

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS

Faculdade de Engenharia de Alimentos

ADRIANA GADIOLI TARONE

**EXTRAÇÃO, BIOACESSIBILIDADE E ENCAPSULAÇÃO DE COMPOSTOS
FENÓLICOS DA CASCA DA JABUTICABA**

**EXTRACTION, BIOACCESSIBILITY AND ENCAPSULATION OF PHENOLIC
COMPOUNDS OF JABUTICABA PEEL**

Campinas – 2021

ADRIANA GADIOLI TARONE

**EXTRAÇÃO, BIOACESSIBILIDADE E ENCAPSULAÇÃO DE COMPOSTOS
FENÓLICOS DA CASCA DA JABUTICABA**

**EXTRACTION, BIOACCESSIBILITY AND ENCAPSULATION OF PHENOLIC
COMPOUNDS OF JABUTICABA PEEL**

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Alimentos e Nutrição.

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food and Nutrition.

Orientador: **Prof. Dr. Mario Roberto Marostica Junior**

ESTE EXEMPLAR CORRESPONDE À REDAÇÃO FINAL
DA TESE DEFENDIDA POR ADRIANA GADIOLI TARONE
E ORIENTADA PELO PROF. DR. MARIO ROBERTO
MAROSTICA JUNIOR.

Campinas – 2021

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

Tarone, Adriana Gadioli, 1985-
T176e Extração, bioacessibilidade e encapsulação de compostos fenólicos da casca da jabuticaba / Adriana Gadioli Tarone. – Campinas, SP : [s.n.], 2021.

Orientador: Mário Roberto Maróstica Junior.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Jabuticaba. 2. Polifenóis. 3. Ultrassom. 4. Bioacessibilidade. 5. Encapsulação. I. Maróstica Junior, Mário Roberto. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Extraction, bioaccessibility and encapsulation of phenolic compounds of jabuticaba peel

Palavras-chave em inglês:

Jabuticaba

Polyphenols

Ultrasound

Bioaccessibility

Encapsulation

Área de concentração: Nutrição Experimental e Aplicada à Tecnologia de Alimentos

Titulação: Doutora em Alimentos e Nutrição

Banca examinadora:

Mário Roberto Maróstica Junior [Orientador]

Lilian Regina Barros Mariutti

Ana Carla Kawazoe Sato

Katia Sivieri

Giovani Leone Zabot

Data de defesa: 11-03-2021

Programa de Pós-Graduação: Alimentos e Nutrição

Identificação e informações acadêmicas do(a) aluno(a)

- ORCID do autor: <https://orcid.org/0000-0002-3812-6472>

- Currículo Lattes do autor: <http://lattes.cnpq.br/7819455955062549>

COMISSÃO EXAMINADORA

Prof. Dr. Mário Roberto Maróstica Júnior – Orientador

Faculdade de Engenharia de Alimentos - UNICAMP

Profa. Dra. Lilian Regina Barros Mariutti

Faculdade de Engenharia de Alimentos - UNICAMP

Prof. Dra. Ana Carla Kawazoe Sato

Faculdade de Engenharia de Alimentos - UNICAMP

Dra. Katia Sivieri

Faculdade de Ciências Farmacêutica de Araraquara - UNESP

Prof. Dr. Giovani Leone Zabot

Universidade Federal de Santa Maria - UFSM

A Ata da Defesa assinada pelos membros da Comissão Examinadora encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

À Deus, ao meu marido pela paciência e dedicação e aos meus pais pelo apoio incondicional, sem eles eu não teria chegado tão longe...

AGRADECIMENTOS

À Deus e aos ensinamentos de Jesus, que me ajudaram a seguir firme no caminho da fé, da perseverança e da paciência, sem nunca me abandonarem! Ao pessoal do Grupo Espírita Aprendizes do Evangelho – GEAE de Barão Geraldo pelo apoio, amizade, incentivo e orações.

