulse 0.4 μs; 333.07 mg GAE/L; Nutrizio et al., 2023) and muscadine grape pomace (2413 mg GAE/L, Noble cultivar, Hoskin et al., 2022) obtained by aqueous extraction using enzyme-assisted and microwave-assisted extractions.

Results show that the TPC of protein-polyphenol particles differed among treatments ($p < 0.05$) and ranged from 68.5 mg GAE/g (IPP-MG) to 88.0 mg GAE/g (IP-MG; **Figure 3A**). Rosemary-derived particles had similar TPC, independent of the type of protein used ($p > 0.05$, **Figure 3A**). As expected, no anthocyanins were detected for rosemary samples. Muscadine-derived treatments, on the contrary, showed remarkable anthocyanin contents (**Figure 3B**). The ANC profile of muscadine samples consisted mainly of diglucosides, which agrees with previous reports with the same cultivar (Wang et al., 2010). Delphinidine-3-5-diglucoside stands out as the predominant anthocyanin in both IP-MG and IPP-MG treatments. Our results for total anthocyanin content are lower than those found by Hoskin et al. (2019), when producing protein-polyphenol particles with buckwheat, pea and rice protein, alone or blended (19.4–20.4 mg/g). The observed differences might be due to different anthocyanin content in the original muscadine grape pomace, since it is variable between harvests and pomace origin (Wang et al., 2010), as well as the different concentration and type of protein used to produce the particles (Grace et al., 2014).

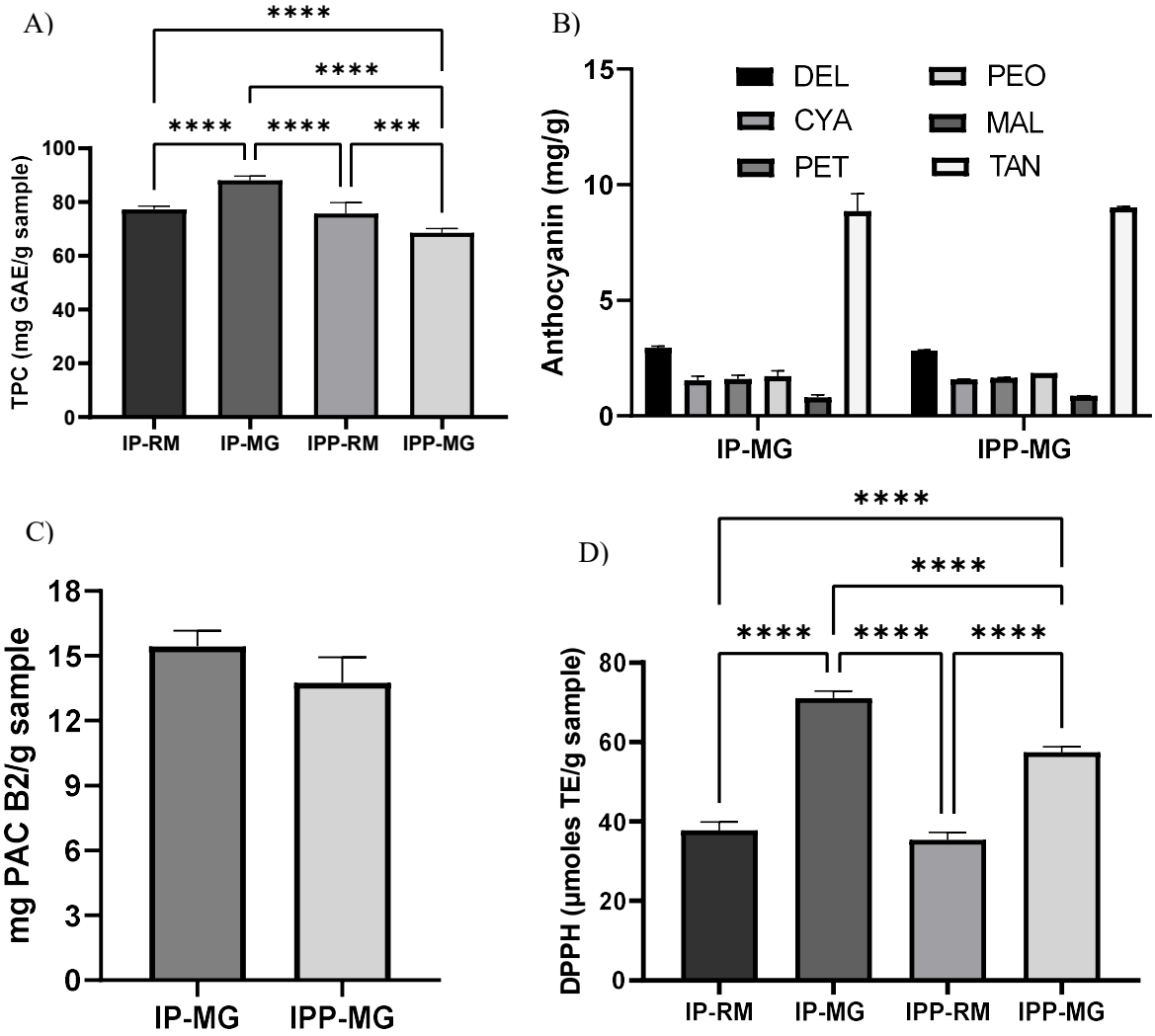


Figure 3. Total phenolic content (TPC, A), anthocyanin compounds (ANC, B), proanthocyanidin (PAC, C), and DPPH radical scavenging activity (D) of spray dried insect protein-polyphenol particles produced with rosemary leaves or muscadine pomace extract. Legend: IP-RM: insect protein with concentrated rosemary extract, IP-MG: insect protein only concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 with concentrated rosemary extract, IPP-MG: blend of insect protein and pea protein 50:50 with concentrated muscadine grape pomace extract. DEL: delphinidine-3,5-diglucoside, CYA: cyanidin-3,5-diglucoside, PET: petunidin-3,5-diglucoside, PEO: peonidin-3,5-diglucoside and MAL: malvidin-3,5-diglucoside, TAN: total anthocyanin content. ***$p < 0.001$ ****$p < 0.0001$.

Proanthocyanidins are polymeric flavonoid molecules (condensed tannins) naturally found in cereals, legumes, seeds, fruits, wine and tea (Santos-Buelga & Scalbert, 2000). High proanthocyanidin content was detected in muscadine extract-derived particles (**Figure 3C**). Hoskin et al. (2019) studied the complexation of buckwheat, pea and rice proteins with blueberry, cranberry and muscadine grape juice and pomace extracts. Similarly, the highest proanthocyanidin contents were observed for muscadine pomace extract complexed with pea protein (16.3 mg PAC B2/g) and buckwheat/pea protein blend (17.7 mg PAC B2/g). Indeed, grape pomace has been reported as an important source of proanthocyanidin (Angeloni et al.,

2022). Rosemary-derived protein-polyphenol particles had negligible PAC (0.3-0.5 mg PAC B2/g), which agrees with previous reports (Vallverdú-Queralt et al., 2014), as common compounds found in rosemary are phenolic acids such as rosmarinic, carnosol and carnosic acid (Bankole et al., 2020; Lešnik et al., 2021).

MG-derived protein-polyphenol particles showed significantly higher ($p<0.05$) antioxidant activity (71.1 and 57.5 μmol TE/g for IP-MG and IPP-MG, respectively) than RM-derived particles (37.7 and 35.4 μmol TE/g for IP-RM and IPP-RM, respectively; **Figure 3D**). Our results suggest a strong influence of MG proanthocyanidins and anthocyanins on the antioxidant activity, a feature previously reported (Chen et al., 2020; Hoskin et al., 2019).

3.3. Water activity, pH, and proximate composition

Spray dried ingredients are popular in the food industry and therefore, it is important to determine key physicochemical features that dictate their incorporation into the formulation of food products (Samborska et al., 2022). Both the protein-polyphenol particles (0.24-0.32) and the protein sources (0.42-0.48) had water activity lower than 0.6 (**Table 1**), accepted as a microbiologically stable range that enables room temperature storage for an extended time (Gomes & Kurozawa, 2021). Except for the insect protein-pea protein blend (5.51 %), the moisture content of all samples was below 5%, considered the desirable threshold to prevent powder agglomeration and microbial contamination (Edris et al., 2016; Gallardo et al., 2013; Gomes & Kurozawa, 2021).

Overall, the protein-polyphenol particles were characterized by high protein content (29.5%-38.3%, **Table 1**). The ash content of spray dried groups increased in the order IP-RM > IPP-RM > IP-MG > IPP-MG (**Table 1**). This indicates that the ash content was influenced by both the polyphenol source (RM-derived had higher ash content than MG-derived) and by the protein type (IP-derived had higher ash content than IPP-derived treatments for the same polyphenol source). The ash content of particles is close to previous report showing fly larvae flour encapsulated with calcium alginate (11.5%) by ionic gelation (Sánchez et al., 2022).

Color is an important parameter for the acceptability of food products and ingredients. IPP-RM showed low and negative a* indicating a greater tendency towards green, while IP-MG had high and positive a* values reflecting reddish color, which agrees with visual sample aspect (**Figure 1**). Higher L* values were obtained for groups containing pea protein (IPP blend) and it is a consequence of the lighter beige color of pea protein used in this study. Browning index results ranged from 14.67 to 75.47 (**Table 1**) and it measures the intensity of samples' brown

hue (Queiroz et al., 2021). It was observed that RM particles showed the highest browning among treatments ($p < 0.05$). Rosemary leaves contain terpenes susceptible to oxidation (Pizani et al., 2022). Our hypothesis is that lipid oxidation may have occurred, leading to the formation of brown α-dicarbonyl derivatives and its products (Charve & Reineccius, 2009; Lu et al., 2014). A similar trend was reported when rosemary leaves were dried by microwave and convective drying at 50 °C, resulting browning values between 42.95-46.68 (Yilmaz & Alibas, 2022). This demonstrates that rosemary leaves are prone to oxidation and consequent browning, even when lower temperatures are applied.

Table 1. Water activity, pH, proximate composition and physicochemical properties of protein sources and spray dried insect protein-polyphenol particles produced with concentrated rosemary leaf extract or muscadine grape pomace extract.

	IP-RM	IP-MG	IPP-RM	IPP-MG	IP	IPP
Water activity	0.260±0.001^{e}	0.270±0.004^{e}	0.237±0.001^{d}	0.321±0.004^{c}	0.423±0.004^{b}	0.486±0.003^{a}
pH	5.80±0.02^{d}	4.26±0.00^{f}	5.91±0.00^{c}	4.37±0.01^{e}	6.56±0.01^{b}	6.71±0.01^{a}
Moisture (%)	4.23±0.10^{bc}	4.35±0.18^{bc}	4.13±0.00^{bc}	4.86±0.06^{ab}	3.58±0.01^{c}	5.51±0.32^{a}
Ash (%)	11.29±0.08^{a}	8.08±0.05^{c}	9.50±0.05^{b}	5.62±0.05^{d}	4.21±0.07^{f}	4.56±0.01^{e}
Fat (%)	3.82±0.10^{c}	7.09±0.65^{b}	1.47±0.00^{c}	3.86±0.62^{c}	18.58±1.26^{a}	9.86±0.13^{b}
Protein (%)	30.3	29.5	37.5	38.3	70**	77***
a*	1.25±0.03^{e}	11.52±0.04^{a}	-0.27±0.04^{f}	10.90±0.01^{b}	5.78±0.11^{c}	4.32±0.26^{d}
b*	28.68±0.14^{a}	-3.32±0.04^{f}	25.85±0.13^{b}	-2.33±0.08^{e}	12.79±0.53^{d}	23.15±0.44^{c}
L*	53.25±0.64^{b}	28.42±0.32^{e}	59.18±0.32^{a}	34.82±0.07^{d}	38.43±0.70^{c}	59.39±0.64^{a}
Browning Index	75.48±0.80^{a}	15.70±0.08^{c}	55.05±0.84^{b}	14.67±0.21^{c}	ND	ND
Hygroscopicity (%)	13.04±0.25^{a}	13.64±0.95^{a}	10.91±0.05^{b}	7.77±0.16^{c}	6.70±0.85^{c}	4.75±0.13^{d}
Solubility (%)	44.00±0.60^{b}	52.83±0.38^{a}	45.13±0.08^{b}	45.35±0.46^{b}	21.60±0.55^{d}	27.75±0.42^{c}
Hausner ratio	1.46±0.01^{ab}	1.72±0.02^{ac}	1.71±0.02^{ac}	1.82±0.11^{c}	1.47±0.07^{ab}	1.50±0.03^{ab}
Carr's index (%)	31.70±0.56^{ab}	41.74±0.57^{ac}	41.55±0.70^{ac}	44.92±3.41^{c}	31.68±3.11^{ab}	33.30±1.48^{ab}

Legend: IP-RM: insect protein with concentrated rosemary leaf extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: insect protein and pea protein 50:50 w/w blend with concentrated rosemary leaf extract, IPP-MG: insect protein and pea protein 50:50 w/w blend with concentrated muscadine grape pomace extract, IP: insect protein, IPP: insect protein and pea protein 50:50 w/w blend. Color parameters: -a*(greenness) or +a* (redness), and -b* (blueness) or +b* (yellowness), L*: lightness. **Protein content declared by the manufacturer. ***Estimated average protein content for the insect protein/pea protein blend. Results are shown as average ± SD. Different letters (a, b, c, d, e, f) in the same row indicate statistical difference according to Tukey post hoc test ($p < 0.05$). ND: not determined.

3.4. Flowability: Hausner ratio and Carr's compressibility index

Food powders flowability is crucial for handling processes such as mixing, packaging, and transportation (Shah et al., 2008). The Hausner ratio and Carr's index (% compressibility) are typically used to evaluate the flow characteristics of powders (Goyal et al., 2015) and constitute important quality control parameters (Moravkar et al., 2022). The Hausner ratio of protein-polyphenol particles varied between 1.46 and 1.82, while the commercial protein sources showed similarly lower values (IP 1.47; IPP 1.50). All polyphenol-protein particles were classified "very difficult flowing" food powders based on a previously reported classification scale (Bhusari et al., 2014). Carr's compressibility index results were between 31.7% and 44.9% and according to the same scale (Bhusari et al., 2014, CI > 25), the particles were classified as poorly flowable particles.

3.5. Hygroscopicity and solubility

Powder hygroscopicity is defined as the powder's ability to absorb moisture from the air, and it is a major parameter that influences the handling and storage of food powders (Saifullah et al., 2016). Protein carriers in general have low hygroscopicity, which enhance the quality of encapsulated powders (Sarabandi et al., 2019). The hygroscopicity of protein-polyphenol particles ranged between 7.77% and 13.04% and IPP-derived groups had lower results ($p<0.05$), because of the lower hygroscopicity of the insect-pea protein blend (**Table 1**). When blackcurrant pomace and cocoa extract were complexed with buckwheat protein and a chia:pea protein blend (Hoskin et al., 2023), particles produced with the pea protein blend showed lower hygroscopicity (below 20%), similarly to the present study.

Solubility is a key quality aspect of food powders, because it impacts several important production parameters such as their reconstitution and miscibility in other food matrices (Daza et al., 2016). In general, high solubility is desired for food ingredients since it enables easier mixing and formulation of products. All protein-polyphenol particles showed good solubility (> 44%) and IP-MG (52.83%) proved to be the most soluble protein-polyphenol treatment ($p<0.05$). Interestingly, all particles were more soluble than the original protein sources (IP: 21.60%; IPP: 27.75%). Solubility changes occur because of the cross-linking phenomenon caused by the binding of phenolics to proteins. Protein-polar polyphenol covalent bond contains phenolic hydroxyl groups capable of altering the protein's charge properties (Rawel et al., 2003) and it may result in either increase or decrease of the solubility of the conjugate (Yan et al., 2021). The solubility of the protein-polyphenol particles is linked to the size and chemical structure of polyphenols, the amino acid sequence and structure of proteins (Strauch & Lila,

2021). Thus, the solubility profile of the protein-polyphenol aggregates is influenced by the type of proteins, type of polyphenols and pH at which their interaction occurs (Quan et al., 2019). Previously, protein-polyphenol aggregates produced with buckwheat and mixture of chia:pea with blackcurrant pomace extract and cocoa extract showed similar results at pH 4 (44-49%) and pH 7 (46-67%) (Hoskin et al., 2023).

3.6. Emulsifying properties

Proteins are excellent emulsifiers due to their amphiphilic properties (Gómez-Mascaraque & López-Rubio, 2016), which allow them to reduce the interfacial tension between water and oil phases during emulsion formation, preventing coalescence and flocculation of the oil droplets (Queiroz et al., 2023). The emulsifying activity index (EAI) describes the interfacial characteristics of protein solutions in oil/water emulsions while the emulsion stability index (ESI) expresses the protein ability to sustain a stable emulsion over time (Li et al., 2022). Understanding the emulsifying properties of ingredients is important to determine which applications are best suited for a particular food ingredient (Klost & Drusch, 2019).

The range of EAI results (1.85 to 16.46 m^2/g) is comparable to the values previously reported for particles produced by the complexation of a protein blend (pea and rice) with muscadine grape pomace extract (Hoskin et al., 2022). As expected, a remarkable influence of the pH was observed for both EAI and ESI. Non-complexed protein sources IP and IPP showed the lowest EAI at pH 4 (**Figure 4A**), which is close to the isoelectric points of pea (pH 4.5, Xu et al., 2020) and insect proteins (differ slightly between species but reported as between pH 4-5, Mishyna et al., 2021). Proteins have the lowest solubility in water at the isoelectric point (Burger & Zhang, 2019) due to the enhanced hydrophobic protein-protein interactions and less electrostatic water-protein interactions. Protein-protein hydrophobic interactions cause them to aggregate and precipitate (Shanthakumar et al., 2022). Therefore, less protein is adsorbed at the oil-water interface because of the formation of large protein aggregates leading to a consequently low EAI (Pan et al., 2021).

The EAI of IP-derived protein-polyphenol particles followed the behavior of the protein source closely and no significant difference ($p > 0.05$) was observed between them. This indicates that the EAI is more dependent on the protein characteristics, and that the polyphenol source did not significantly alter the emulsifying activity of the insect protein. All treatments' EAI showed an increasing tendency at alkaline conditions (pH 10), with IPP-derived groups presenting higher

EAI ($p < 0.05$) when compared to IPP alone or IP treatments (**Figure 4A**). The emulsifying activity of protein-derived products is dependent on their ability to form a layer on the surface of the oil droplets and stabilize this water-oil interface, which, in turn, is dependent on their solubility and adequate dispersion in the solution (Hadidi et al., 2022; Mishyna et al., 2021). Although the solubility of all protein-polyphenol aggregates was found to be within a relatively close range (44-52%, **Table 1**), the higher protein content (**Table 1**) of IPP-based groups (37.5-38.2%), when compared to IP-based groups (29.5-30.3%) might have had an influence on the solubility and emulsifying ability of the samples in alkaline conditions. Strauch and Lila (2021) demonstrated that the solubility of pea protein was significantly dependent on the pH, and that alkaline conditions (pH 8-10) favored protein solubility for being close to the pK_a (negative log base ten of the acid dissociation constant) of amine groups. Our hypothesis is that the higher protein content of IPP-derived treatments (resulting from the higher protein content of PP blended with IP) might have positively influenced the solubility of the protein-polyphenol particles in alkaline conditions (pH 10) and contributed to their higher EAI. Moreover, the EAI of IPP-MG and IPP-RM treatments were significantly different ($p < 0.05$) in the whole pH range evaluated. However, in acidic environments (pH 2 and 4), the EAI of IPP-MG was higher ($p < 0.05$), while in neutral and alkaline environments (pH 7 and 10), EAI of IPP-RM was higher ($p < 0.05$). Our hypothesis is that, in this case, the polyphenol source might have influenced the solubility of the IPP-based samples. Analogously, acidic pH 2-4 is near to the pK_a of acid groups, which are more concentrated in the MG-derived samples (pH 4.26-4.37, **Table 1**), than on RM-derived samples (pH 5.80-5.91, **Table 1**), which can positively contribute to the solubility of IPP-MG and to its higher emulsifying performance in acidic environments (pH 2-4).

Most protein-polyphenol particles showed ESI above 80%, with less pH-dependent variation compared to EAI. In addition, the protein-polyphenol particles showed greater results than their protein source counterparts (not complexed, IP or IPP), with the exceptions of IPP-MG at pH 2 and IPP-RM at pH 7 (**Figure 4B**). This behavior indicates a clear influence of the protein-polyphenol complexation on ESI. In fact, the positive influence of protein-polyphenol complexation on ESI was also described by Sui et al. (2018) for soy protein isolate complexed with anthocyanins derived from black rice extract. Similarly, Hao et al. (2022) showed the same trend for when pea protein was complexed with resveratrol and chlorogenic acid. Both covalent and non-covalent modification of polyphenols resulting from protein-polyphenol complexation alter the hydrophobic/hydrophilic balance of the protein and lead to enhanced ESI (Li et al.,

2020; Lin et al., 2022; Sui et al., 2018). Moreover, higher protein surface hydrophobicity resulting from protein-polyphenol conjugation is associated with stronger repulsion between oil droplets coated with such protein-polyphenol particles, which leads to lower oil droplet coalescence and, in turn, higher emulsion stability (Quan et al., 2019). Taken altogether, our results show that protein-polyphenol particles obtained from insect protein, alone or blended with other alternative protein source, are functional ingredients capable of stabilizing food emulsions.

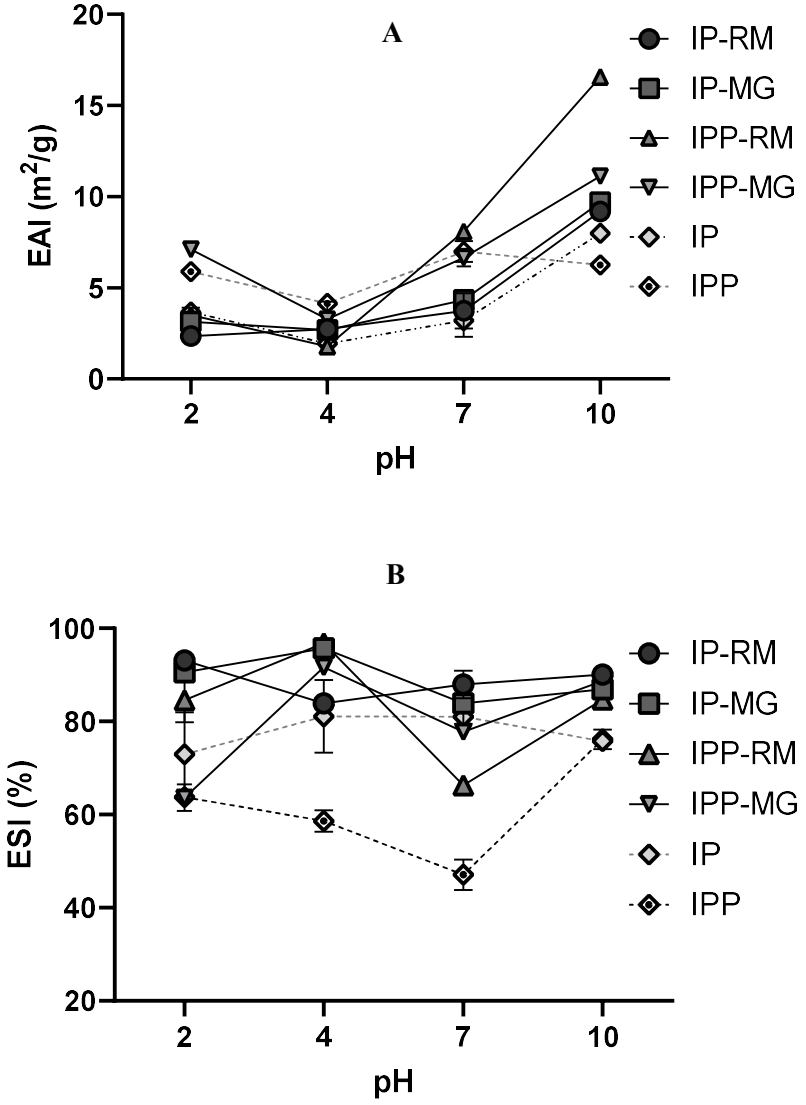


Figure 4. (A) Emulsion activity index (EAI, %) and (B) emulsion stability index (ESI, %) of protein sources (IP and IPP) and spray dried insect protein-polyphenol particles produced with concentrated extracts of rosemary leaves or muscadine grape pomace. Legend: IP-RM: insect protein with concentrated rosemary leaf extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 w/w with concentrated rosemary leaf extract; and IPP-MG: blend of insect protein and pea protein 50:50 w/w with concentrated muscadine grape pomace extract, IP: insect protein, IPP: insect-pea protein 50:50 w/w blend.

3.7 Foaming properties

The ability of a protein solution to efficiently trap air bubbles within a continuous liquid phase is known as foaming capacity (FC), whereas foam stability (FS) expresses the rate at which foam volume is sustained over time. Thus, higher FC and FS are desired to create cohesive, elastic films stably wrapped around air bubbles for extended time, to achieve aerated, fluffy food foams (Li et al., 2023).

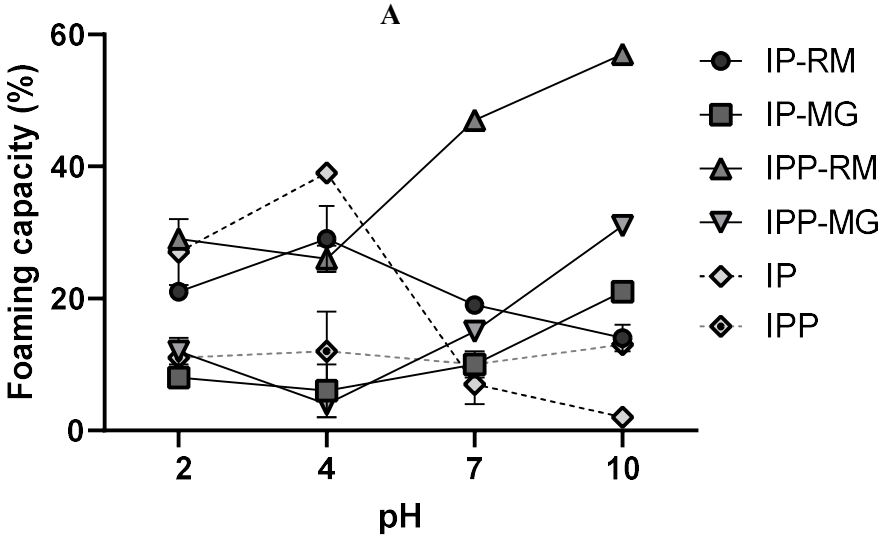
In an acid environment (pH 2-4), MG-derived protein-polyphenol particles presented consistently lower FC ($p < 0.05$) when compared to RM-derived groups and non-complexed IP (Figure 5A), showing a clear dependence on pH for the polyphenol source used in protein-polyphenol complexation. The performance of protein-derived materials to create and sustain stable foams is highly dependent on protein flexibility, which allows the formation of a film wrapping that stabilize air bubbles in aqueous medium (Gravel & Doyen, 2020). Wang et al. (2023) recently showed that the complexation of protein with anthocyanins is highly dependent on the pH of the solution, especially due to the pH-dependent charge modifications that the anthocyanins undergo. In their study, the authors showed that in acidic environment (pH 3-5), the electrostatic repulsion between the positively charged forms of anthocyanins became much stronger. Our hypothesis is that stronger intra- and intermolecular repulsion resulting from the presence of anthocyanins in the MG-derived groups (**Figure 3B**) may reduce the protein flexibility from these groups in acidic environments, preventing them from effectively migrating to the air-water interface and jeopardizing the foam formation.

Previously, it has been reported that the protein net charge increases in alkaline pH, decreasing hydrophobic interactions while enhancing protein flexibility. Higher protein flexibility makes proteins diffuse to the air-water interface more quickly to encapsulate air, which improves foam production (Malik & Saini, 2016). In pH 7-10, MG-derived protein-polyphenol particles presented a slight increase in FC but were still outperformed by RM-derived groups (**Figure 5A**). Although strong electrostatic repulsions from anthocyanins are not expected to play a significant role on neutral to alkaline pH (Wang et al., 2023), the higher fat content ($p < 0.05$) of MG-derived groups compared to their RM-derived counterparts (**Table 1**), may have compromised the performance of IP-MG and IPP-MG groups, since lipids generally present a negative effect on foaming formation (Gravel & Doyen, 2020).

In acidic pH 2-4, the highest FS among protein-polyphenol particles was observed for RM-derived particles ($p < 0.05$). Similarly to what was observed for FC, MG-derived groups performed poorly under acid conditions, which might indicate the influence of stronger

electrostatic interactions resulting from anthocyanins protonation (Wang et al., 2023) in promoting low protein flexibility and poor stabilization capacity. At neutral environment (pH 7), all treatments behaved similarly ($p > 0.05$), but at pH 10, they outperformed their respective protein source (IP or IPP, not complexed), with the highest FS ($p < 0.05$) being observed for IPP-derived particles (**Figure 5B**). The better FC and FS results of protein-polyphenol particles under alkaline conditions (pH 10) could be attributed to greater protein solubility resulting from an increased net charged protein molecules and from the proximity to the pK_a of amine groups (Strauch & Lila, 2021). Moreover, the higher protein content of IPP-derived groups (**Table 1**) and the lower fat content ($p < 0.05$, **Table 1**) of IPP-RM justify its better performance for both FC and FS.

Cross-linked structures resulting from protein-polyphenol complexation improve protein unfolding and influence interfacial elasticity, thus providing better foam formation (Jia et al., 2016; Li et al., 2021). Previous reports demonstrated that when protein (WPI or casein) is complexed with chlorogenic acid, enhanced foaming properties are observed (Jiang et al., 2018). Overall, pH affects the foaming behavior of insect-derived protein-polyphenol aggregates. However, the complex nature of the interactions between proteins and polyphenols, as well as the type of protein and polyphenol source/type also seem to influence the final foaming properties of these food ingredients (Cao et al., 2018), and should be taken into consideration when designing a final application.



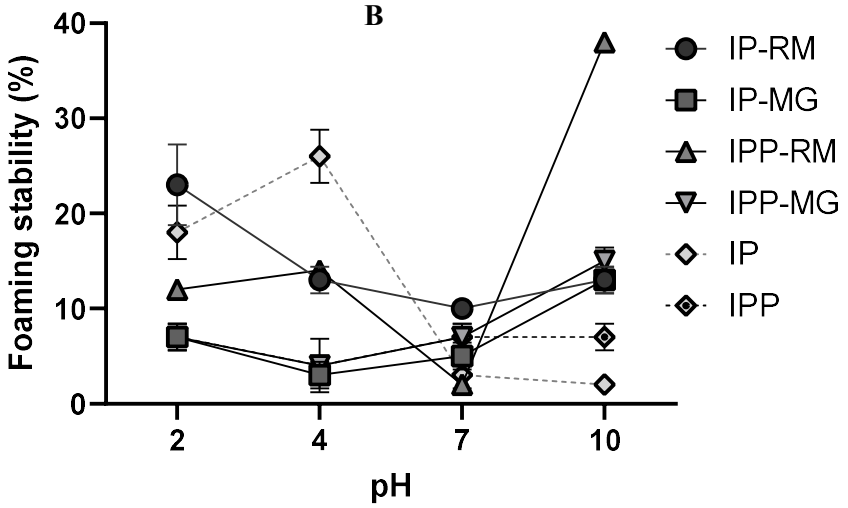


Figure. 5. Foaming capacity (A) and foaming stability (B) of protein sources (IP and IPP) and spray dried insect protein-polyphenol particles produced with concentrated extracts of rosemary leaves or muscadine grape pomace. Legend: IP-RM: insect protein with concentrated rosemary leaf extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 w/w with concentrated rosemary leaf extract; and IPP-MG: blend of insect protein and pea protein 50:50 w/w with concentrated muscadine grape pomace extract, IP: insect protein, IPP: insect-pea protein 50:50 w/w blend.

3.8 Thermal stability

The demand for bioactive-rich foods has skyrocketed in recent years. However, processing techniques have to be designed taking into account that many bioactive molecules are compounds susceptible to thermally-induced modifications and therefore, prone to degrade when exposed to temperature ranges often used in industrial and culinary operations (Bodbodak et al., 2022).

Therefore, the thermal stability of protein-complexed polyphenols was evaluated by comparing the TPC of particles exposed to temperatures commonly found in food-related industrial operations (65 °C, 100 °C and 135 °C) to non-thermally treated counterparts at 25°C.

The TPC retention of IP-RM and IP-MG treatments exposed to 65°C and 135°C ranged between 70% and 80% (Figure 6). Greater TPC losses were found by Akbas et al. (2017) when freeze-dried wheatgrass juice with whey protein and maltodextrin were heat-treated at 70°C for 60 min (TPC retention of 65%). When exposed to 65°C, MG-derived particles showed similar TPC retention to the control group (25°C, $p>0.0001$), while RM-derived treatments showed decreased TPC retention (Figure 6). Rosemary extracts are rich in heat-sensitive phenolic acids (carnosol, carnosic acid, rosmarinic acid, rosmanol, epirosmanol) (Grace et al., 2021; Tzima et al., 2021). Multiple hydroxyl groups found in the rosemary phenolic acids increase their susceptibility to thermal degradation (Pang et al., 2014). Thus, we hypothesize that the different phytochemical composition of muscadine grape extracts (Hoskin et al., 2019) and rosemary

extracts play an important role in the observed results.

An interesting trend of increased TPC retention was observed for particles exposed to 100 °C, even when compared to 25°C (IPP-MG, $p < 0.0001$). This behavior might be explained by the formation of Maillard reaction products between reducing sugars and free amino acids in the protein-polyphenol particles. This is especially true for MG-derived treatments, in which the concentration of reducing sugars is expected to be higher than in RM. In addition, the presence of melanoidins and other Maillard reaction products may interfere with the ability of the Folin-Ciocalteu method to detect polyphenols, since they can be responsible for increasing the metal chelation potential of the samples (Brudzynski & Miotto, 2011; Medeiros et al., 2019; Wang et al., 2011). Overall, insect protein-polyphenol particles were heat stable with lower TPC retention observed only when they were exposed to harsh 135°C ($p<0.001$). This demonstrates the protective effects of encapsulation materials on reducing thermal degradation of polyphenols, either by acting as a thermal buffer or by shifting the degradation temperature up (Castro-López et al., 2021; Dicastillo et al., 2019; Hanuka-Katz et al., 2022; Yan et al., 2022).

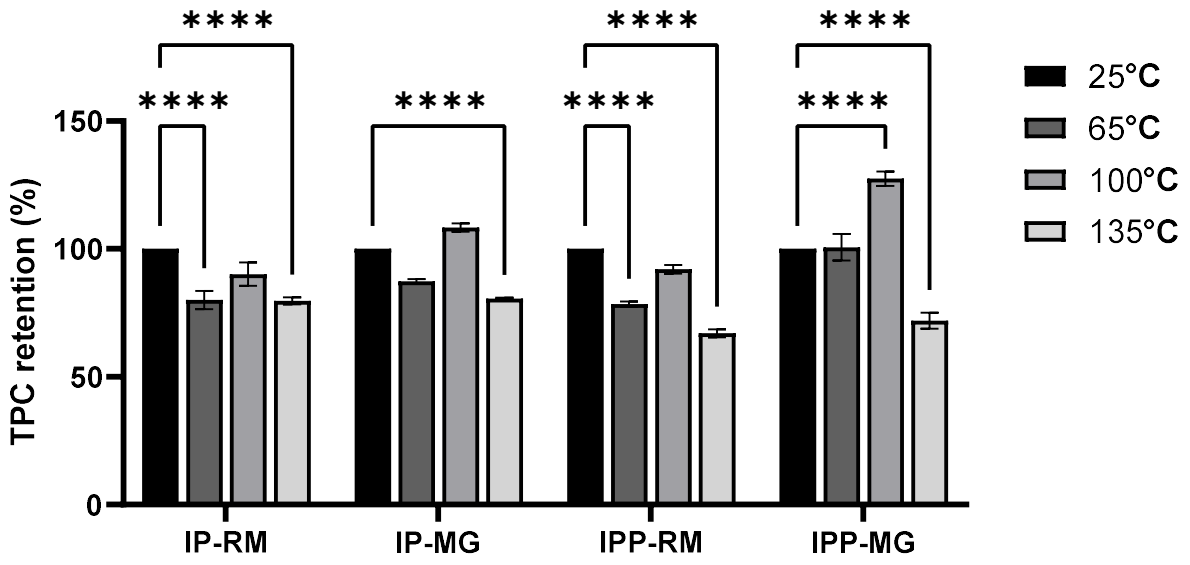


Figure 6. Thermal stability of polyphenols (measured as retention of total polyphenol content (TPC), %) in spray dried insect protein-polyphenol particles submitted to temperatures 25°C, 65°C, 100°C and 135°C. Legend: IP-RM: insect protein with concentrated rosemary leaf extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 w/w with concentrated rosemary leaf extract; and IPP-MG: blend of insect protein and pea protein 50:50 w/w with concentrated muscadine grape pomace extract. Samples marked with an asterisk are significantly different: ****$p < 0.0001$.

3.9 Bioaccessibility

The biological benefits associated with the ingestion of polyphenols and other phytoactive compounds depend not only on their presence, concentration, and chemical profile in foods, but also on their capacity to be effectively absorbed by gut microbiome in the gastrointestinal tract (Lila et al., 2022). To be absorbed, polyphenols are hydrolyzed by digestive enzymes or intestinal microbiota, with fractions being digested in the small intestine (48%), large intestine (42%) and the remaining (10%) is estimated to keep intact in the food matrix (Wojtunik-Kulesza et al., 2020). Although polyphenols are easily oxidized and damaged when subjected to the harsh conditions of the digestion process, their combination with carrier agents such as proteins may alter the interactions and modifications that they undergo during digestion making them more concentrated and available for absorption (Qie et al., 2022). Polyphenol-protein interactions might improve the solubility and, consequently, increase the bioavailability of polyphenols (Li et al., 2021; Liao et al., 2022). However, the metabolic outcomes of protein-polyphenol aggregate binding on the ultimate bioavailability of proteins are still unclear (Lila et al., 2022). In this sense, bioaccessibility is a key preliminary predictor to evaluate the effectiveness of the polyphenol delivery to the gastrointestinal tract, where they should be potentially absorbed (Thakur et al., 2020).

In vitro digestion models are popular research strategies to simulate human digestion of experimental products. They are less expensive, faster, and more straightforward strategies, with no ethical limitations when compared to in vivo options (Grace et al., 2021). Recovery (RI%) and bioaccessibility (BI%) indexes were used to evaluate how the complexation with insect proteins (alone or blended with pea protein isolate) would affect the digestive fate of polyphenols from concentrated extracts of rosemary leaves and muscadine pomace after going through simulated digestion. After being subjected to the three in vitro digestion phases (oral, gastric and intestinal), the final bioaccessible fraction (supernatant) from the intestinal phase was separated from the residue (residual solids) by centrifugation, a reliable technique for simulating the bioaccessible fraction of food protein (Grace et al., 2021; Hoskin et al., 2023). Therefore, the intestinal RI% represents the share of the initial TP (standardized at 10 mg/mL) retained by the residue (not bioaccessible fraction) after in vitro digestion, while the BI% evaluates the share of the initial TP found in the supernatant after intestinal phases (bioaccessible fraction), that could potentially be absorbed by enterocytes.

Figure 7 shows the results for both RI% and BI% of all protein-polyphenol particles as well as

the non-complexed RM and MG concentrated extracts. Overall, low RI% (≤ 10%) and high BI% (> 50%) were obtained for all groups. Low intestinal RI% may indicate either that the polyphenols were effectively transferred to the bioaccessible fraction (supernatant) or that they were degraded by the digestion process. Indeed, the observed high BI% levels show that a significant share of the initial polyphenols was present in the bioaccessible fraction, which points to an effective release of the polyphenols from the protein-polyphenol aggregates into the bioaccessible fraction.

Moreover, rosemary polyphenols complexed to insect protein-polyphenol particles (alone or blended with pea protein) were more bioaccessible than in non-complexed rosemary extract ($p<0.05$). In contrast, for MG-derived particles, similarly high bioaccessibility was observed in complexed and non-complexed polyphenols ($p > 0.05$; **Figure 7**). This indicates that the type of polyphenols influence bioaccessibility. Phenolic acids are more easily absorbed, but larger polyphenolic chains such as PAC abundantly found in MG-derived particles may have reduced bioavailability, and may be degraded before absorption (Yang et al., 2022). In addition, the stability of anthocyanins, also found in MG-derived particles, is highly dependent on the pH, which is a major factor influencing digestion (Oliveira & Pintado, 2015). Neutral to alkaline environments usually found on intestinal phase of digestion (here it was carried out at pH 7) promote anthocyanin instability and increase degradation, especially for those containing hydroxyl and methoxyl groups in the anthocyanidin ring B (Nagar et al., 2021). In fact, delphinidine-3,5-diglucoside, cyanidin-3,5-diglucoside, petunidin-3,5-diglucoside, peonidin-3,5-diglucoside and malvidin-3,5-diglucoside, all containing hydroxyl and/or methoxyl groups in the anthocyanidin ring B, were detected in MG-derived protein polyphenol particles (**Figure 3B**). Although still delivering highly bioaccessible polyphenols (BI% > 50%), we hypothesize that the different polyphenol composition of MG-derived protein-polyphenol particles (higher content of PAC and anthocyanins compared to RM-derived counterparts) plays an important role on the observed bioaccessibility differences.

Previously, Grace et al. (2021) showed that rosemary phenolic acids complexed in spray dried protein-polyphenol microparticles prepared with whey and soy protein isolates have higher solubility, bioaccessibility index and bioavailability compared to free, uncomplexed polyphenols. Likewise, when blueberry and muscadine grape pomace polyphenols were complexed with rice and pea protein, increased post-digestion BI (69% and 62%, respectively) was observed (Xiong et al., 2020). Protein-curcumin complexes (protein-phenolic interaction; pH 7) using WPI and pea protein also showed good BI (78% and 72%, respectively; Vijayan et

al., 2021). On the contrary, maqui juice anthocyanins microencapsulated with maltodextrin and soy protein isolate by spray drying and freeze drying after in vitro digestion, showed lower BI compared to non-encapsulated forms (44.1–43.8%; Fredes et al., 2018).

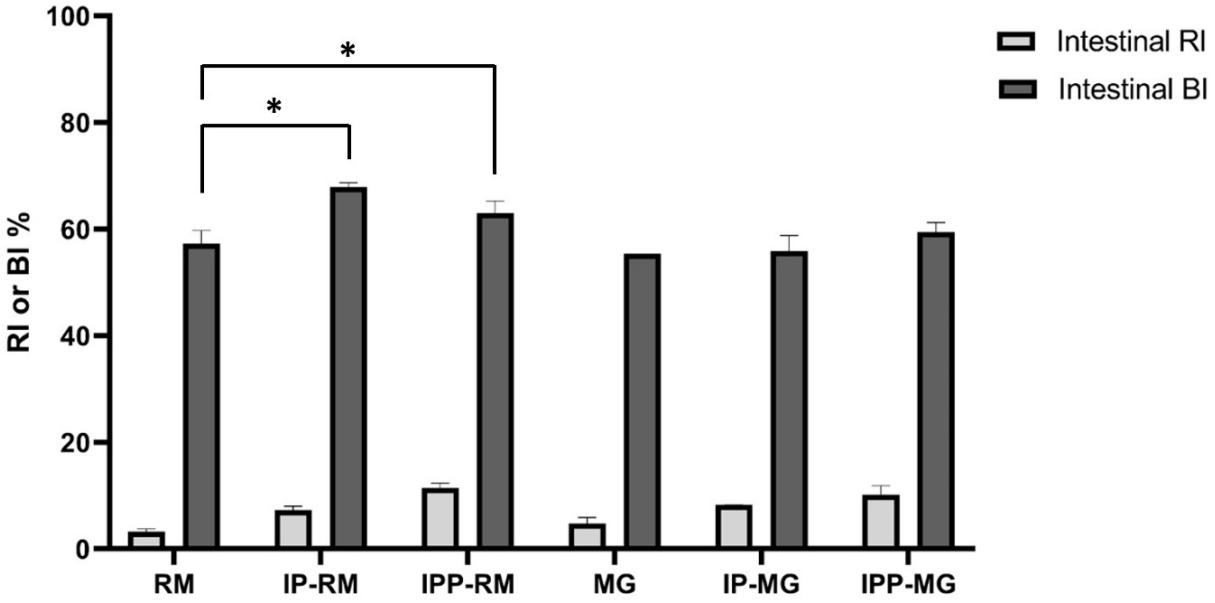


Figure 7. Recovery index (RI, %) and bioaccessibility index (BI, %) for total phenolic content (TPC) of concentrated extracts of rosemary leaves or muscadine pomace and spray dried insect protein-polyphenol particles. Legend: RM: concentrated rosemary leaf extract; MG: concentrated muscadine grape extract; IP-RM: insect protein with concentrated rosemary leaf extract, IP-MG: insect protein with concentrated muscadine grape pomace extract, IPP-RM: blend of insect protein and pea protein 50:50 w/w with concentrated rosemary leaf extract; and IPP-MG: blend of insect protein and pea protein 50:50 w/w with concentrated muscadine grape pomace extract. Samples marked with an asterisk are significantly different: *p > 0.05.

4. Conclusions

Insect protein is a nutritious alternative protein source, with a much lower environmental footprint compared to traditional livestock and other popular protein sources consumed in Western countries nowadays. Here we demonstrated that functional food ingredients produced by spray drying microencapsulation of insect protein and naturally occurring polyphenol-rich sources is an efficient strategy to overcome the appearance and functionality attributes of insect protein, which are major issues that hamper its use in food formulations. The protein-polyphenol ingredients produced with insect protein and rosemary leaf or muscadine grape pomace extracts had desirable characteristics such as protein-rich composition, attractive colors, good solubility, and overall physicochemical attributes that warrant adequate

performance into food matrices. Overall, we presented a rational and efficient strategy to valorize insect protein and diversify its utilization by establishing an industrially friendly technological route to produce versatile, pleasantly colored and convenient products.

Declaration of Competing Interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this scientific manuscript.

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CAPÍTULO 6

CONCLUSÃO GERAL

Capítulo 6 - Conclusão geral

A presente tese de doutorado demonstrou a viabilidade técnica de agregar valor a resíduos de frutas e proteínas alternativas como ervilha e inseto através de técnicas de processamento racionais e favoráveis ao meio ambiente.

Foi apresentado protocolo de processamento a partir do uso de técnicas emergentes para a recuperação eficiente de bioativos em resíduos de fruta, com menor impacto ambiental que as técnicas tradicionais, através de um processo conduzido em menor tempo e com menor consumo de energia. Esse tipo de metodologia abre novas perspectivas para satisfazer a demanda crescente por protocolos de processamentos ecológicos e econômicos. E ainda, através da técnica de microencapsulação de bioativos por atomização do *American elderberry*, foi confirmado o potencial da polpa e resíduo de uma fruta até agora pouco valorizada no mercado para produzir ingredientes alimentares de alto valor agregado. Esse estudo apresenta novas rotas de produção que visam estimular o interesse de produção e comercialização de novos produtos na indústria de alimentos. A estrutura de trabalho apresentada aqui pode ser aplicada para diversas outras matérias-primas alimentares abundantes no Brasil, mas ainda com seu potencial não devidamente explorado.

O que diz respeito a proteína de inseto, a definição de estratégias para produzir alimentos nutritivos e sustentáveis com esse tipo de matéria-prima alimentar é uma necessidade real para o mercado ocidental. Transformar um produto como insetos em ingredientes coloridos e atraentes, ricos em compostos fitoquímicos é sem dúvida uma abordagem promissora para amenizar obstáculos na aceitação do consumo desta proteína em mercados ocidentais como no Brasil e em outros países. Portanto análises mais detalhadas bem como o uso do ACV na produção desses ingredientes são uma perspectiva para trabalhos futuros.

Assim sendo, a presente tese de doutorado abre possibilidades para o estudo e aplicação de estratégias de processamento estruturadas e fundamentadas em revalorizar matérias-primas abundantes e ricas em fitoquímicos, mas ainda subutilizadas, para produzir produtos sustentáveis com alto valor agregado e funcionais.

ANEXO 1

CARACTERÍSTICAS DA PROTEÍNA DE ERVILHA

CHARACTERISTICS	PP
Water activity	0.483±0.002
Moisture (%)	6.61±0.24
Ash (%)	15.26±1.27
Fat (%)	0.62±0.13
Protein (%)	84
pH	6.88±0.01
a*	1.44±0.03
b*	17.17±0.36
L*	76.38±0.15
Hygroscopicity (%)	6.04±0.15
Solubility (%)	37.48±0.38
Hausner ratio	1.31±0.02
Carr's index (%)	23.75±1.25

	PP			
	EAI (m^2/g)	**ESI (%)**	**FC (%)**	**FS (%)**
pH_2	7.29±0.29	73.35±2.42	49.00±1.00	11.00±1.00
pH_4	7.47±0.73	36.85±5.74	56.00±2.00	51.00±5.00
pH_7	7.50±0.65	88.87±6.01	54.00±2.00	20.00±0.00
pH_{10}	7.27±0.42	69.37±3.93	57.00±3.00	52.00±2.00

PP: PEA PROTEIN

EAI: EMULSION ACTIVITY INDEX

ESI: EMULSION STABILITY

FC: FOAMING CAPACITY

FS: FOAMING STABILITY

ANEXO 2

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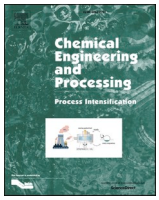

Ultrasound-assisted polyphenol extraction of acerola and jambolan pomaces: comparison of extraction protocols, kinetic modeling, and life cycle assessment

Edilene Souza da Silva [a], Andréa Oliveira Nunes [a], Roberta Targino Hoskin [a,b]

[a] Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Natal, RN. 59078-970, Brazil
[b] Plants for Human Health Institute, North Carolina State University. Kannapolis, NC. 28081, USA

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ABSTRACT

In this study, tropical acerola and jambolan pomaces were submitted to four water-based polyphenol extraction methods: conventional solid-liquid extraction CSLE; heated conventional solid-liquid extraction HCSLE; static ultrasound-assisted extraction SUAE; and ultrasound-assisted extraction and mechanical stirring UAES. Our objective was to evaluate and compare the extraction protocols regarding their performance, extraction kinetics, mathematical modelling, and environmental viability using the life cycle assessment (LCA) tool. The highest total polyphenol content was obtained by UAES after 90 min (1,606.8 mg GAE/100g for acerola and 1,580.7 mg GAE/100g for jambolan). These results are significantly higher ($p \leq 0.05$) compared to CSLE (1,296.4 mg GAE/100g for acerola, 644.1 mg GAE/100g for jambolan). The Power Law model showed the best experimental fit compared to Peleg's and second-order models. Regarding the environmental viability, the LCA tool revealed that UAES had the lowest environmental impact among all extraction protocols, mainly due to its lower energy consumption. Overall, the combination of mechanical stirring and ultrasound improved water-based polyphenol extraction rates with reduced energy consumption. This study shows UAES as an environmentally friendly strategy to achieve efficient extraction of naturally occurring polyphenols from tropical fruit pomaces.

1. Introduction

Fruit pomaces are by-products of the fruit processing industry and consist of peels, residual pulp, and seeds. The pomace composition varies within fruits, but generally speaking, they are a complex mixture of macronutrients (carbohydrates, minerals, and vitamins) [1,2] with significant residual content of bioactive compounds such as phenolics and carotenoids that sometimes exceed the amount found in the fruit pulp [3]. For instance, acerola fruit (Malpighia *emarginata*) is one of the main natural sources of ascorbic acid in the world, and its residue retains significant amounts of bioactive compounds [4,5]. This "superfood" is cultivated in Central and South Americas, and used for the production of supplements and nutraceuticals [6,7]. Jambolan (*Syzygium cumini* (L.)) is another phytochemical-rich tropical fruit reported as a superfood due to its high content of phenolics, mainly anthocyanins, potent antioxidant activity [6], and health-relevant biological effects [8]. However, differently from acerola, its consumption and production are modest and not commercially relevant yet [9].

There is an increasing market for natural, clean label, environmental-friendly food products [10–12]. This trend has encouraged the investigation of new ways to manage industrial secondary streams and recover residual phytochemicals from fruit pomaces using extraction protocols that cause minimum impact to the environment, using less solvents and maximizing the use of natural resources [13]. Among upcycleable phytochemicals, the polyphenol extraction from agricultural pomaces consist of a major, increasingly important research field due to the well-established scientific evidence showing the health relevance of polyphenol compounds and their potential applications in the pharmaceutical and food industries [14,15]. In addition, finding ways to optimize the use of fruit pomaces is a rational strategy to add value to a widely abundant natural resource that would be, otherwise, discarded in the environment, incinerated or destined to less profitable end uses [16]. This is especially true for tropical fruit pomaces, that have their production concentrated in low-income countries that would benefit from increased income generation and stronger economic growth [17].

Extraction techniques have been referred as conventional (solid-liquid, liquid-liquid extractions using heat and/or organic solvents or water) and green technologies, such as ultrasound-assisted extraction

E-mail address: rtcorrei@ncsu.edu (R.T. Hoskin).

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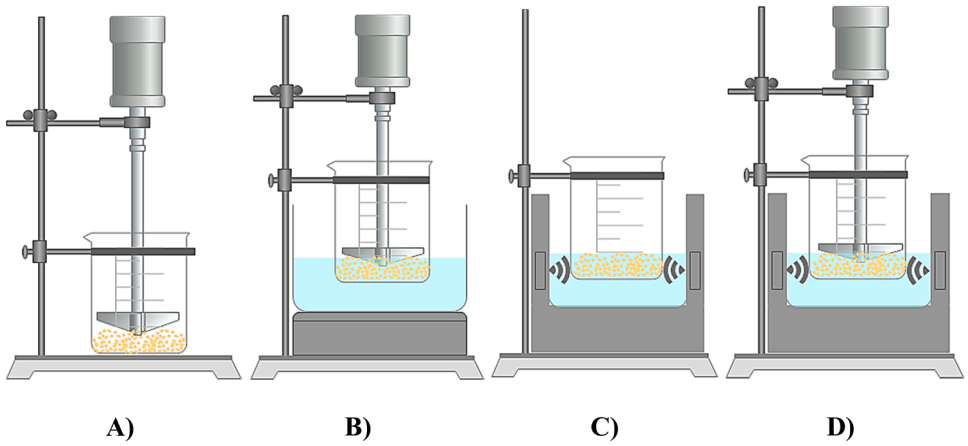


Fig. 1. Representation of performed extractions: A) Conventional solid-liquid extraction (CSLE); B) Heated conventional solid-liquid extraction (HCSLE); C) Static ultrasound-assisted extraction (SUAE); and D) Ultrasound-assisted extraction and mechanical stirring (UAES).

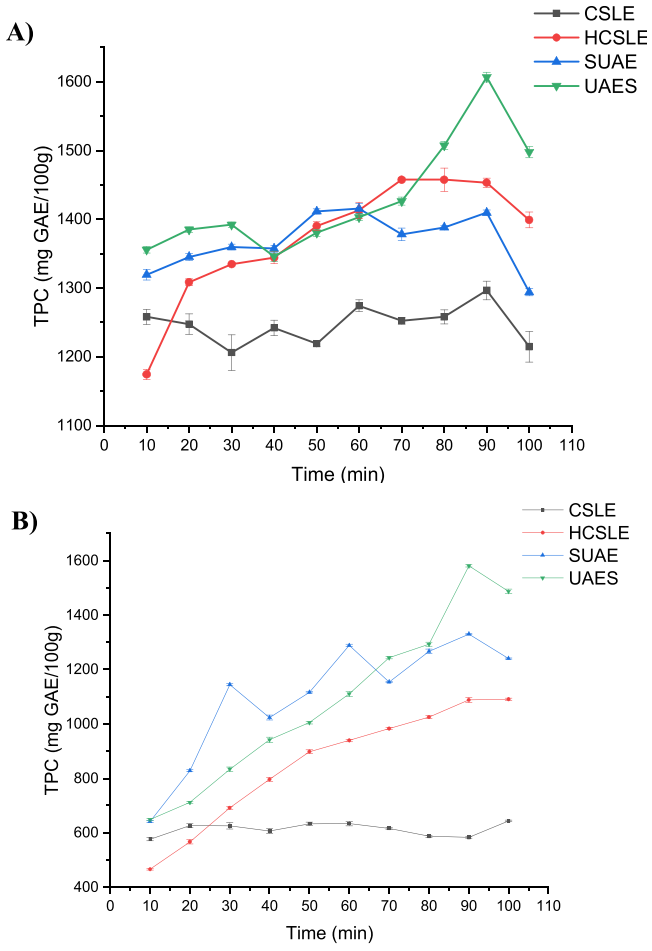


Fig. 2. Polyphenol extraction of acerola pomace (A) and jambolan pomace (B) submitted to conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES) for 100 minutes. TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g). Bars represent standard deviation.

(UAE) [18]. Extraction processes can be intensified with the use of the ultrasound technique, a more efficient and ecologically correct method to extract phytochemicals from natural resources [20]. In addition to using less solvents, it requires shorter lead time, less energy, and can be combined with other extraction methods to enhance extraction efficiency [19]. This emerging extraction technology is based on an acoustic cavitation mechanism that uses compression and decompression cycles to cause fragmentation, erosion, and inter-particle shocks to rupture cellular structures, facilitating the release of entangled compounds such as phenolics, carotenoids and other phytomolecules [21]. It has proved to be particularly useful for fruit pomaces where rigid cell walls entrap compounds of interest and make it inefficient and/or not economically viable to use conventional solid-liquid extraction to recover phytomolecules [22]. For example, UAE has been successfully applied to several by-products such as jambolan bark [20], acerola pomace [23], grape pomace [24], peanut peels [25], green sweet lime peels [26], apple pomace [27], mango peels [28], lime [29] and passion fruit rinds [30].

Several studies have established the importance of the type of solvent for the recovery of bioactive compounds from natural plant materials using ultrasound-assisted extraction [27,31]. Water is a cheap, readily available food-grade solvent aligned with the current green chemistry principles valued by both consumers and industry [32]. Additionally, water can produce efficient polyphenol extraction yields, in comparison to organic solvents such as ethanol, acetone or methanol [33]. When ultrasound-assisted aqueous extraction of polyphenol was performed in kinetic study from brewer's spent grain it showed the best performance among pure solvents tested [34]. Mathematical modeling is an essential engineering technique used to simulate, describe, design and control a target process and it has been applied to study the kinetics extraction of multiple food materials such as olives [35], cocoa [36], basil leaves [37], soybeans [38] and yerba mate [39,40].

However, UAE can be conducted using multiple protocols and to determine their environmental friendliness, it is necessary to analyze the impact of UAE regarding several environmental factors. Life Cycle Assessment (LCA) is a powerful tool used by industries and production sectors to analyze and compare the potential environmental impacts during the life cycle of a product system. It helps to generate reliable data to assist in the decision-making process, design improvement strategies, and establish sustainability policies and ecological labeling [41,42].

In this research study, we investigated four polyphenol extraction protocols applied to acerola and jambolan pomaces using only water as the extraction solvent: conventional solid-liquid extraction, heated conventional solid-liquid extraction, static ultrasound-assisted extraction and ultrasound-assisted extraction with mechanical stirring. Overall, the objective of this research study was to evaluate and compare these extraction strategies regarding their a) performance, b) extraction kinetics and mathematical modelling of experimental extraction data and, finally, c) environmental viability using the LCA tool. This research work evaluates ultrasound intensified polyphenol extraction to provide

Table 1
Kinetic models constants and regression statistical parameters for acerola and jambolan pomaces submitted to different water-based polyphenol extraction protocols.

Acerola pomace	Model constant					Statistical parameters				
		CSLE	HCSLE	SUAE	UAES		CSLE	HCSLE	SUAE	UAES
Peleg model	K_1	-7.01E-05	1.79E-03	5.69E-04	7.77E-04	R^2	0.996	0.997	0.998	0.978
	K_2	8.03E-04	6.74E-04	7.13E-04	6.79E-04	NRMSD	0.020	0.016	0.013	0.042
	C_{eq}	1244.8	1483.1	1403.1	1492.9					
Second-order model	H	1480.6	529.7	1866.6	1253.4	R^2	0.996	0.997	0.998	0.978
	C_e	1253.6	1484.5	1408.0	1493.6	NRMSD	0.020	0.015	0.012	0.042
Power law model	B	1204.8	962.5	1233.5	1149.4	R^2	0.996	0.998	0.999	0.984
	n	0.01	0.09	0.03	0.06	NRMSD	0.019	0.012	0.011	0.036
Jambolan pomace	Model constant					Statistical parameters				
		CSLE	HCSLE	SUAE	UAES		CSLE	HCSLE	SUAE	UAES
Peleg model	K_1	6.36E-04	1.83E-02	9.02E-03	1.75E-02	R^2	0.994	0.993	0.984	0.962
	K_2	1.62E-03	7.54E-04	6.81E-04	5.47E-04	NRMSD	0.031	0.033	0.053	0.075
	C_{eq}	616.7	1325.7	1467.9	1829.0					
Second-order model	H	461.1	53.2	109.6	54.9	R^2	0.989	0.988	0.970	0.925
	C_e	617.9	1345.7	1476.3	1882.8	NRMSD	0.035	0.067	0.053	0.075
Power Law model	B	599.9	180.2	367.2	182.7	R^2	0.988	0.997	0.963	0.965
	n	0.004	0.400	0.287	0.455	NRMSD	0.033	0.017	0.059	0.051

Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES). R^2: Correlation coefficient; NRMSD: Normalized root means squared deviation. K_1 is the Peleg rate, K_2 is the Peleg capacity constant and C_{eq} is the equilibrium concentration of total extracted TPC; H is the initial extraction rate and C_e is the TPC at equilibrium; n is the power law exponent (<1) and B is constant related to extraction rate.

novel, meaningful scientific data regarding the recovery of bioactive compounds from underexplored fruit pomaces abundantly found in the tropical world and help unveil their potential as raw materials for multiple industries.

2. Materials and methods

2.1. Acerola (Malpighia emarginata) and jambolan (Syzygium cumini (L.)) pomaces

Acerola pomace resulting from industrial fruit pulp extraction was donated by a local fruit processing plant (Delicia da Fruta, Natal, RN, Brazil). Jambolan pomace resulted from processing jambolan fruits locally harvested (5° 24′ 9.2880′’ S, 36° 57′ 14.7780′’ W) in January 2019, using a fruit pulping machine (Juicer RI1858 650W, Philips Walita, Shanghai, China). Both pomaces consisted of skins, seeds, and residual pulp remaining after fruit pulp separation. They were kept frozen at -6 °C until further use and contained 78.0 % and 54.7 % of moisture, respectively.

2.2. Extraction protocols

Four different extraction protocols (Fig. 1) were investigated using only water as the extraction solvent. A fruit pomace:water ratio of 1g:50 mL defined in preliminary experiments (data not shown) was used for all experiments.

2.2.1. Conventional solid-liquid extraction (CSLE)

The pomace:water solution was continuously stirred using a mechanical stirrer (TE-139 TECNAL, Piracicaba, Brazil) at room temperature (25°C) and 150 rpm.

2.2.2. Heated conventional solid-liquid extraction (HCSLE)

The pomace:water solution was placed in a temperature-controlled water bath using a heating base (TE-0853 TECNAL, Piracicaba, Brazil) and continuously stirred (TE-139 TECNAL, Piracicaba, Brazil) at 150 rpm. The temperature was monitored during extraction, and a gradual temperature increase was applied until 45°C was reached (protocol established to match temperature increase observed for SUAE and UAES protocols described below).

2.2.3. Static (no stirring) ultrasound-assisted extraction (SUAE)

Indirect ultrasonication was applied to the pomace:water solution by placing the aqueous mixture in an ultrasonic bath (ALTSONIC Clean 3IA, 3L). The temperature of the solution increased progressively with time reaching a maximum of 45 ± 2°C within 100 min. The temperature increase resulted only from the UAE process and no external heat was applied. The ultrasound treatment was applied continuously using 40 kHz frequency and 100 W power.

2.2.4. Ultrasound-assisted extraction and mechanical stirring (UAES)

Indirect ultrasonication was applied to the pomace:water solution by placing the aqueous mixture in an ultrasonic bath (ALTSONIC Clean 3IA, 3L), using similar conditions described for SUAE treatment. However, the solution was mechanically stirred at 150 rpm. The ultrasound treatment was applied continuously using 40 kHz frequency and 100W power. The temperature of the solution increased progressively with time reaching a maximum of 45 ± 2°C within 100 min.

All extraction protocols were conducted for 100 min with samples collected every 10 min. The samples were vacuum filtered at 4°C using qualitative filter paper and centrifuged for 10 min at 4000 rpm. The supernatants were collected, immediately frozen and kept protected from the light until further analysis.

2.3. Total polyphenol content (TPC)

Samples were analyzed for TPC using a previously published protocol with modifications [43]. Briefly, sample extracts (25 μL), distilled water (75 μl) and Folin-Ciocalteau reagent (1N, 25 μL) solutions were transferred to 96-well microplates and received 100 μL of Na_2CO_3 solution 7.5 %. Experiments were performed in triplicate. Results were calculated using a standard curve of gallic acid (0-500 mg/L, $R^2 = 0.99$) and expressed as mg of gallic acid equivalent per 100 g of sample (mg GAE/100 g).

2.4. Kinetic models

The kinetic modelling of all four polyphenol extraction protocols was evaluated using three different mathematical models (Peleg, Second-order and Power law models), commonly used for the modelling of solid-liquid extraction of plant-based natural products [44]:

a) **Peleg's model.** The Peleg's model uses a non-exponential model equation based on the extraction rate constant (k_1) and equilibrium concentration of total extracted TPC (C_{eq}) (Equation 1), where *t* (min) is the extraction time; *Ct* is the TPC (mg/100g) at a given time

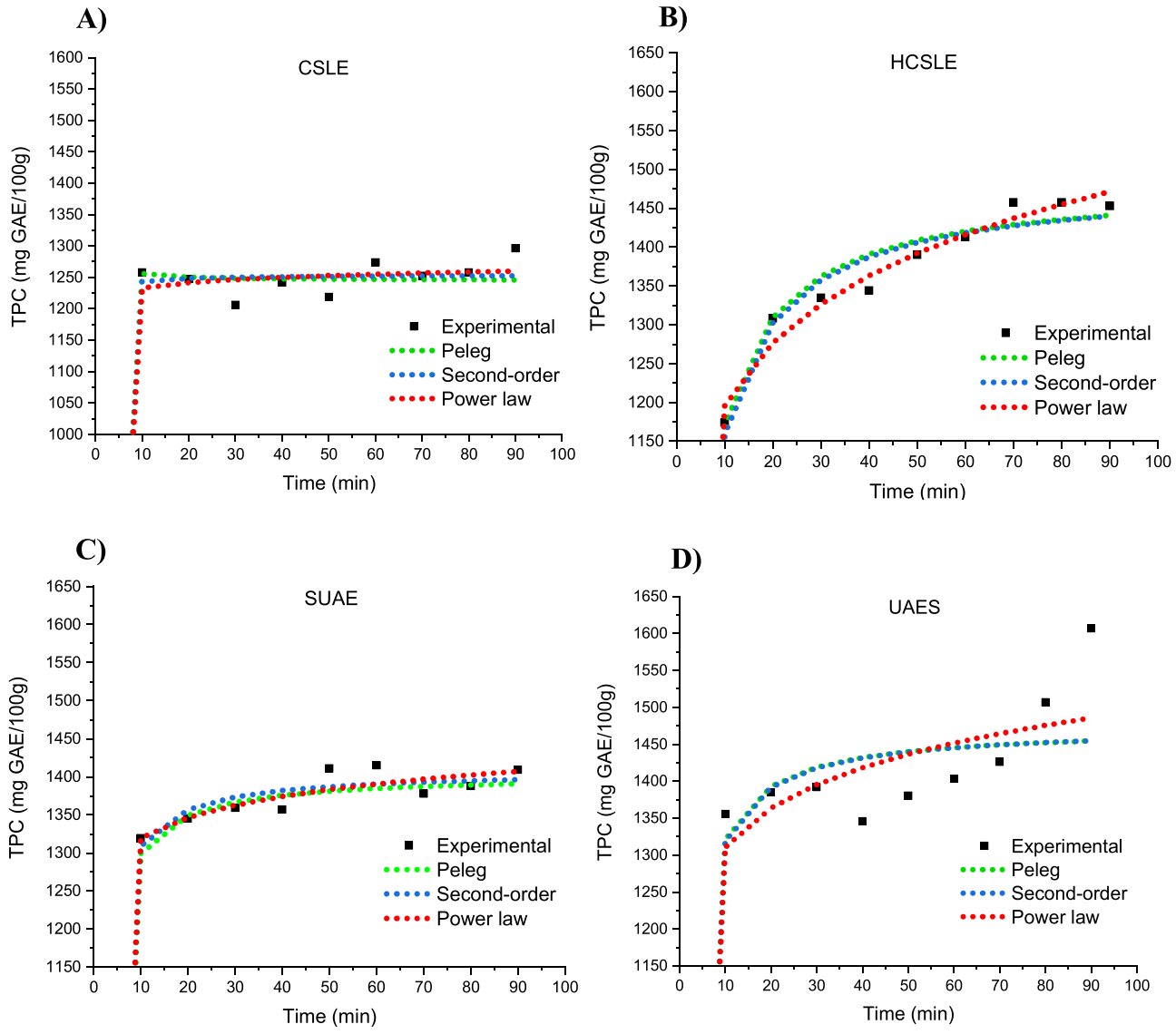


Fig. 3. Kinetic modeling for acerola pomace submitted to conventional solid-liquid extraction (CSLE) (A), heated conventional solid-liquid extraction (HCSLE) (B), static ultrasound-assisted extraction (SUAE) (C) and ultrasound-assisted extraction and mechanical stirring (UAES) (D). TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g).

t; k_1 is the Peleg rate (100g/mg min) and k_2 is the Peleg capacity constant (100 g/mg); C_{eq} is the equilibrium concentration of total extracted TPC when $t \rightarrow \infty$ (C_{eq}, 100g/mg) defined as $C_{eq}=1/k_2$ [45].

$$C_t = \frac{t}{k_1 + k_2.t} \quad (1)$$

b) **Second-order kinetic model**. It approaches the rate dynamics of the solid-liquid extraction process by considering parameters such as the initial extraction rate (H) and the content of polyphenols present when the extraction reaches equilibrium (Ce). A general second-order law [46] is described in Equation 2, where *t* (min) is the extraction time; *Ct* is the TPC (mg/100g) at a given time *t*; *h* is the initial extraction rate (mg/100g min) when t approaches 0, C_e is the TPC at equilibrium (mg/100g) and *k* is the second-order extraction constant (100g/mg min) described as $k = h/Ce^2$ [45].

$$C_t = \frac{t}{\frac{1}{h} + \frac{t}{C_e}} \quad (2)$$

c) **Power Law model**. The Power law model is typically used to assess the diffusion of target molecules and it is based on the extraction rate constant (B) and the diffusional exponent involved in transport mechanisms (n) [47] as defined by Equation 3, where *Ct* is the TPC (mg/100g) at a given time *t* (min) *n* is the power law exponent (<1) and *B* is constant related to extraction rate (100 g / mg.min^{-1}).

$$C_t = B.\ t^n \quad (3)$$

2.4.1. Model evaluation

To determine the best fit to the experimental data, the concordance between experimental data and calculated values was established by means of correlation coefficient (R^2) and normalized root means squared deviation (*NRMSD*) criteria defined as (Equation 4):

$$NRMSD = \frac{RMSD}{Exp_{max}} = \frac{\sqrt{\left(\frac{1}{n}\right).\sum_{p=1}^{n}\left(Exp_p - Pred_p\right)^2}}{Exp_{max}} \quad (4)$$

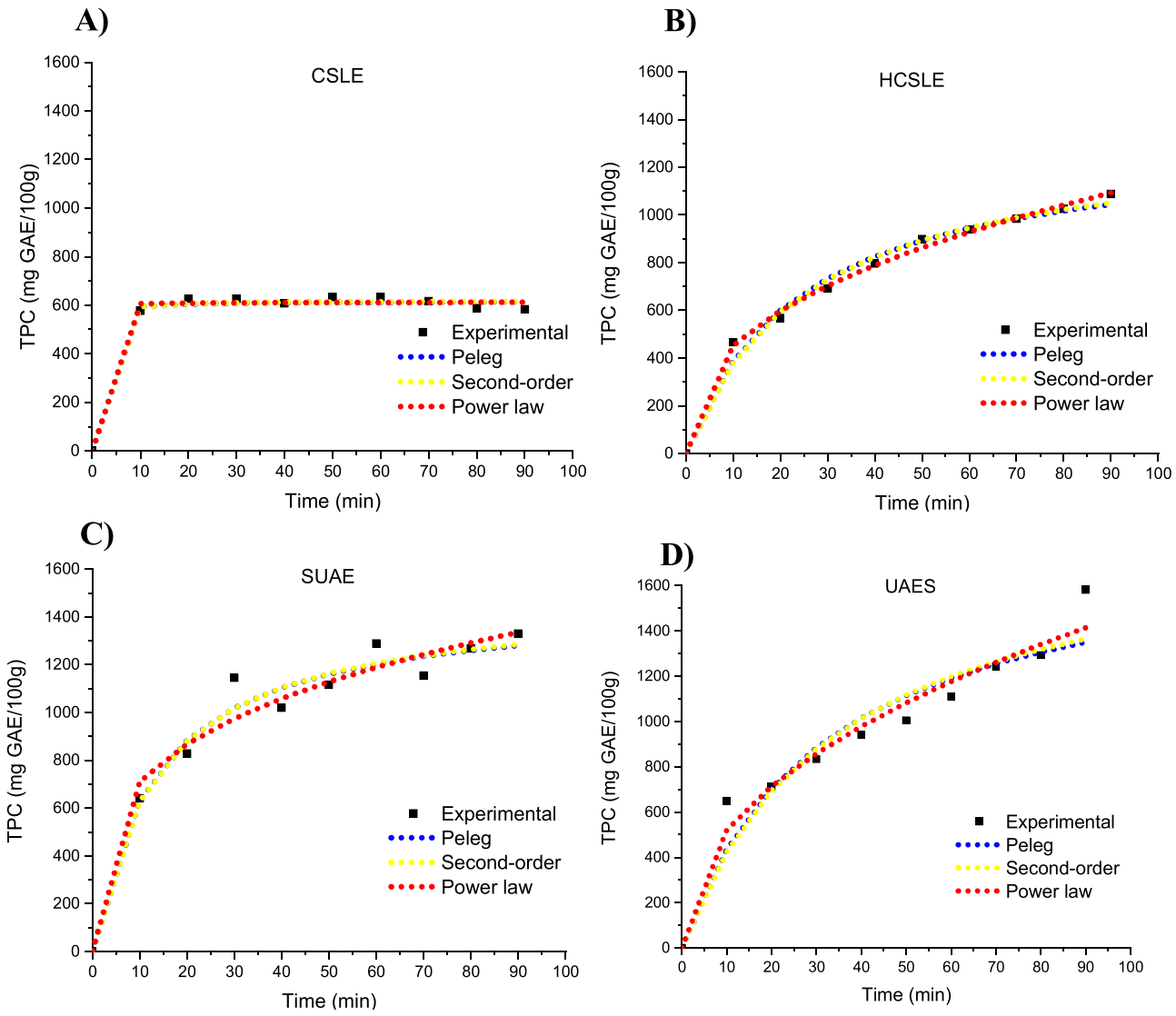


Fig. 4. Kinetic modeling for jambolan pomace submitted to conventional solid-liquid extraction (CSLE) (A), heated conventional solid-liquid extraction (HCSLE) (B), static ultrasound-assisted extraction (SUAE) (C) and ultrasound-assisted extraction and mechanical stirring (UAES) (D). TPC is expressed as mg of gallic acid equivalent per 100g of sample (mg GAE/100g).

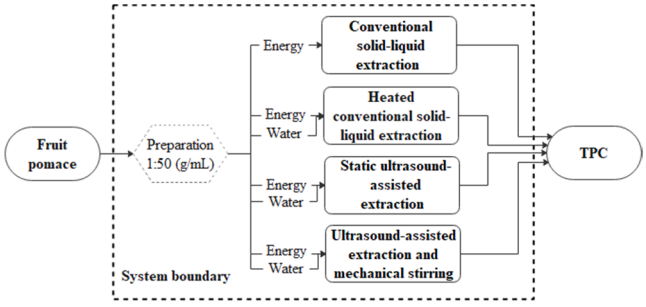


Fig. 5. Flowchart and system boundaries used for the LCA evaluation of polyphenol extraction of acerola and jambolan pomaces. TPC: total polyphenolic content.

where n is the number of kinetic experimental points, Exp_p is the experimental value at point p; $Pred_p$ is the predicted model value at point p and Exp_{max} is the maximum result within n experimental values.

2.5. Life cycle assessment (LCA)

The methodology conducted according to ISO 14040:2006 and 14044:2006 [48,49] consisted of four stages: a) Goal and scope definition; b) Life cycle inventory analysis (LCI); c) Life cycle impact assessment (LCIA) and d) Interpretation of results.

The LCA goal was to determine and compare the environmental impacts caused by the different polyphenol extraction protocols investigated in this study. All process inputs and outputs were based on a functional unit that standardized and guided all quantitative analysis. The functional unit (FU) was defined as 1400 mg of polyphenols, taking into account the average adult daily consumption in the United States of America [50]. The scope considered in this study was the cradle-to-gate approach which considers the extraction process and resources used in each one of the investigated protocols, such as energy and water [51].

The life cycle inventory analysis (LCI) consisted of primary and secondary data. The primary data consisted of the amount of water (L) used in the water bath and the energy consumption (kWh) of each extraction protocol measured by a standard digital AC wattmeter BR110 (220V). Secondary data such as electricity and water production were obtained from EcoInvent v.3.6 database.

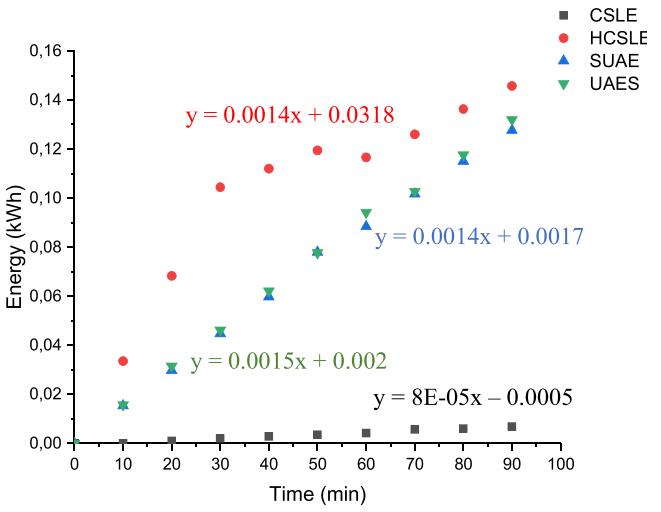


Fig. 6. Graphical representation of time (min) x energy (kW/h) during the polyphenol extraction of acerola or jambolan pomaces submitted to conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES).

Finally, the environmental impact assessment (LCIA) was evaluated using the *SimaPro*® 9.1 software using the impact assessment methodology implemented (CML-IA) baseline LCIA method V3.06/EU25, considering the following 12 impact categories: abiotic depletion, abiotic depletion (fossil fuels), global warming (GWP100a), ozone layer depletion (ODP), human toxicity, freshwater aquatic ecotoxicity, marine aquatic ecotoxicity, terrestrial ecotoxicity, photochemical oxidation, acidification and eutrophication.

2.6. Statistical analysis and model evaluation

All experiments were conducted in triplicate and results were expressed as mean ± standard deviation (SD). The analysis of variance (one-way ANOVA) and Tukey's test ($p \leq 0.05$), as well as the calculation of kinetic model parameters and correlation analysis were performed by *Statistica® v.10* software.

3. Results and discussion

3.1. Effect of the extraction protocol on polyphenol extraction

Results obtained by solid-liquid extraction (CSLE and HCSLE) and ultrasound-assisted extraction protocols (SUAE and UAES) were evaluated and compared. For acerola pomace, the highest TPC concentration was observed for UAES at 90 min (1607 mg GAE/100g) and represents a TPC increase of 24 % compared to CSLE results obtained at similar time ($p \leq 0.05$; Fig. 2A). For jambolan pomace (Fig. 2B), a gradual TPC increase was observed between 0-90 min for all extraction methods, except CSLE. Once again, the highest TPC was found after 90 min for UAES extraction (1581 mg GAE/100g), which means an increase of 171 % compared to CSLE ($p \leq 0.05$) at the same extraction time. For jambolan, the TPC results increased in the following order UAES > SUAE > HCSLE > CSLE. For acerola, UAES also yielded the highest TPC, however HCSLE and SUAE came in second and third places. For both pomaces, CSLE yielded the lowest TPC among extraction protocols, while UAES was the most efficient method to extract polyphenols from both fruit pomaces (Fig. 2).

For acerola pomace (Fig. 2A), TPC fluctuations were observed over time for all groups. Our hypothesis is that these variations occur because of an enhanced extraction/phenolic degradation cycle during acerola pomace extraction. Initially, the mechanical shearing resulting from both ultrasound or mechanical mixing ruptures cell walls (in the case of ultrasound), promotes the dissolution of active components in the media, and improves the polyphenol extraction rate. However, over time, degradation of acerola polyphenolic structures is likely to occur due to strong mechanical effects, reducing the TPC in solution. This cycle might occur over and over at different rates for each type of extraction, leading to the observed behavior. Similar trend was observed for pitahaya peels [52] and pomegranate flowers [53].

Also, it was observed that TPC declined abruptly after 100 min of extraction of acerola pomace, mainly for SUAE and UAES. Indeed, ultrasonication time has been reported as a crucial factor influencing the extraction yield [54]. As the ultrasound-assisted extraction progresses, greater contact of the solvent with the sample (acerola pomace) is achieved, however, phenolic compounds can be oxidized and damaged, which reduces yield during longer times of extraction [20,55,21].

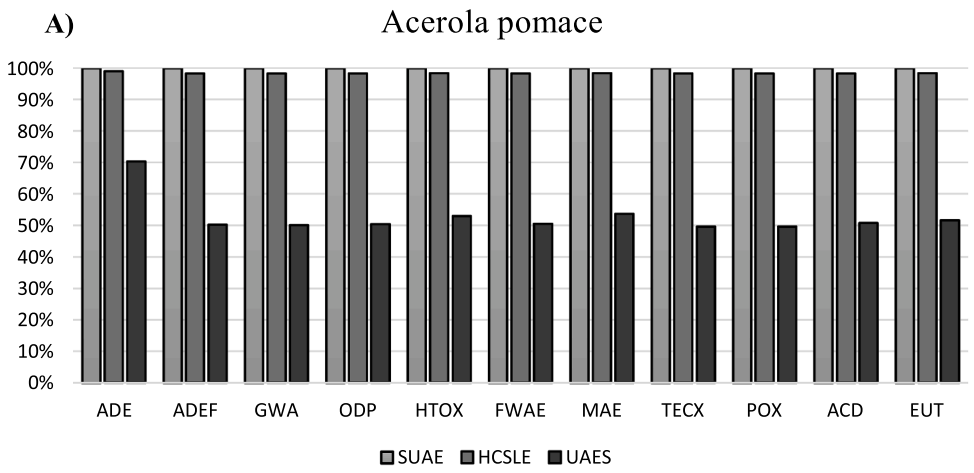
The extraction rate is controlled by mass transfer resistance and by intraparticle diffusion. Mechanical stirring decreases the mass transfer resistance by creating turbulence, but it has no effect on the intraparticle diffusion. Differently, ultrasonication intensifies both the mass transfer and the intraparticle diffusion [56], and enhances the mass transfer rate during the first extraction stage [57]. The ultrasound energy generates impact waves that cyclically compress/decompress the fibrous pomace structure, creating cavitation bubbles that induce fragmentation, pore formation and erosion and promote solubilization of the target compounds in the solvent, increased mass transfer rates in the solid:liquid interface, that ultimately leads to enhanced extraction yields [58,59]. Our hypothesis is that the synergistic combination of sonication and stirring (UAES treatment) provided greater cell wall disruption, facilitating mass transfer at both external and internal particle levels [26], which resulted in better polyphenol extraction. This configuration (stirring and sonication) has promising industrial use due to the intensifying synergistic effects on polyphenol extraction [60].

On the other hand, CSLE (stirring only, no heating or ultrasound) led to less efficient extraction, being necessary the combined use of ultrasound and stirring to enhance the extraction performance. Similar result was observed when phenolic compounds were extracted from dehydrated chicory by-products using agitation and sonication under various temperatures [61]. Green technologies like ultrasound-assisted technology has been referred as sustainable extraction strategies to achieve efficient, clean-label recovery of natural phytochemicals and pigments with reduced use or even elimination of harmful organic solvents and lower energy consumption yield [62,54]

Table 2
Data inventory of polyphenol extraction protocols for acerola (AP) and jambolan (JP) pomaces submitted to different polyphenol extraction protocols.

	Input					**TPC output (mg)**
	Volume of water bath (liters)	**Time (min)**		**Electricity (kWh)**		1400
	Both AP and JP	AP	JP	AP	JP	
CSLE	0	3.59E+06	5.30E+82	287.099	4.24E+78	
HCSLE	3	53.18	167.49	0.106	0.266	
SUAE	3	75.61	106.15	0.108	0.15	
UAES	3	31.81	88.1	0.05	0.134	

Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES). kWh: kilowatt-hour; TPC: total phenolic content (expressed in mg); AP: acerola pomace; JP: jambolana pomace.



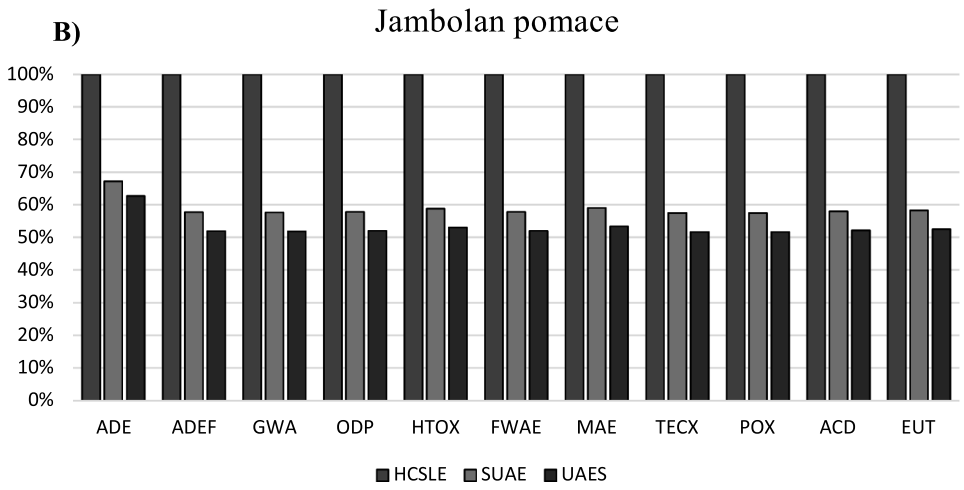


Fig. 7. Impact evaluation of polyphenol extraction of acerola pomace (A) and jambolan pomace (B) using the CML-IA baseline LCA method assessed for 12 impact categories: Abiotic depletion (ADE); Abiotic depletion (fossil fuels) (ADEF); Global warming (GWP100a) (GWA); Ozone layer depletion (ODP); Human toxicity (HTOX); Fresh water aquatic ecotoxicity (FWAE); Marine aquatic ecotoxicity (MAE); Terrestrial ecotoxicity (TECX); Photochemical oxidation (POX); Acidification (ACD) and Eutrophication (EUT). Conventional solid-liquid extraction (CSLE), heated conventional solid-liquid extraction (HCSLE), static ultrasound-assisted extraction (SUAE) and ultrasound-assisted extraction and mechanical stirring (UAES).

The temperature for UAES and SUAE treatments was monitored, and a sharp increase was observed during the first 30 min, and then it reached a plateau around 45°C. Similar temperature increase has been reported previously for sonication-assisted extraction [34]. For HCSLE (use of stirring and heating), a gradual temperature increase was applied to simulate what was observed with the use of ultrasound. HCSLE led to better polyphenol extraction results compared to CSLE, and we hypothesize that this is due the enhancement of polyphenol diffusion, leading to higher polyphenol solubility in the solvent and more efficient extraction [37]. Heating facilitates solvent penetration and accelerates the mass transfer rate, leading to increased polyphenol extraction [23, 63].

In this study, we used low frequency and high intensity (40 kHz, 100W) ultrasound treatments that have been reported as able to produce strong shear and mechanical forces to efficiently extract bioactive molecules from natural plant sources. In a study using aqueous ultrasonic-assisted extraction applied to grape pomace, González-Centeno et al. [64] reported that maximized phenolic content and antioxidant activity were observed when using similar sonication parameters shown here.

When conventional and ultrasound-assisted extraction (50 kHz) were applied to red araca peels, approximately 25 % higher TPC (589.49 mg GAE/100g) was observed for ultrasound-assisted extraction compared to conventional maceration method (477.53b ± 3.09 mg GAE/100g) after 90 min [65]. Our results for acerola pomace are higher than study by Rezende et al. [66], that used ultrasound-assisted extraction (50 kHz) applied to acerola pomace using ethanol/water 1:1 (1046 mg GAE/100) and just water (777.7 mg GAE/100g) as solvents. Similarly, our results after 90 min of extraction for both acerola and jambolan pomaces are superior than previous ultrasound-assisted extractions of grape pomace (Gonzáles-Centeno et al. [67], water-based, ultrasound probe, 55 kHz, 230 mg GAE/100g) and jambolan bark (Bhadange et al. [20], methanol-based, ultrasound probe, 20kHz, 13 mg GAE/g).

3.2. *Extraction kinetics and mathematical modelling of polyphenol extraction*

The kinetic extraction process involves two stages. The initial mixing of solute and solvent is the washing stage, while the second stage involves diffusion, a much slower solute transport process [44]. Because the extraction peak was observed at 90 min for both acerola and jambolan pomaces, the time interval 0-90 min was chosen for modelling the extraction kinetics. Table 1 presents the kinetic parameters for each empirical model investigated in this study. The criteria to determine the model that best represented the experimental values were higher values of R^2 and lower values of NRMSD. Generally speaking, all three models showed good fit to the experimental data ($R^2 > 0.9$, NRMSD < 0.1), however, when all extraction protocols were considered, the Power law model showed the best fit to the experimental TPC data for both acerola and jambolan pomaces (Figs 3 and 4, respectively). Indeed, it displayed the highest R^2 (0.984-0.999 for acerola pomace; 0.963-0.997 for jambolan pomace) and the lowest NRMSD (0.011-0.036 for acerola pomace; 0.017-0.059 for jambolan pomace). The NRMSD was the determining

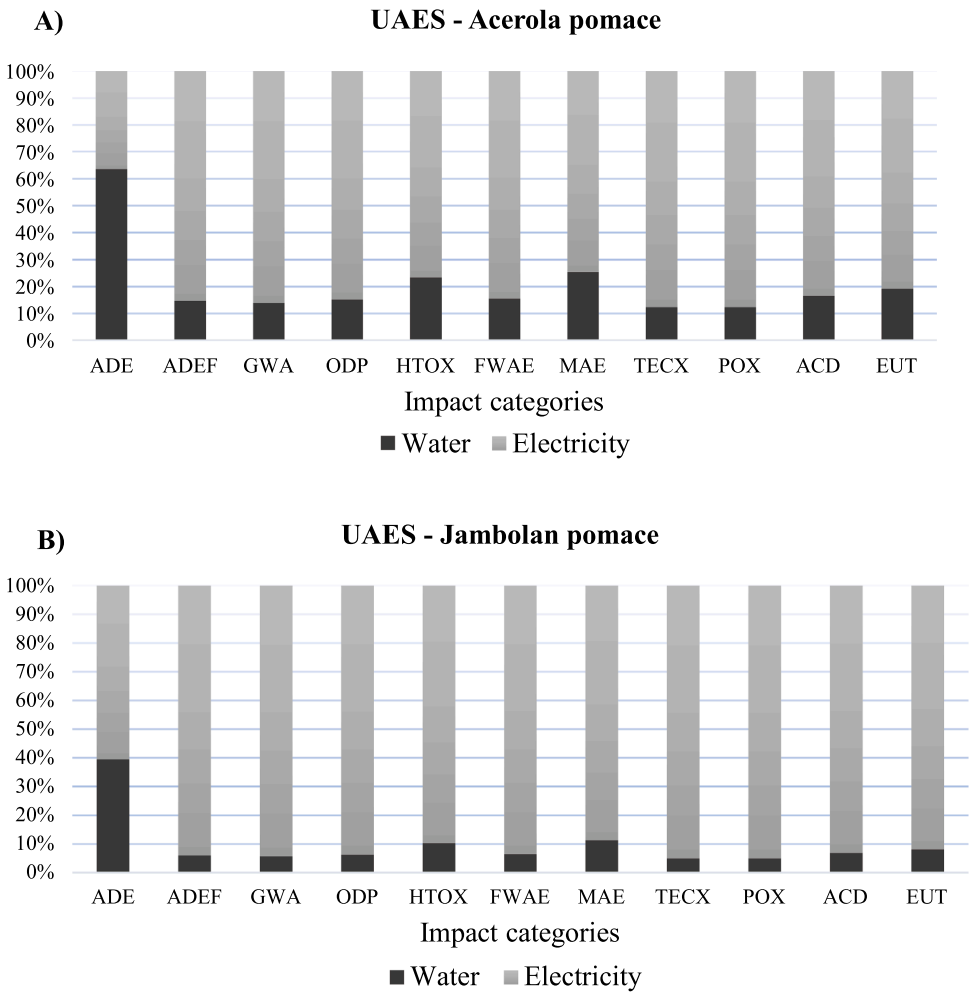


Fig. 8. Contribution of water and energy for the impact evaluation of ultrasound-assisted and mechanical stirring polyphenol extraction (UAES) of acerola pomace (A) and jambolan pomace (B) conducted by CML-IA baseline LCA method assessed for 12 impact categories: Abiotic depletion (ADE); Abiotic depletion (fossil fuels) (ADEFF); Global warming (GWP100a) (GWA); Ozone layer depletion (ODP); Human toxicity (HTOX); Fresh water aquatic ecotoxicity (FWAE); Marine aquatic ecotoxicity (MAE); Terrestrial ecotoxicity (TECX); Photochemical oxidation (POX); Acidification (ACD) and Eutrophication (EUT).

criterion for choosing the model, because low values suggest that the model properly describes the extraction progress with a good quality of fit and greater accuracy of the experimental data [47]. Overall, acerola pomace extraction had higher constant B and lower time exponent n (also referred as diffusion coefficient), when compared to jambolan pomace. (Table 1). Higher B values were found for ultrasonication-assisted extraction protocols SUAE and UAES (Table 1), which indicates that the diffusion stage was positively affected when ultrasound-assisted extraction was performed. The time exponent n of Power Law model, also called the diffusion coefficient, showed low values for TPC extraction ($n \leq 0.6$), which indicates that the polyphenol extraction was controlled by Fickian diffusion [34]. A poor model fit was observed for experimental UAES data (Fig. 3D) of acerola pomace. We hypothesize that this might have happened due to the substantial TPC decrease at 40 min followed by increased TPC at 50 min. Apparently the models were not able to capture this abrupt change, which explains the lowest R^2 observed for UAES data (Table 1).

3.3. Environmental viability of extraction protocols using the LCA tool

In this study, acerola and jambolan pomaces were not submitted to drying before extraction, like in other extraction studies [68–70]. The polyphenol extraction (Fig. 5) was evaluated under a perspective of cradle-to-gate approach, considering only the extraction protocols and processing inputs such as energy and water. The preparation of the extraction solution (water mixed with pomace) was not included in the inventory, since it is identical for all investigated extraction protocols, therefore it would add similar impact for all treatments.

The experimental energy consumption data was plotted against time and linear equations were generated for each extraction protocol (Fig. 6). The time necessary to extract 1400 mg of polyphenols and the respective energy consumption are presented in the inventory table (Table 2). The lowest and highest energy consumption during the first 100 minutes of extraction were observed for CSLE and HCSLE, respectively. It was observed that the UAES protocol (mechanical stirring and sonication) exhibited similar energy consumption requirements compared to extraction using sonication only (SUAE) during this specific length of time (Fig. 6). It became evident that, despite the lower energy consumption during the first 100 minutes of extraction, the CSLE protocol required much longer extraction time to reach the functional unit (established as 1400 mg of polyphenols). Because of this, the estimated total energy consumption required to achieve the functional unit was the highest for CSLE among all tested extraction protocols. On the other hand, because of the enhanced extraction capacity, UAES required the shortest time and lowest energy consumption to achieve the same functional unit for both fruit pomaces among all extraction protocols (Table 2).

The time and energy consumption data were used to determine the environmental impact (LCIA). The CML-IA baseline LCIA method was applied to the data collected from each one of the four different extraction protocols to evaluate the 12 impact categories (Fig. 7A, acerola pomace and Fig. 7B, jambolan pomace). Results were calculated as percentage (%) relative to the greatest impact (set as 100 %). The time needed to extract 1400 mg of polyphenols followed the order CSLE > SUAE > HCSLE > UAES for acerola pomace and CSLE > HCSLE > SUAE > UAES for jambolan pomace (Table 2). The CSLE results were not

included here, due to the long time and high energy requirements (Table 2) needed to extract 1400 mg of polyphenols (functional unit used in this study) for both fruit pomaces. This result is corroborated by the highest K_2 (Peleg capacity constant) values obtained for CSLE, resulting in a low maximum extraction capacity.

For acerola pomace, a similar high environmental impact measured by LCA was observed for both HCSLE and SUAE protocols (Fig. 7A). Indeed, both extraction protocols applied to acerola residue needed longer times compared to UAES to reach the FU, which leads to higher energy consumption (Table 2). The application of heat during HCSLE extraction protocol substantially increased the environmental impact in all assessed categories for jambolan pomace (Fig. 7B). The UAES protocol generated the lowest impact among all treatments for both pomaces and all 12 categories considered in this study. UAES applied to both pomaces had around 50% of the impact of HCSLE and SUAE for most of the categories, obtaining the highest value for the abiotic depletion category (70% for the acerola pomace and 63% for the jambolan pomace). The shortest extraction time required by UAES to reach the FU (1400 mg of phenolics) is a decisive factor that leads to lower energetic consumption and consequently, lower environmental impact compared to all other extraction protocols.

One of the main objectives of LCA is to compare the contributions of process parameters and conditions for the environmental impact and identify hotspots [72]. Results show that the UAES protocol had the best performance regarding time, water and electricity requirements for both acerola and jambolan pomaces. Based on these findings, the UAES protocol was further investigated to clarify the contributions of specific parameters to the impact evaluated by the LCA tool. The electrical energy was a major impact contributor for both pomaces, influencing the results in a more pronounced way when compared to water requirements (Figs 8A and 8B). Overall, the water category had an impact below 30 % for AP and 20 % for JP, but the abiotic depletion (related to mineral resources and fossil fuels utilization) category presented a higher impact for both fruit pomaces - around 65 % for AP (Fig. 8A) and 40 % for JP (Fig. 8B).

It is noteworthy that water was the only extraction solvent used in this study. Even though methanol, ethanol and other organic solvents can be successfully applied to polyphenol extraction, their use might incur in higher environmental impact and may require an additional solvent elimination step [71]. Besides being a food-grade, accessible and low-cost solvent, water was chosen for its good performance [72] and suitability to food processing operations [70,73].

Moreover, ultrasound-assisted extraction naturally leads to temperature increase in the system. Because of this, thermostatic baths or external heat application were not necessary and allowed for an ecofriendlier extraction protocol for both SUAE and UAES. In this regard, Vauchel et al. [71] showed that higher temperature (60°C) and the use of ethanol as the solvent extraction led to a higher impact in all LCA categories when analyzing the antioxidant phenolics extraction from chicory waste using UAE.

Few research studies have used the LCA tool to compare extraction techniques protocols in the scientific literature [74]. The ones that did use it, were mainly focused on operating conditions such as time, temperature and solvent composition. The results obtained in this study strongly agree with previous results that showed ultrasound-assisted extraction protocols as effective strategies to reduce extraction time, energy consumption and environmental impact [71,74,75].

4. Conclusions

Indirect ultrasound coupled to mechanical stirring (UAES) proved to be an efficient polyphenol extraction protocol for both acerola and jambolan pomaces. The polyphenol content of acerola and jambolan polyphenol extracts obtained by UAES after 90 minutes were 1.2 and 3-fold compared to conventional solid-liquid extraction (CSLE), respectively. The Power law model showed the best fit to experimental kinetics extraction data for both acerola and jambolan pomaces and was used for the calculation of LCA. UAES extraction protocol demonstrated the lowest environmental impact among all extraction methods, mainly due to shorter extraction time and reduced energy consumption for both fruit pomaces. Our results highlight the importance of the use of green technologies for proper waste reutilization, biomolecules recovery and environmental protection. Overall, this study reveals that UAES is a rational and environmentally friendly strategy for obtaining polyphenol-rich extracts from fruit pomaces for multiple uses.

Author contributions

Edilene Silva: Original draft preparation, conceptualization, data collection and analysis; Andrea Nunes: Conceptualization and design, methodology, writing – reviewing and editing; Roberta Targino Hoskin: Conceptualization and design, writing – reviewing and editing, supervision.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Spray drying to produce novel phytochemical-rich ingredients from juice and pomace of American elderberry

K.S. Ravichandran [a], E.S. Silva [c,f], M. Moncada [c], P. Perkins-Veazie [c], M.A. Lila [c], C.M. Greenlief [d], Andrew L. Thomas [e], R.T. Hoskin [c], K. Krishnaswamy [a,b,*]

[a] Division of Food, Nutrition and Exercise Sciences, University of Missouri, Columbia, MO, USA
[b] Department of Chemical and Biomedical Engineering, University of Missouri, Columbia, MO, USA
[c] Food, Bioprocessing & Nutrition Sciences Department, Plants for Human Health Institute, North Carolina State University, Kannapolis, NC, USA
[d] Charles W. Gehrke Proteomics Center, University of Missouri, Columbia, MO, USA
[e] Division of Plant Science and Technology, Southwest Research, Extension & Education Center, University of Missouri, Mt. Vernon, MO, USA
[f] Laboratory of Food Bioactive Compounds, Chemical Engineering Department, Federal University of Rio Grande do Norte (UFRN), Campus Central, s/n, Natal, RN, 59078-970, Brazil

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ABSTRACT

The cultivation and commercialization of American elderberries (*Sambucus nigra* subsp. *canadensis*), rich in acylated anthocyanins, is nascent. In this study, American elderberry juice and pomace extract were spray dried using soy protein isolate (SPI) or tapioca starch (TS) as carriers to develop functional food ingredients. Physicochemical, morphological, and bioactive properties were analyzed, and an *in vitro* gastrointestinal digestion model was used to study polyphenol bioaccessibility. An efficient spray drying process (solids recovery >60%) was established. Elderberry particles produced with SPI had higher solubility (60%–64%), lower porosity (69%–70%), and better flowability (22% Carr index, 1.29 Hausner ratio). Spray dried particles produced with tapioca starch showed significantly higher total polyphenol content (42–49 mg gallic acid equivalent/g sample), proanthocyanidin content (0.76–2.86 mg proanthocyanidin-B2/g sample), and anthocyanins (7.86–33.80 mg/g sample) for both elderberry juice and pomace extract, compared to SPI-derived ones. Particles of encapsulated elderberry juice or pomace extract with SPI had higher bioaccessibility compared to non-encapsulated elderberry juice or TS-derived particles. Overall, spray drying American elderberry juice and pomace extract is an effective and sustainable strategy to create novel ingredients for multiple food applications. These findings offer an industry-friendly technological solution to develop value-added ingredients for the emerging American elderberry market.

1. Introduction

Elderberry (*Sambucus* spp.), a deciduous shrub belonging to the family *Viburnaceae*, produces a small dark-purple fruit that is well-known in Europe, North Africa, Asia, and the USA. The fruit and flowers of elderberry are used in a variety of dietary supplement products, in part due to their flavonoids, phenolic acids, terpenoids, lipids, and alkaloids. Elderberry has been used in traditional medicine to treat various diseases due to its antioxidant, anti-bacterial, anti-carcinogenic, anti-allergic, immune-stimulating, and anti-viral properties (Domínguez et al., 2020; Liu et al., 2022; Thomas et al., 2020). Moreover, during the respiratory syndrome SARS-CoV-2 (COVID-19) pandemic, the interest in elderberry supplements skyrocketed to over $320 million (American Botanical Council report) (Osman et al., 2023), as the populations across the world looked for alternatives and complementary therapies to support the prevention and/or treatment of upper respiratory symptoms, complications, and adverse events caused by this severe illness (Wieland et al., 2021).

The cultivation and commercial utilization of American elderberry (*Sambucus nigra* subsp. *canadensis*) are emerging, in contrast to the well-established European elderberry (*Sambucus nigra* subsp. *nigra*). The chemistry of the two species is different, as American elderberries contain more than 50% acylated anthocyanins (known for imparting color stability) while European elderberries have little or no acylated

* Corresponding author. Division of Food, Nutrition and Exercise Science, University of Missouri, Columbia, MO, 65211, USA
E-mail address: krishnaswamyk@umsystem.edu (K. Krishnaswamy).

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anthocyanins (Osman et al., 2023).

European elderberries have been used to prepare several products (Terzić et al., 2023), but the technological potential of American elderberries has not been fully explored yet, as they are traditionally used to prepare a very limited number of products such jams, jellies, syrups, juice, and wine (Thomas et al., 2015). Further, the by-product of elderberry juice processing (pomace consisting of seeds and skin, and residual pulp) is a good source of fatty acids, dietary fibers, and polyphenols that can be used to produce value-added ingredients to formulate functional foods and nutraceuticals (Costa et al., 2021). Indeed, the rational use of fruit pomaces can enhance the nutritional value of food products and reduce food waste which aligns with one of the important targets of sustainable development goals laid down by the FAO (Food and Agriculture Organization) for the 2030 agenda (FAO publications catalogue, 2021).

Berries are highly susceptible to spoilage due to their high moisture content and delicate structure, making them more prone to microbiological contamination and enzymatic degradation. Spray drying, one of the most used drying techniques in the food and pharmaceutical industries, has multiple advantages such as high energy efficiency, scalability, and short drying time (Kandasamy & Naveen, 2022). Spray drying encapsulation is a one-step drying technique that enables the production of food particles with reduced moisture, extended shelf-life, preserved and concentrated phytochemical content, all in a convenient particulate format (Correia et al., 2017). Spray drying operates with a short residence time of the product inside the drying chamber that allows for sufficient removal of moisture content while protecting the degradation of heat-sensitive compounds (Jafari et al., 2023; Bassani et al., 2022; Ravichandran & Krishnaswamy, 2021). However, spray drying of sugar-rich materials, such as fruit juices, is challenging because of the low glass transition temperature and consequent stickiness associated with the presence of low molecular weight sugars and organic acids in the solution (Sobulska & Zbicinski, 2021).

Carriers, also referred to as drying aids and/or wall materials, are used to minimize excessive stickiness and consequent loss of product and to provide better retention of bioactive compounds (Grace et al., 2021). Polysaccharides and proteins are the two major carriers used for the encapsulation of food-bioactive compounds. Protein-based carriers exhibit superior film forming, surface activity, and emulsifying capabilities and provide a good source of energy with high nutritional value. In particular, plant-based proteins are more affordable options with better hydrophobic properties, lower toxicity, and less allergenicity compared to animal proteins (Akbarbaglu et al., 2021). On the other hand, polysaccharide-based carriers have lower cost, neutral flavors, and low viscosity at higher concentrations (Shishir & Chen, 2017).

Our research team has demonstrated that the spray drying microencapsulation of berry juice and pomace extracts is an efficient method to produce dried fruit ingredients with preserved phytochemical content, enhanced functional attributes, and extended storage while maintaining desirable organoleptic and biofunctional properties (Correia et al., 2017; Hoskin et al., 2019; Hoskin et al., 2022). In this study, we use spray drying encapsulation of American elderberry juice and pomace extract to obtain novel and convenient elderberry powdered ingredients. We investigated and compared the spray drying performance and quality attributes of spray dried particles (physicochemical content, bioactive properties, and bioaccessibility) produced with both elderberry juice and pomace extract using two carriers – polysaccharide-based (tapioca starch) and protein-based (soy protein isolate). Our results provide technical insights to create novel, value-added ingredients for the incipient market of American elderberry products, as an effort to diversify and increase the market opportunities of this underexploited, rapidly emerging specialty crop.

2. Materials and methods

2.1. Materials

American elderberry fruits were harvested (August 2022) from research orchards located at the University of Missouri's Southwest Research, Extension, and Education Center, near Mt. Vernon, MO, USA. Ripe fruits were sealed in zippered plastic storage bags, immediately cooled, then promptly frozen (−20 °C). Berries from the cultivar 'Bob Gordon' were pressed to obtain elderberry juice, whereas the cultivars 'Bob Gordon', 'Kelly 7–14', 'Ozark', 'Pocahontas', 'Rogersville', and 'Wyldewood' were used to produce a concentrated liquid extract from pomace. Soy protein isolate (SPI, 90% protein) was obtained from Bulk Supplements (Henderson, NV, USA) and organic tapioca starch (TS) from PuroRaw (Brooklyn, NY, USA) and Anthony's Goods (CA, USA). Gallic acid (G7384), L-ascorbic acid (A7506), 2,2-diphenyl-1-picrylhydrazyl (DPPH, D9132), and 4-dimethylamino cinnamaldehyde (DMAC, D4506) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used for chromatographic analyses were HPLC grade and all other reagents were of analytical grade.

2.2. Production of elderberry juice and concentrated pomace extract

Frozen elderberries were thawed at room temperature (25 °C) and rinsed with water to remove impurities or foreign materials before being pressed. The elderberry juice (EJ) was produced using a pulper (C80 Automatic Sieve, Ridgeland, MS, USA). The resulting pomace and juice were then stored at −20 °C until further use. The elderberry pomace (EP) was used to prepare a concentrated pomace extract according to an adapted extraction protocol (Hoskin et al., 2019). First, the freeze-dried elderberry pomace was mixed with a 50% ethanol solution (1:3 extraction ratio w/v) and kept in a water bath at 80 °C for 2 h. Next, the mixture was filtered through double layer cheesecloth under vacuum, then centrifuged at 24,400 × g (20 min, 25 °C). Finally, the ethanol was removed using a rotary evaporator (Buchi Labortechnik, New Castle, DE, USA), and the final concentrated extract was kept at −20 °C (no longer than 1 week) until further use.

2.3. Production of spray dried particles using carbohydrate and protein carriers

The frozen elderberry juice was thawed at room temperature (25 °C and centrifuged at 15,552 × g (10 min, 20 °C; Sorvall LYNX 6000 centrifuge, Waltham, MA, USA). The supernatant was collected, mixed with either soy protein isolate, or tapioca starch at a concentration of 8% (w/v) then homogenized at 3,200 × g for 10 min (IKA, Ultra Turrax T25 homogenizer, Wilmington, NC, USA). Similarly, the concentrated liquid elderberry pomace extract was mixed with either soy protein isolate or tapioca starch (8% w/v) and homogenized for 2 h using a magnetic stirrer to allow complete hydration.

The drying procedure was performed using a B-290 spray dryer (Buchi Labortechnik, New Castle, DE, USA), with a concurrent flow and a nozzle diameter of 0.7 mm. The feed flow was controlled at 10 mL/min by a peristaltic pump and the drying air had an inlet temperature of 120 °C and an outlet temperature ranging from 67 to 77 °C. The elderberry juice or pomace extract mixed with tapioca starch or soy protein isolate was kept at constant magnetic stirring at 30 °C during the drying process. The resulting spray dried elderberry particles were collected, weighed, sealed, and kept frozen at −20 °C (no longer than 1 month) until further use. As a result, four experimental groups of aggregate particles were produced and tested: EJ-TS (elderberry juice with tapioca starch), EJ-SPI (elderberry juice with soy protein isolate), EP-TS (elderberry pomace extract with tapioca starch), and EP-SPI (elderberry pomace extract with soy protein isolate).

2.4. Determination of total soluble solids, pH, and berry size

The pH of elderberry juice and pomace extract was determined at room temperature (25 °C) using a digital pH meter (SevenCompact S220, Mettler Toledo, Columbus, OH, USA). Similarly, the total soluble solids (TSS) content was measured at room temperature using a digital refractometer (HI96800, Hanna Instruments, Smithfield, RI, USA) and recorded as °Brix. Berry size was measured in length (diameter, mm) using ImageJ software (National Institutes of Health, 2023).

2.5. Solids recovery

Solids recovery was calculated as the relationship between the solids content of the spray dried powder recovered after spray drying to the total solids in the feed solution according to Equation (1) (Grace et al., 2021).

$$Yield\ \% = \frac{mass\ of\ powder}{total\ solids\ in\ feed\ solution} \times 100 \quad (1)$$

2.6. Physicochemical properties of spray dried particles

2.6.1. Moisture content and water activity

The moisture content was determined using a halogen moisture analyzer (HE53, Mettler Toledo, Columbus, OH, USA) at 105 °C. The water activity (Aw) was determined using a water activity meter (Cx-2, Decagon Devices, Inc., Pullman, WA, USA) at room temperature (25 °C).

2.6.2. Color parameters

The color measurements were performed using a Colorimeter (Konika Minolta CR-410, Ramsey, NJ, USA) with a 0° viewing angle and a pulsed xenon lamp as the light source. The instrument displays readings in terms of color coordinates (L*: whiteness to darkness, a*: redness to greenness, and b*: yellowness to blueness). Instrument calibration was performed using the standard white tile, and samples were placed on a Petri dish during each measurement. The total color change (ΔE), hue angle (H°), and chroma (C) were calculated by Equations (2)–(4) (Correia et al., 2017):

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (2)$$

where L_0, a_0, b_0 denote the color coordinates of elderberry juice, used as a standard reference to compare the color change of the powder.

$$H^\circ = 360 + tan^{-1}\left(\frac{b^*}{a^*}\right), when\ \ b^* < 0 \quad (3)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$

2.6.3. Density and porosity

Approximately 2 g of each sample was transferred into a 10 mL graduated cylinder and the volume occupied by the powder was used to calculate the bulk density (ρ_b, ratio of mass to bulk volume, Equation (5)). A gas pycnometer (Quantachrome ultra pycnometer 1000 Anton Paar, Graz, Austria) was used to measure the true density. A sample cell containing approximately 1 g of powder was filled with a known volume of compressed helium gas. The volume measured by the pycnometer was used to calculate the true density (ρ_T) (Nani & Krishnaswamy, 2023). The porosity (ε) values were obtained based on the relationship between bulk and true densities (Equation (6)).

$$Bulk\ density\ \left(\frac{g}{cm^3}\right) = \frac{sample\ \text{mass}}{\text{bulk volume}} \quad (5)$$

$$Porosity\ (\%) = \frac{true\ density - bulk\ density}{true\ density} \times 100 \quad (6)$$

2.6.4. Particle size distribution and zeta potential

The particle size and zeta potential of spray dried elderberry particles were analyzed using a dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern, MA, USA and DelsaNano C, Beckman Coulter, CA, USA respectively). For this, 0.5 g of the sample was dispersed in water and diluted to a final concentration of 0.005% (w/v) according to Nani and Krishnaswamy (2023) with slight modifications.

2.6.5. Glass transition temperature (T_g)

The glass transition temperature (T_g) was determined using a differential scanning calorimeter (Q200 DSC, TA Instrument, Schaumburg, IL, USA). Each sample, weighing approximately 7–8 mg, was placed in a sealed aluminum pan. The samples were cooled to 20 °C and then heated to 180 °C at a rate of 10 °C per minute in a nitrogen-purged environment with a flow rate of 50 mL/min. A reference aluminum pan was used for comparison. The midpoint values of T_g were determined using Universal V4 5A TA Instruments analysis software from Schaumburg, IL, USA (Singh et al., 2022).

2.6.6. Morphology

The powder morphology was examined by scanning electron microscopy (FEI Quanta 600F ESEM). The spray-dried elderberry particles were mounted using a double-sided carbon adhesive and coated with a 25 nm platinum layer in a vacuum. The examination was conducted at 5 kV with an 8 mm working distance, 30 μm objective aperture, and 3.5 spot size, under a magnification of 1000x.

2.6.7. Flowability

The Hausner ratio (HR, Equation (7)) and Carr index (CI, Equation (8)) were used to estimate the cohesiveness and flow characteristics of spray dried particles, respectively (Correia et al., 2017). Flowability of spray dried particles is classified based on a previous report (Supplementary Table 1, Goyal et al., 2015).

$$Hausner\ ratio\ (HR) = \frac{tapped\ density}{bulk\ density} \quad (7)$$

$$Carr's\ compressibility\ index\ (\text{CI}) = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100 \quad (8)$$

2.6.8. Hygroscopicity

The hygroscopicity of spray dried elderberry particles was determined as described by Nani and Krishnaswamy (2023). Approximately 0.5 g of sample was placed in a desiccator with a NaCl saturated solution (75% relative humidity at 30 °C) for 7 days. The hygroscopicity was calculated as a percentage based on the ratio of the mass of absorbed moisture to the initial mass of powder (Equation (9)).

$$Hygroscopicity\ \% = \frac{mass\ of\ powder\ after\ 7\ days - initial\ mass\ of\ powder}{initial\ mass\ of\ powder} \times 100 \quad (9)$$

2.6.9. Solubility

Solubility was determined based on Correia et al. (2017) with slight modifications. Approximately 0.5 g of powder was mixed with 10 mL of water and placed in a circulatory water bath at 37 °C for 30 min. Next, the mixture was centrifuged at 3074×g for 10 min, and the supernatant was poured on a pre-weighed Petri dish, then oven dried at 105 °C until a constant weight was obtained (equation (10)).

$$Solubility\ \% = \frac{weight\ of\ dried\ supernantant}{weight\ of\ powder} \times 100 \quad (10)$$

2.7. Phytochemical analyses

2.7.1. Sample preparation

Briefly, 20 mg of spray dried elderberry particles was suspended in 1 mL of 1% acetic acid in 80% methanol in water, followed by 5 min of sonication at 55 °C and 10 min centrifugation at 21,130×*g*. Next, the filtered supernatant was collected, and the process was repeated. Finally, the eluates were pooled together and used for phytochemical analysis.

2.7.2. Total polyphenol content (TPC) and proanthocyanidin content (PAC)

An adapted Folin-Ciocalteau method was used to measure the total polyphenol content spectrophotometrically in microplates (Spectramax Plus 384, Molecular Devices, Sunnyvale, CA). Samples were read at 765 nm against a gallic acid standard curve, and results were expressed as gallic acid equivalent (mg GAE/mL or mg GAE/g sample) (Hoskin et al., 2019).

The total proanthocyanidin (PAC) content was determined using an adaptation of the DMAC (4-dimethylamino cinnamaldehyde) assay with a calibration curve of PAC-B2 (3.125–100 μg/mL) and results were expressed as mg PAC-B2/100 g sample (Prior et al., 2010).

2.7.3. Ascorbic acid content

Ascorbic acid was determined following a previous protocol (Jiang et al., 2017). Filtered samples (20 μL) were injected into a Hitachi Elite LaChrom high-performance liquid chromatograph (Hitachi Ltd., San Jose, CA) equipped with a UV–Vis diode array detector, controlled temperature autosampler (4 °C), and column compartment (30 °C). Ascorbic acid detection and quantification were performed using a C18 column (Synergi 4μ Hydro-RP 80 Å, 6 × 250 μm; Phenomenex Inc., Torrance, CA). The mobile phase consisted of 0.0065N H_2SO_4 with a flow rate of 1 mL/min. Total ascorbic acid content was calculated from standard curves generated by injecting 20 μL of L-ascorbic acid and reported as mg/100g fresh weight.

2.7.4. Anthocyanin profile

The anthocyanin profiles of elderberry juice, pomace extract, and spray dried elderberry particles were determined by double extraction. For this, 0.2 g of elderberry juice or pomace extract and 0.02 g of spray dried elderberry particles were mixed with 1.5 mL of acidified methanol following a previous protocol (Perkins-Veazie et al., 2016). Filtered samples (20 μL) were injected into an ultra-high performance liquid chromatograph (Waters Acquity, Milford, MA), equipped with a diode array detector, controlled temperature autosampler (4 °C), and column compartment (45 °C). Anthocyanin and phenolic separation were performed using a C18 column (Waters Acquity UPLC BEH 1.7 μm, 2.1 × 50 mm). The mobile phase consisted of 5% formic acid in water (A), and 100% methanol (B) with a flow rate of 0.3 mL/min using a step gradient of 0 min, 10% B; 5 min, 15% B; 15 min, 20% B; 20 min, 25% B; 25 min, 30% B; 45 min, 60% B; 47 min, 10% B; 60 min, 10% B. Compound concentrations were estimated using standard curves generated by injecting 20 μL of 0.0625–0.5 mg/mL preparations of cyanidin 3-O-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside-5--glucoside, and cyanidin 3-sambioside as external standards. Compound identification was performed based on retention time compared to authentic standards and those reported by Lee and Finn (2007). Samples were reported as mg/g dry weight (DW) and sums of anthocyanins were calculated to obtain total anthocyanin and phenolic contents.

2.7.5. Antioxidant activity - 2.2-diphenyl-1-pricrylhydrazil (DPPH) assay

The radical scavenging activity measured by DPPH assay was conducted according to our previous protocol (Correia et al., 2017). Briefly, 20 μL of eluted samples were added to 180 μL of DPPH solution (150 μmol/L) in methanol-water 80% (v/v) using 96-well microplates. After 40 min in the dark at room temperature, the absorbance was measured at 515 nm. Results were calculated using a standard curve built with different concentrations (100–500 μM) of Trolox (6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid) and expressed as μM Trolox equivalents/g.

2.8. In vitro simulated gastrointestinal digestion

A modified standardized static *in vitro* gastrointestinal digestion (GID) method was adapted from Minekus et al. (2014). Simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) electrolyte stock solutions were added to elderberry-derived samples with previously standardized total phenolic content (7.8–9.2 mg TP). The mixtures were suspended in 0.5 mL water and thoroughly mixed with 0.35 mL of SSF, followed by sequential addition of 50 μL of 1500 U/mL porcine pancreas α-amylase solution, 25 μL of 0.03 M $CaCl_2$ and 75 μL of water (pH 7.0). For the gastric phase, the resulting oral bolus (1 mL) was mixed with 0.64 mL of SGF electrolyte stock solutions, 160 μL porcine pepsin stock solution of 25,000 U/mL, 5 μL of 0.03 M $CaCl_2$, 20 μL of 1 M HCl, and 175 μL of water (pH 3.0). The reaction vessel was placed into an incubator at 37 °C for 2 h under agitation. For the intestinal phase, 2 mL of gastric chyme was mixed with 1.1 mL of SIF electrolyte stock solution, 0.5 mL of pancreatin solution 800 U/mL, 0.25 mL fresh bile (160 mM in fresh bile), 40 μL of 0.3 M $CaCl_2$, 15 μL of 1 M NaOH and 95 μL of water (pH 7.0) and shaken once again for 2 h at 37 °C. After the intestinal digestion, samples were centrifuged to obtain the soluble fraction (supernatant) and the residual fraction, then frozen and freeze-dried. The bioaccessibility index (BI) was calculated as

$$\text{BI}\ (\%) = (\text{TP post-digestion of supernatant/TP pre-digestion}) \times 100$$

where TP post-digestion is the total phenolic (μg/mg sample) quantified in the intestinal supernatant after the complete digestion process, and TP pre-digestion is the total phenolic (μg/mg sample) quantified before the *in vitro* digestion (Grace et al., 2021; Xiong et al., 2020).

2.9. Statistical analyses

Two-way analysis of variance of mean values was carried out using JMP 14.0 statistical software (SAS Institute Inc, Cary, NC, USA). *In vitro* simulated gastrointestinal results were analyzed by Prism 8.0 (GraphPad Software, San Diego, CA, USA) to perform ordinary one-way ANOVA analysis. Means were compared by Tukey test with statistical significance $p < 0.05$ at a 95% confidence interval unless stated. Principal component analysis (PCA) was conducted and cross-verified using both Origin Pro, 2021, and Jmp 14.0, and the PCA graphs from Origin Pro, 2021 were used. Results are presented as the mean ± SD. All physical and chemical analyses were performed in triplicate unless noted.

3. Results and discussion

3.1. Spray drying process evaluation

The solids recovery, also referred to as spray drying yield, is an important indicator of the feasibility and efficiency of the spray drying process (Hoskin et al., 2019). The solids recovery of EJ-SPI, EJ-TS, EP-SPI and EP-TS treatments were similar ($p > 0.05$) and averaged 68.80 ± 3.24, 62.18± 2.47, 68.8 ± 5.60, 71.7± 2.90% respectively. Our results are within the successful range for lab-scale spray drying (solids recovery >50%; Tontul and Topuz (2017) and are considerably higher than those obtained for the spray drying of blueberry with SPI (50.1%), jussara (*Euterpe edulis*) pulp with SPI (39.8%) and chokeberry (*Aronia* spp.) with tapioca starch (35.3%) (Correia et al., 2017; Gawałek & Domian, 2020; Santana et al., 2016). One of the primary reasons for reduced product recovery in fruits is the stickiness caused by the presence of low molecular weight sugars and organic acids (Leyva-Porras

et al., 2019). Indeed, elderberries contain high sugar concentrations (fructose, glucose, sucrose) and a variety of organic acids (citric, malic, shikimic, and fumaric acids) (Thomas et al., 2015b; Veberic et al., 2009) which might jeopardize the spray drying operation when no carriers are used (Verma & Singh, 2015). The elderberry juice and pomace extract had a pH of 4.61 and 3.72 respectively and total soluble solids of 8.33 and 5.9 °Brix, respectively. Hence, the findings of this study suggest that the addition of high molecular weight carriers, such as SPI or tapioca starch at a relatively low concentration (8%) enabled the efficient spray drying of elderberry juice and pomace extract, making it an efficient strategy to obtain powdered elderberry ingredients.

3.2. Physicochemical characterisation of spray dried elderberry particles

3.2.1. Morphology

Fig. 1 shows the morphology of spray dried elderberry juice and pomace extract particles. Those produced with SPI (EJ-SPI, EP-SPI) have a predominantly spherical shape with varying sizes, with rough or wrinkled surfaces. The observed wrinkled surface on SPI-derived particles can be attributed to uneven shrinkage during drying or cooling, a common characteristic of powders produced using proteins as a carrier. The increased flexibility of the protein film formed on the droplet surface leads to particle shrinkage without ruptures, resulting in particles with more wrinkles and rough surfaces (Muzaffar & Kumar, 2016). On the other hand, elderberry particles produced with tapioca starch (EJ-TS, EP-TS) had a smoother surface with oval-shaped particles and fewer wrinkles, or dents. Particle morphology can also directly influence the stability of the microencapsulated core, as well as the flowability, and bulk density of powders. For instance, particles with more wrinkles or rough surfaces could be more susceptible to oxidation due to increased surface area, and the reduced surface contact results in a more free-flowing powder (Correia et al., 2017; Zhang et al., 2020).

3.2.2. Moisture content and water activity

Both the moisture and water activity of spray dried particles play a crucial role in determining the flowability, stickiness, and storage stability of powdered food products due to their influence on the glass transition temperature and crystallization properties of food materials (Righi da Rosa et al., 2021). The moisture content of spray dried elderberry samples was between 2.68% and 4.31% (Table 1), consistent with previous reports for spray dried black mulberry juice and chokeberry extract (Fazaeli et al., 2012; Vidović et al., 2019). Reducing the moisture level can minimize the agglomeration and caking in spray dried particles, which are undesirable phenomena that can jeopardize the proper storage of the final product. Therefore, it is crucial to maintain a moisture content of less than 6% for a food powder intended for long-term storage (Bajac et al., 2022). Water activity (A_w) measures the availability of free water responsible for deteriorative reaction and provides an important index to estimate the shelf life of particulate products. Powders with a water activity of less than 0.3 are considered as microbiologically and chemically stable (Shishir & Chen, 2017). The water activity of spray dried elderberry particles was between 0.16 and 0.21 (Table 1), typical values for spray dried products (Santhalakshmy et al., 2015).

3.2.3. Density and porosity

The density of food powders influences a product's processing, packaging, storage, and shipping. Higher bulk density is desired during

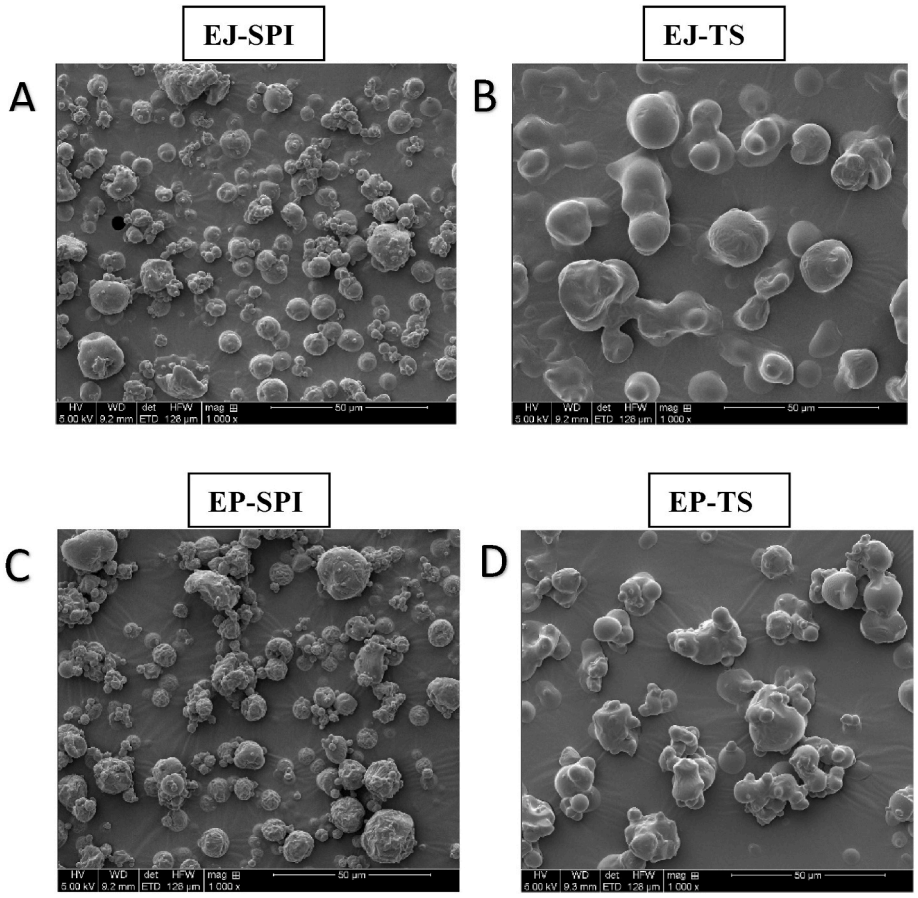


Fig. 1. Scanning electron micrographs (1000× magnification) of spray dried American elderberry juice and elderberry pomace extract particles. A) EJ-SPI: elderberry juice with soy protein isolate; B) EJ-TS: elderberry juice with tapioca starch; C) EP-SPI: elderberry pomace extract with soy protein isolate; D) EP-TS: elderberry pomace extract with tapioca starch.

Table 1
Physical properties of spray dried American elderberry juice and elderberry pomace extract particles.

Parameters	EJ-SPI	EJ-TS	EP-SPI	EP-TS
Moisture content (%)	4.31 ± 0.21[a]	2.68 ± 0.05[b]	3.06 ± 0.13[b]	3.04 ± 0.15[b]
Water activity	0.218 ± 0.005[a]	0.169 ± 0.003 [c]	0.174 ± 0.001[c]	0.176 ± 0.005[b]
Bulk density (kg/m^3)	416.67 ± 11.22[a]	373.49 ± 15.37[b]	404.29 ± 17.19[a]	415.41 ± 17.29[a]
True density (kg/m^3)	1362.17 ± 10.55[b]	1415.18 ± 7.27[b]	1364.20 ± 4.40 [b]	1471.53 ± 17.56[a]
Porosity %	69.36 ± 0.75[b]	73.18 ± 0.76[a]	70.73 ± 1.13[b]	72.48 ± 1.21[a]
Carr Index (%)	22.62 ± 1.52[b]	31.18 ± 3.88[a]	22.33 ± 2.99[b]	34.79 ± 2.54[a]
Hausner ratio	1.29 ± 0.03[b]	1.46 ± 0.08[a]	1.29 ± 0.05[b]	1.54 ± 0.06[a]
Particle diameter (μm)	1.14 ± 0.95	8.13 ± 6.72	0.51 ± 0.33	0.64 ± 0.48
Polydispersity index (PDI)	0.697	0.684	0.416	0.568
Zeta potential	−18.57 ± 2.26[a]	−15.96 ± 0.91[a]	−15.85 ± 2.45[a]	−15.04 ± 3.27[a]
Glass transition temperature (Tg, °C)	73.66 ± 3.72[b]	62.32 ± 0.95[c]	62.25 ± 1.46[c]	120.93 ± 3.17[a]
Hygroscopicity %	22.72 ± 0.65[a]	21.26 ± 1.25[a]	21.22 ± 3.77[a]	18.77 ± 0.72[a]
Solubility %	60.60 ± 2.16[a]	51.16 ± 1.70 [b]	64.38 ± 0.41[a]	49.40 ± 1.93[b]

Results are shown as average ± SD. Different letters in the same row indicate statistical difference by Tukey's test (2-way ANOVA, $p < 0.05$). EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

transport as it requires less storage and transportation space and contains fewer cavities in the powder for oxygen penetration, thereby increasing storage stability (Bajac et al., 2022). Spray dried elderberry juice and pomace extract particles had a bulk density between 290 and 416 kg/m^3, where EJ-TS treatment had a significantly lower value ($p < 0.05$) compared to other treatments. Similar values were observed for spray dried blueberry juice and chokeberry extract (Darniadi et al., 2018; Tzatsi & Goula, 2021). True densities of spray dried elderberry juice and pomace extract particles were 1362 and 1471 kg/m^3, respectively. EP-TS treatment resulted in significantly higher true density compared to other treatments. Encapsulation of blackberry juice using maltodextrin and gum Arabic gave similar results of true densities (1487–1512 kg/m^3) (Ferrari et al., 2012) as those reported in our work.

High porosity implies a large interparticle void space containing oxygen available for degradation reactions (Bajac et al., 2022). Additionally, Lu et al., (2021) reported that the major drawback of carbohydrate-based wall materials is their high porosity, which can lead to decreased storage stability. The carrier type dictated porosity values, and tapioca starch treatments showed higher values ($p < 0.05$) compared to SPI. The porosity of elderberry spray dried particles was similar to those reported for blackberry powder using maltodextrin and gum Arabic (70%–73%) (Ferrari et al., 2012).

3.2.4. Flowability

Low-cost physical analyses such as bulk and tapped densities are used to estimate the Hausner ratio (HR) and Carr index (CI), parameters that enable the prediction of the flow behavior of powders (Goyal et al., 2015). The CI and HR values ranged from 22% to 34% and 1.29–1.54, respectively (Table 1). Similar results were obtained when blueberry pomace extract was spray dried with SPI (Correia et al., 2017) and chokeberry juice spray dried with tapioca starch (Gawałek & Domian, 2020). Overall, the type of carrier had a significant influence on the flowability of powders, as elderberry particles produced with SPI (EJ-SPI, EP-SPI) showed significantly lower ($p < 0.05$) CI and HR, indicative of better flowability. According to Muzaffar and Kumar (2016), powder flowability depends on moisture content, particle size, and shape. Higher moisture content leads to increased cohesiveness because of the plasticizing effect of water, which makes the particle surface more viscous and reduces flowability. Nonetheless, the presence of significantly higher moisture content in EJ-SPI did not negatively affect its flow properties (Table 1). In addition, smaller particle sizes can increase cohesion and reduce flowability by providing more contact points for interparticle bonding. However, roughness on the surface can obstruct the particles from approaching each other resulting in better flowability. Our study revealed that particles produced with SPI (EJ-SPI, EP-SPI) were smaller than those produced with tapioca starch (EJ-TS, EP-TS), but the rough or wrinkled surface of the former might play an important role in the observed better flowability. Fig. 1 revealed that EJ-SPI and EP-SPI particles had a greater degree of sphericity compared to EJ-TS and EP-TS. Generally, particles with a higher degree of sphericity tend to exhibit better flow properties (Gagneten et al., 2019) as observed for SPI treatments. Similar behavior was observed with spray dried raspberry, blackcurrant, and elderberry extract powders. Raspberry powders had a smoother surface with less shrinkage and showed less flowability compared to others (Gagneten et al., 2019).

3.2.5. Particle size and zeta potential

The particle size of food powders influences their handling, stability, transportation, and storage requirements. The wall material was the main factor that affected the size of spray dried elderberry particles, as SPI treatments (EJ-SPI, EP-SPI) showed smaller particle size compared to tapioca starch treatments (EJ-TS, EP-TS; Table 1). This finding could be related to the shrinkage of spherical particles during drying. In this regard, ESEM images (Fig. 1) show more shrinkage for SPI treatments compared to tapioca starch treatments, which might play a role in the observed smaller particle size. Further, the PDI values (Table 1) indicate that the elderberry particles have a polydisperse (PDI >0.1) particle size distribution (Raval et al., 2019). Comparable behavior was observed when raspberry and elderberry extracts were spray dried with maltodextrin. Elderberry particles showed a smaller particle size (6 μm) which was related to their shrinkage, while raspberry powders showed less shrinkage and increased particle size (8.5 μm) which decreased their flowability (Gagneten et al., 2019).

Zeta potential is linked to the electrostatic repulsion between particles and it was measured to determine the stability of spray dried elderberry particles in an emulsion. Bhattacharjee (2016) classifies particles with zeta potential values of ±0–10 mV, ±10–20 mV, ±20–30 mV, and > ±30 mV as highly unstable, relatively stable, moderately stable, and highly stable, respectively. The obtained microparticles had negative zeta potential values ranging from −15 to −18 mV (Table 1) which indicates that the powders would be relatively stable when dispersed in an aqueous solution. However, our results indicate lesser stability in comparison to spray dried chokeberry particles produced with maltodextrin and skimmed milk (−35 to −39 mV) (Ćujić-Nikolić et al., 2019).

3.2.6. Glass transition temperature (T_g)

The glass transition temperature (T_g) is the temperature at which amorphous materials transform from a high-viscosity, glass-like state to a lower-viscosity, rubber-like state due to the increased molecule mobility. Powders stored below their T_g have an increased viscosity, hindering molecular mobility, whereas storage above T_g results in decreased viscosity causing structural changes such as stickiness and product collapse (Daza et al., 2016; Santhalakshmy et al., 2015). Table 1 shows T_g values for spray dried elderberry juice and pomace extract particles ranging from 62 to 120 °C, similar to what was previously observed for blackberry (51–60 °C) and black mulberry (40–76 °C) powder using maltodextrin or gum Arabic as carriers (Fazaeli et al., 2012; Ferrari et al., 2013). Interestingly, EP-TS treatment showed

significantly higher T_g values compared to other treatments. In this study, the T_g values of wall materials SPI and tapioca starch were 39.07 ± 2.31 and 55.54 ± 10.67, respectively. Also, the expected lower sugar level of elderberry pomace extract, compared to elderberry juice, may explain the significantly higher T_g values of the EP-TS particles.

3.2.7. Color parameters

Color parameters are the direct indicators of quality and deeply influence the consumers' desire to purchase a certain food product. Both factors (carrier type and polyphenol source) and their interactions significantly influenced ($p < 0.05$) L*, a*, and b* values (Table 2) (Fig. 2). EJ-TS particles had a significantly lower ($p < 0.05$) lightness (L*), indicating that this treatment looks darker than the others. According to Murugesan and Orsat (2011), higher L* does not necessarily mean the quality deterioration of a product; rather, it could be the dilution effect of wall materials. The products of Maillard reactions (furfural and hydroxymethylfurfural) condense with anthocyanin to form brown pigments which could lead to lower lightness (Ferrari et al., 2013). Parameter b* shows that elderberry pomace extract particles are more towards the yellow coordinate while the juice particles are closer to the blue coordinate. The coordinate a* is the most sensitive color measurement as it directly correlates to the anthocyanin content. The a* and chroma values (Table 2) are significantly higher ($p < 0.05$) for EJ-TS and EJ-SP suggesting higher anthocyanin content in these treatments compared to EP-TS and EP-SPI. Ferrari et al. (2012) spray dried blackberries with gum Arabic and maltodextrin and found that increased a* and chroma directly correlated with the anthocyanins and antioxidant capacity, which agrees with the present study.

The hue angle reflects the characteristic color of the powder. A hue angle of 0°, 90°, 180°, and 270° represents red, yellow, green, and blue colors, respectively (Santhalakshmy et al., 2015). All elderberry particles showed very distinct hue angles ($p < 0.05$), as elderberry juice-derived particles were in the range of 330–350° (purple-red coordinate) and elderberry pomace extract particles presented values between 14 and 18° (red coordinate). Previously, a much lower hue angle (270°), closer to the blue coordinate, was observed for European elderberry spray dried with SPI (Murugesan & Orsat, 2011). These significant differences could be attributed to the different cultivars and sub-species, mainly regarding the diverse anthocyanin profile of subsp. *nigra* (rich in non-acylated compounds) that can influence the color stability and hue of the product (Jokioja et al., 2021). The total color change (ΔE) is a significant color parameter to analyze the color variation between fresh and processed products. Significant differences ($p < 0.05$) in ΔE values (Table 2) were observed for both factors (elderberry source, carrier type) and their interactive effects. In general, elderberry particles produced with SPI (EJ-SPI, EP-SPI) had a higher ΔE compared to particles produced with tapioca (EJ-TS, EP-TS), which could be due to the inherent color of SPI (Murugesan & Orsat, 2011).

Table 2
Color analysis of spray dried American elderberry juice and elderberry pomace extract particles.

Parameters	EJ-SPI	EJ-TS	EP-SPI	EP-TS
L*	36.76 ± 0.21^{c}	32.27 ± 2.14^{d}	53.48 ± 0.31^{a}	45.99 ± 0.02^{b}
a*	33.05 ± 0.11^{b}	34.67 ± 0.91^{a}	11.35 ± 0.15^{d}	17.6 0± 0.15^{c}
b*	−14.66 ± 0.04^{d}	−6.69 ± 0.25^{c}	3.86 ± 0.06^{b}	4.59 ± 0.07^{a}
Chroma (C)	36.15 ± 0.11^{a}	35.7 0± 0.56^{a}	11.99 ± 0.16^{c}	18.19 ± 0.16^{b}
Hue angle (°)	336.09 ± 0.06^{b}	349.19 ± 0.34^{a}	18.78 ± 0.04^{c}	14.62 ± 0.09^{d}
Total color change (ΔE)	28.74 ± 0.12^{c}	21.12 ± 0.5^{d}	40.88 ± 0.40^{a}	32.30 ± 0.17^{b}

Results are shown as average ± SD. Different letters in the same row indicate statistical difference by Tukey's test (2-way ANOVA, $p < 0.05$). EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

Fig. 2. Spray dried elderberry particles from juice (A, B) and pomace extract (C, D). Legend: (A) EJ-SPI: elderberry juice with soy protein isolate; (B) EJ-TS: elderberry juice with tapioca starch; (C) EP-SPI: elderberry pomace extract with soy protein isolate; (D) EP-TS: elderberry pomace extract with tapioca starch.

3.2.8. Hygroscopicity

Hygroscopicity refers to a product's ability to absorb moisture from the environment. The hygroscopicity values of spray dried elderberry juice and pomace extract were similar ($p < 0.05$) and between 18.77 and 22.72%. However, according to GEA Niro, (2023) (15.1%–20.0% - hygroscopic; 20.1%–25% - very hygroscopic) they receive different classifications, with EP-TS being classified as hygroscopic and all other treatments classified as very hygroscopic. In general, high hygroscopicity causes particles to clump together, which can alter the nutritional value and flow characteristics of the powder and impair handling and storage (Bajac et al., 2022). Despite EP-TS particles showing lower hygroscopicity compared to the other treatments, they exhibited the least flowability among them, as indicated in Table 1. This implies that while EP-TS particles had a lower tendency to absorb moisture from the surroundings, they had a poorer ability to flow smoothly. The results in the present study are comparable to those obtained for spray dried beetroot powder with whey protein isolate (20.15%–23.18%) but lower than spray dried tamarind pulp with SPI (20%–34%) and lulo (*Solanum quitoense*) pulp with maltodextrin or gum Arabic (54%) (Igual et al., 2014; Muzaffar & Kumar, 2015).

3.2.9. Solubility

The practical application of particulate products in the food industry is also influenced by the extent of their solubility in water. Poorly soluble powders may cause difficulties in processing, formulation, and incorporation, causing economic losses (Bajac et al., 2022). Ideally, food powders would wet quickly and sink rather than float (Santhalakshmy et al., 2015). Table 1 shows that the solubility of spray dried treatments ranged from 49.40% to 77.84%. Elderberry particles with SPI (EJ-SPI, EP-SPI) showed statistically higher solubility ($p < 0.05$) than particles produced with tapioca starch (EJ-TS, EP-TS). The decreased solubility of EJ-TS and EP-TS treatments can be explained by the structure and functional properties of tapioca starch. Tapioca starch has a ratio of

approximately 1:5 between amylose and amylopectin, with highly branched amylopectin forming clusters held together by hydrogen bonds, creating a tightly packed granular structure. This hinders water penetration and starch dissolution (Mukerjea et al., 2007; Stephen & Phillips, 2006). Additionally, a study by Babic et al. (2006) showed that the solubility of tapioca starch in water was around 7% at 65 °C and it increased with temperature. In the present study, tapioca starch was homogenized with elderberry juice or pomace extract at room temperature, which may explain its lower solubility. Relative to other studies, the solubility of both SPI and tapioca starch-derived treatments in this study was superior to spray dried blueberry pomace extract with SPI and similar to tamarind pulp with SPI (Correia et al., 2017; Muzaffar & Kumar, 2015). In contrast, the solubility of elderberry extract spray dried with beta-glucan, maltodextrin, and gum Arabic (Sobieralska & Kurek, 2019) was higher (89.14%–90.18%) than the spray particles in this study (49.40%–64.38%).

3.3. Phytochemical content of spray dried elderberry particles

Processing of foods, particularly thermal processing, may result in decreased nutritional value and bioactive content. Therefore, finding processing methods that provide end products with undegraded phytochemical molecules is a primary challenge for the food industry (Zhang et al., 2020). Elderberries are a rich source of natural polyphenols and anthocyanins with relevant biological activities, potent coloring attributes, and antioxidant capacities. The elderberry juice has TPC (7623 mg GAE/L), with an expressive part of this being anthocyanins (8418 mg/L) and proanthocyanidin (238 mg/L). The elderberry pomace extract has a TPC of 9216 mg/L, and anthocyanins of 1012 mg/L, with a remarkable concentration of proanthocyanidins (562 mg/L). Both the elderberry juice and pomace extract showed high antioxidant activity (2945 and 5413 μM Trolox equiv/L) measured by the DPPH method. The elderberry pomace extract has a lower concentration of anthocyanins compared to the juice but is extremely high in proanthocyanidins with greater antioxidant activity than the juice (Hoskin et al., 2019).

The phenolic compounds present in spray dried elderberry particles come primarily from the elderberry juice or pomace extract since the TPC of both carriers (SPI and tapioca starch) was negligible (<2 mg/g). Overall, particles produced with tapioca (EJ-TS, EP-TS) showed significantly ($p < 0.05$) higher TPC (42–49 mg GAE/g sample) compared to those produced with SPI (EJ-SPI, EP-SPI) (32–39 mg GAE/g sample) (Fig. 3A). Moreover, the elderberry pomace extract particles had higher TPC than the juice particles ($p < 0.05$), indicating that the pomace, composed of skins, seeds, and pulp, is a rich source of phytochemicals and may have the potential to produce functional ingredients. TPC of spray dried elderberry juice with SPI and tapioca (EJ-SPI, EJ-TS) in this study was between 32 and 42 mg GAE/g, which is comparable to spray dried elderberry juice powders (subsp. *nigra*) obtained with different wall materials in a 1:1 ratio of total solids to wall material (Murugesan & Orsat., 2011). These wall materials include soy milk powder (46 mg GAE/g), soy protein powder (36 mg GAE/g), isolated soy protein (44 mg GAE/g), gum acacia (48 mg GAE/g), and maltodextrin (40 mg GAE/g), as reported by Murugesan & Orsat. (2011). Furthermore, the TPC content of spray dried elderberry juice particles shown here is higher than previously shown for freeze dried elderberry juice powder (12.42 mg GAE/g, Casati et al., 2019).

Table 3 shows the detected anthocyanins in liquid elderberry juice and elderberry pomace extract, as well as in the spray dried particles produced from them by UPLC (Ultra-performance liquid chromatography) analysis. Nine major peaks were identified, similar to those reported by Lee & Finn. (2007) for various American elderberry cultivars. The composition of anthocyanins in subsp. *canadensis* is more diverse than that in subsp. *nigra* because it contains both acylated and non-acylated anthocyanins. Table 3 shows that the primary pigment is cyanidin 3-(E)-p-coumaroyl-sambubioside-5-glucoside, which is an acylated anthocyanin, and the secondary pigment is cyanidin 3-sambubioside-5-glucoside, which is a non-acylated anthocyanin. These results agree well with (Lee & Finn., 2007) and with two of the three major anthocyanins detected by Johnson et al. (2015). Cyanidin-based compounds are the major anthocyanins present in subsp. *canadensis* and more than 75% of the total pigments detected were acylated anthocyanins in this study, which is consistent with the findings of Lee & Finn. (2007). In contrast, the two anthocyanins constitute the majority of subsp. *nigra* species are non-acylated cyanidin 3-glucoside and cyanidin 3-sambubioside (Osman et al., 2023), which are found only in trace amounts in subsp. *canadensis* (Table 3). The presence of acylated anthocyanins in subsp. *canadensis* gives these pigments more color stability due to intramolecular co-pigmentation and self-association (Zhou et al., 2020).

Overall, the anthocyanin profiles of spray dried juice and pomace

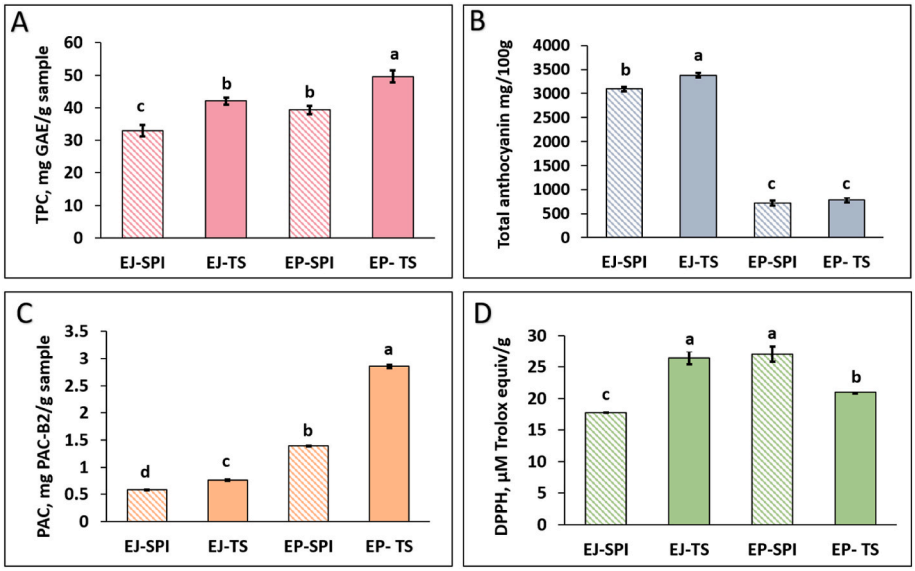


Fig. 3. (A) Total polyphenol content, (B) Total anthocyanin content, (C) Proanthocyanidin content (PAC), and (D) Antioxidant capacity for spray dried particles. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch. Bars with different letters are significantly different by Tukey's test (2-way ANOVA) test, $p < 0.05$.

Table 3
Concentration of anthocyanins identified by UPLC (Ultra Performance Liquid Chromatograph) analysis of American elderberry juice, pomace extract and their spray dried particles [a].

Anthocyanin	Elderberry juice mg/100 mL	Elderberry pomace extract mg/100 mL	EJ-SPI mg/100 g	EJ-TS mg/100 g	EP-SPI mg/100 g	EP-TS mg/100 g
cyanidin 3-sambubioside-5-glucoside	144.77 ± 40.40	16.12 ± 0.88	734.26 ± 13.64	685.92 ± 28.73	115.62 ± 18.37	112.77 ± 13.64
cyanidin 3-glucoside	ND	ND	6.07 ± 0.60	6.58 ± 0.56	ND	ND
cyanidin 3-sambubioside	0.87 ± 0.09	ND	17.41 ± 0.77	16.61 ± 0.81	ND	ND
cyanidin-based anthocyanin	3.11 ± 0.39	ND	6.70 ± 0.03	7.12 ± 0.28	ND	ND
delphinidin 3-rutinoside	11.09 ± 3.50	ND	51.69 ± 0.78	56.07 ± 2.71	10.52 ± 0.46	9.49 ± 1.09
cyanidin 3-(Z)-p-coumaroyl-sambubioside-5-glucoside	21.07 ± 1.91	3.42 ± 0.09	74.20 ± 0.72	79.65 ± 1.11	22.06 ± 2.04	24.29 ± 1.93
cyanidin 3-p-coumaroyl-glucoside	6.00 ± 1.00	0.60 ± 0.16	19.77 ± 0.19	21.23 ± 0.12	5.98 ± 0.32	6.40 ± 0.25
cyanidin 3-(E)-p-coumaroyl-sambubioside-5-glucoside	654.09 ± 33.10	81.08 ± 4.89	2181.78 ± 3.61	2500.99 ± 9.35	564.66 ± 37.46	633.14 ± 33.47
cyanidin 3-p-coumaroyl-sambubioside	ND	ND	ND	6.05 ± 0.58	ND	0.76 ± 1.32
Total anthocyanins	841.81 ± 80.29	101.22 ± 4.37	3091.88 ± 43.07	3380.22 ± 41.75	718.84 ± 57.97	786.86 ± 50.70

[a] Results are shown as mean ± SD. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch.

extract particles mirrored the anthocyanins originally present in the elderberry juice and pomace extract. For instance, cyanidin 3-(E)-p-coumaroyl-sambubioside-5-glucoside was the major anthocyanin in juice and pomace extract, and a similar trend was observed in the resulting spray dried particles (Table 3). Both factors (elderberry source and carrier type) and their interaction significantly affected the total anthocyanin content of spray dried particles (Fig. 3B). Notably, spray dried juice particles (EJ-SPI, EJ-TS) had almost four times the concentration of anthocyanins found in the counterpart pomace extract particles (EP-SPI, EP-TS), which indicates that the elderberry fruit pulp is a major source of anthocyanins. Notably, the total anthocyanins of spray dried American elderberry juice with carriers (3091–3380 mg/100g) in this study is significantly higher than the total monomeric anthocyanins in freeze dried European elderberry pulp using different carriers such as maltodextrin (1059 mg/100g), corn-based soluble fiber (1200 mg/100g), waxy maize modified starch (1251 mg/100g), k-carrageenan (1363 mg/100g) (Baeza et al., 2021). Therefore, spray drying is a viable alternative to deliver elderberry phytochemicals in a stable format with increased shelf life (Ferrari et al., 2013).

The proanthocyanidin content (PAC) of spray dried particles derived from elderberry pomace extract (EP-SPI, EP-TS) is significantly ($P < 0.05$) higher than juice particles (EJ-SPI, EJ-TS) (Fig. 3C). Similar to what was observed for TPC and anthocyanins, tapioca starch-derived particles showed higher PAC compared to SPI. Lower levels of PAC were observed for elderberries compared to blueberries, cranberries, chokeberries, and blackcurrant (Hoskin et al., 2019; Sidor & Gramza--Michałowska, 2015). Proanthocyanidins are characterized by their degree of polymerization, which has only been reported in the range of dimeric to hexameric forms in elderberries (Wu et al., 2004). Further, as the degree of polymerization increases, astringent yellow to brown compounds are formed (Krenn et al., 2007). Because of the negligible detection of oligomers and polymers of proanthocyanidin in elderberries, they have less astringent taste than chokeberries (Wu et al., 2004).

A serving size of ½ cup (73 g) of American elderberries contains approximately 434 mg of TPC and 183 mg of total anthocyanins (Perkins-Veazie et al., 2015). Thus, small amounts of spray dried juice samples (10–14 g and 5–6 g) would yield equivalent amounts of TPC and anthocyanins respectively. It is noteworthy that the elderberry pomace constituted 32% of the original weight of elderberries used in this study. Although fruit pomaces are often discarded as processing waste, the elderberry pomace proved to be a valuable source of phytochemicals as 8–11 g and 22–26 g of spray dried pomace extract samples provide the same amounts of TPC and anthocyanins as in ½ cup serving size of elderberry fruits, respectively. The higher equivalence required for anthocyanins is probably because they are less prevalent in the initial pomace material. It is possible that anthocyanins are found in lower quantities in the tissue types comprising pomace, or they might belong to a polyphenol class that is more susceptible to degradation during the pomace preparation process (Strauch & Lila, 2021).

The small size of elderberries (2.3 mm diameter; 50 mm^3 volume) and their delicate structure make it challenging to handle the fruits without causing damage. A study by Johnson et al. (2015) showed that frozen storage of American elderberry juice over 3, 6, and 9 months resulted in a significant decrease in total phenolics and anthocyanins. While fruit juice concentrates are natural FDA-approved food colorants, the limited stability and color fading of juice anthocyanins restrict their use. Nevertheless, the American elderberry juice particles obtained in this study are rich in acylated anthocyanins (Table 3), and therefore, enhanced stability against pH, light, and heat might be expected (Osman et al., 2023).

3.4. Antioxidant capacity

The radical scavenging activity of spray dried elderberry juice and pomace extract is shown in Fig. 3D. The antioxidant capacity refers to substances that delay or mitigate oxidative reactions by free radicals that cause structural or functional damage to cell structures (Lobo et al., 2010). The DPPH method is a popular and effective assay for evaluating the antioxidant properties of samples due to its simplicity, low cost, and efficacy in hydrophilic environments. This method measures the sample's ability to reduce a stable DPPH radical in the presence of an antiradical compound (Chedea & Pop, 2019).

Greater antioxidant capacity was observed for EJ-TS and EP-SPI treatments (Fig. 3D). Pearson's correlation coefficient was employed to examine the relationship between antioxidant capacity and TPC, anthocyanin, and PAC values. For spray dried elderberry juice particles, a strong and significant positive correlation was observed between DPPH and TPC, PAC, and anthocyanin content ($r = 0.974$, $p < 0.05$; $r = 0.995$, $p < 0.05$; $r = 0.986$, $p < 0.05$, respectively), which confirm that these bioactive compounds contributed to the observed free radical scavenging activity. Likewise, for spray dried elderberry pomace extract particles, DPPH showed a positive correlation with TPC and PAC ($r = 0.995$ $p < 0.05$; $r = 0.98$, $p < 0.05$ respectively, while no such correlation was observed for anthocyanin ($r = 0.73$, $p > 0.05$). This demonstrates that both TPC and PAC play an important role in the antioxidant activity exerted by particles produced with elderberry pomace extract. However, compared to the elderberry juice, the elderberry pomace extract produced in this study is not a relevant source of anthocyanins (Fig. 3B, Table 3) and therefore, this class of phenolic compounds did

not contribute to the observed DPPH results of EP-SPI or EP-TS.

3.5. Ascorbic acid content

No ascorbic acid was detected in juice, pomace extract, or spray dried elderberry particles in this study. Sobieralska & Kurek. (2019) reported ascorbic acid in both elderberries (subsp. *nigra*) extract (179 ± 3.257 mg/100g) and the spray dried elderberry powder produced with beta-glucan (9.4–21.37 mg/100g). According to Młynarczyk et al. (2018), the presence of ascorbic acid is inconsistent across studies. Depending on the cultivar and location, the ascorbic acid in subsp. *nigra* ranged from 6 to 25 mg/100g (Kaack & Austed, 1998). In that study, when European elderberry juice was purged with oxygen, the levels of selected anthocyanin and quercetin increased as the ascorbic acid concentration decreased. This suggests that oxygen can promote the interaction of ascorbic acid with flavonoids which would lead to ascorbic acid depletion in the final product. Hence, a possible reason for the negligible detection of ascorbic acid in the elderberry juice or pomace extract could be their oxidation and consequent decrease during pulping.

3.6. In vitro bioaccessibility assay

To exert pharmacological activity, bioactive substances must be effectively absorbed from the intestinal system into the bloodstream, and transported to the appropriate location in the body (Gullon et al., 2015). *In vitro* digestion models are popular research strategies to simulate and predict the *in vivo* digestion of target phytochemicals. Compared to *in vivo* models, they are more affordable, simpler, and efficient and do not require ethical committee approvals (Grace et al., 2021).

In this study, the bioaccessibility of spray dried particles was evaluated based on the percentual bioaccessibility index using the total polyphenol content as the marker phytochemical group. Bioaccessibility ranged from 12.26% (elderberry juice, liquid, non-encapsulated) to 26.86% (spray dried EJ-SPI) and important differences among elderberry groups were observed. Similarly low BI was observed for non-encapsulated elderberry juice and pomace extract ($p > 0.05$, Fig. 4). For both elderberry juice and pomace extract, the bioaccessibility was enhanced when elderberry polyphenols were complexed and encapsulated with SPI ($p < 0.05$), resulting in more than a 2-fold increase in the bioaccessibility of EJ-SPI, compared to liquid, non-encapsulated elderberry juice. Previous studies on the *in vitro* GID of elderberry (*S. nigra* subsp. *nigra* and *S. lanceolata*) polyphenols reported significant loss or degradation post-digestion process (Olejnik et al., 2016; Pinto et al., 2017).

Phenolic compounds during GID are unstable due to dietary components, pH changes, and digestive enzymes, leading to conversion, and degradation (Pinto et al., 2017). The potential of using protein-polyphenol complexation to enhance the bioavailability of dietary bioactives was demonstrated by Diaz et al. (2020). In a study by Grace et al. (2021), the bioaccessibility of polyphenols from rosemary (*Rosmarinus officinalis*) extract increased significantly after spray drying with whey protein and soy protein isolates as wall materials, compared to the uncomplexed rosemary extract. The bioaccessibility increased from 20.2% in the rosemary extract without protein carrier to 56.7% and 53.8% in rosemary-protein particles produced with whey and soy protein isolates, respectively. Protein-bound polyphenols exhibit greater bioavailability than unbound polyphenols, primarily due to the protein-polyphenol interactions occurring via hydrogen bridge binding, van der Waals, and hydrophobic interactions Lila et al. (2022). Hydrogen and hydrophobic interactions were predominant in soybean protein-polyphenol binding; SPI is mainly composed of glycinin, β-conglycinin, and lipophilic proteins (Ao et al., 2021). The hydroxyl group of flavan-3-ol polyphenol can form hydrogen bonds with protein polar groups, while non-polar aromatic rings of polyphenols bind to the non-polar surface of soy proteins through hydrophobic interactions (Lila et al., 2022).

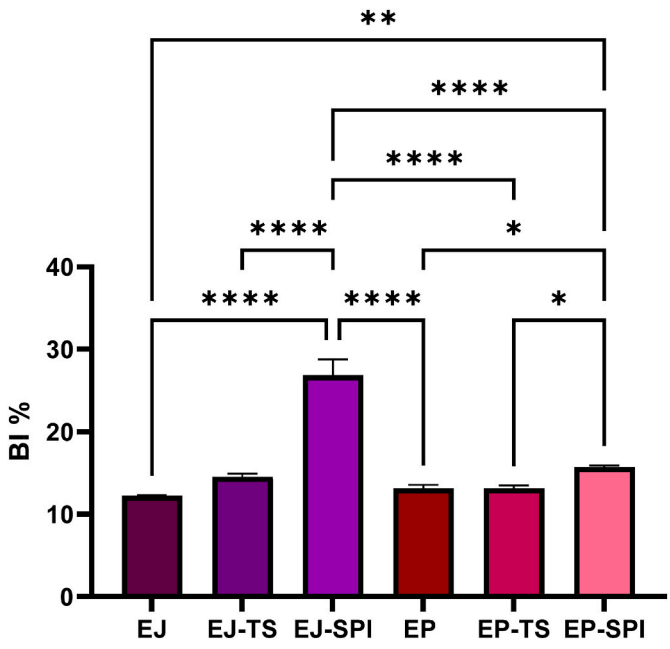


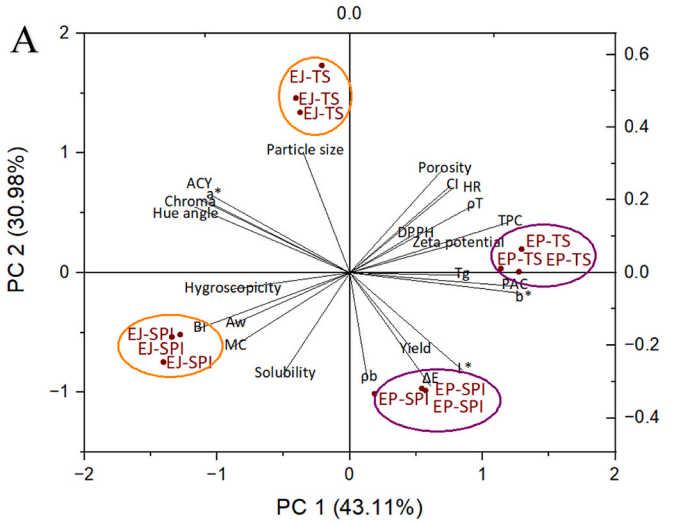
Fig. 4. Bioaccessibility index (%) for spray dried American elderberry particles after simulated *in vitro* gastrointestinal digestion. EJ: elderberry juice (non-encapsulated); EJ-TS: elderberry juice with tapioca starch; EJ-SPI: elderberry with soy protein isolate; EP: elderberry concentrated pomace extract (non-encapsulated); EP-TS: elderberry pomace extract with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate. Asterisks denote statistical differences among mean values according to Turkey's test: *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$; ****$p < 0.0001$.

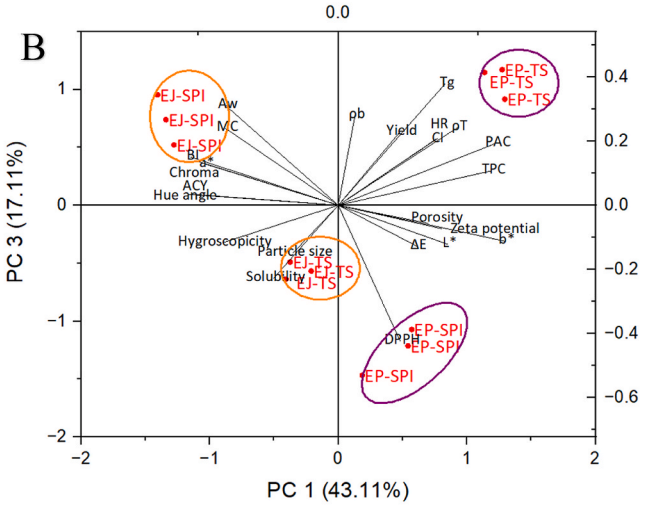
3.7. Principal component analysis (PCA)

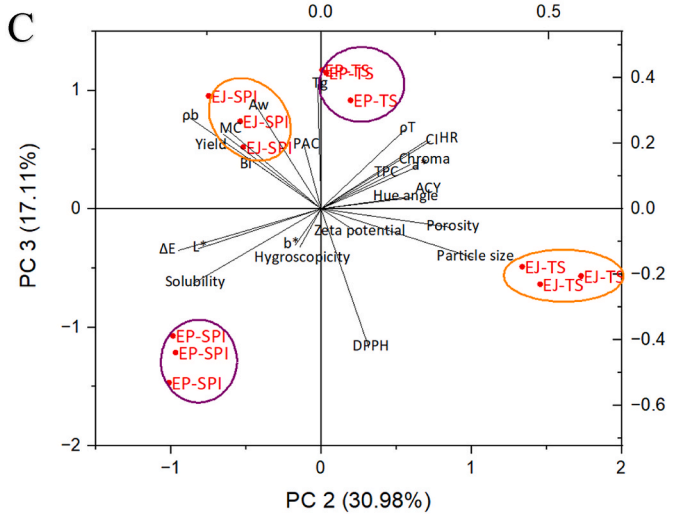
To better understand the trends and relationships among the different variables and factors studied, principal component analysis (PCA) was performed. In the PCA biplot (Fig. 5), PC1, PC2, and PC3 accounted for 43.11%, 30.98%, and 17.11% of the variance, respectively. The cumulative PC (91.20%) is high enough to explain the total variance in the data set. PC1 is mainly positively contributed by TPC, PAC, b*, and true density. On the other hand, PC1 was negatively contributed by bioaccessibility index, anthocyanins, a*, chroma, hue angle, and hygroscopicity. For PC2, particle size, porosity, Carr index, and Hausner ratio showed positive contributions while L*, bulk density, solubility, and ΔE had negative contributions. PC3 was mainly positively contributed by T_g, moisture, A_w, and yield, while it was mainly contributed negatively by DPPH. The biplot shows that particles of different treatments are clearly separated based on the PCA scores, revealing that the processing method and the addition of wall material influenced the properties of the spray dried particles. On PC1, the negative side displays spray dried juice particles, whereas the positive side shows pomace extract particles. Conversely, on PC2, particles produced with tapioca starch are located on the positive side, while those obtained with SPI are on the negative side (Fig. 5A).

4. Conclusion

Elderberry juice and pomace extract were spray dried with tapioca starch and soy protein isolate. The process proved to be efficient as satisfactory solids recovery was obtained for all treatments and phytochemical-rich and stable elderberry particles were produced. Furthermore, higher bioaccessibility was observed for spray dried







(caption on next column)

Fig. 5. A-C Two-dimensional biplot ordination on PCA of (A) PC 1 and 2; (B) PC 1 and 3; (C) PC 2 and 3 of spray dried American elderberry juice or elderberry pomace extract particles displaying the relationship among treatments and variables. Aw: water activity, MC: moisture content, T_g: glass transition temperature, b*: yellowness/blueness, a*: redness/greenness, L*: lightness/darkness, ACY: anthocyanin, BI: bioaccessibility index, CI: Carr index, HR: Hausner ratio, ΔE: total color change, DPPH: antioxidant capacity, ACY: total anthocyanins, TPC: total polyphenol content, PAC: proanthocyanidins, ρ_b: bulk density, ρ_T: true density. EJ-SPI: elderberry juice with soy protein isolate; EJ-TS: elderberry juice with tapioca starch; EP-SPI: elderberry pomace extract with soy protein isolate; EP-TS: elderberry pomace extract with tapioca starch. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

elderberry particles produced with both juice and pomace extract compared to non-encapsulated elderberry sources. Therefore, in this study, we show that spray dried elderberry particles are a convenient and versatile format to deliver polyphenols from underexplored American elderberries to be used in multiple food applications. These phytochemical-rich fruit ingredients can be efficiently prepared both from elderberry juice or pomace, creating sustainable, diversified, and profitable avenues for fruit growers and processors. The spray drying encapsulation of polyphenol-rich juice and pomace extract obtained from American elderberries is a commercially sound and industrially friendly strategy to maximize the marketability and overall value of this underutilized American crop.

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CRediT authorship contribution statement

K.S. Ravichandran: Formal analysis, Investigation, Methodology, Data curation, Writing – original draft, Writing – review & editing, Visualization. **E.S. Silva:** Investigation, Formal analysis. **M. Moncada:** Writing – review & editing. **P. Perkins-Veazie:** Methodology, Writing – review & editing. **M.A. Lila:** Writing – review & editing. **C.M. Greenlief:** Writing – review & editing. **Andrew L. Thomas:** Funding acquisition, Resources, Writing – review & editing. **R.T. Hoskin:** Conceptualization, Methodology, Writing – review & editing. **K. Krishnaswamy:** Conceptualization, Methodology, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.102981.

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