Ao Conselho Nacional de Pesquisa Científica e Tecnológica (CNPq) pela bolsa de estudos concedida durante o doutorado (processo nº 140942/2016-5). O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001. À Universidade Estadual de Campinas - UNICAMP pela oportunidade concedida para a realização deste doutorado. A todos os funcionários e docentes da FEA que de alguma maneira contribuíram para o meu trabalho.

Aos professores do Programa de Pós Graduação em Alimentos e Nutrição pelos ensinamentos e conhecimentos transmitidos. Ao professor Jorge Herman Behrens pelo apoio. À professora Lilian Regina Barros Mariutti pelo apoio, carinho e ajuda em muitos aspectos diferentes, essencial para minha jornada até aqui. À professora Cinthia Bau Betim Cazarin pelo carinho, amizade e pela coorientação, mesmo que informal, também essencial para minha jornada e finalização desta tese. À Dra. Soely Reis, por toda a ajuda, amizade e bons momentos passados no LANUM. A todos os técnicos e funcionários do DEPAN, em especial à Adriana e à Suzana, que, além da ajuda, tornaram os dias mais alegres! Ao professor Mario Roberto Marostica Junior pela orientação e apoio em todos os momentos, permitindo a conclusão dessa tese.

Aos meus amigos do LANUM e às minhas amigas do LAFOP (Miriam, Dani e Amanda). Obrigada por fazerem parte dessa jornada e torná-la mais leve e divertida. À turma da marmita (Marina, Mirian, Amanda, Helena e todos os que se agregavam) pelas seções de terapia em grupo cruciais para chegar ao fim do dia. Nossos cafés, almoços, piqueniques, festinhas e conversas nas bancadas eram fontes de informação, conforto, amparo, carinho e amizade, e farão muita falta! À Jú, amiga e pesquisadora do IAC, pelas conversas de incentivo e sincera amizade. Às queridas amigas Helena e Cintia por me ajudarem a manter a sanidade nos momentos mais difíceis, não sei o que faria sem vocês!

A tous les membres de l'INRAE-PACA à Avignon, pour leur accueil et leur aide, mon court séjour a été inoubliable. Merci à tous d'avoir participé à mon éternelle évolution dans les études en sciences alimentaires. Quelques remerciements particuliers: au Dr Pascale Goupi pour toutes les connaissances partagées et amitié; au Dr Beatrice pour l'amitié et l'aide scientifique et personnelle, pour les cartes, les traductions et la patience avec mes mimer; au Dr Christian Ginies pour toutes les connaissances partagées, des dessins explicatifs, et pour les cours de français; au Dr Frédéric Carlin pour le «bom dias», il m'a fait sentir le bienvenu; au Dr Marie-Jose pour m'apprendre des choses fondamentales en sciences alimentaires; au Dr Claire Dufour pour avoir ouvert les portes de son laboratoire. Merci pour le dévouement avec moi et mon travail, pour les cours, l'attention, l'affection et l'amitié, et pour les connaissances techniques, scientifiques et historiques que vous avez partagées avec moi, ils étaient très importants pour faire de moi une professionnelle et une personne meilleure! Merci beaucoup mes amis!

E por fim, obrigada a toda a minha família que me acompanhou nessa jornada! As minhas avós Shirley e Walderez pelo amor e orações. A minha tia Walderez pela preocupação e carinho. A minhas primas Marcela e Daya pelas conversas e desabafos. Aos meus pais, pelo amor incondicional, apoio e carinho. E ao meu marido, pelo amor incondicional, pela paciência, por me apoiar à distância nos 3 meses que passei na França, e por segurar a minha barra nos momentos mais difíceis (te amo)! Se não fossem vocês, não teria chegado até aqui...

A todos os que de alguma forma contribuíram para a realização deste trabalho e não foram aqui citados, os meus sinceros agradecimentos.

RESUMO

A jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) é uma fruta típica brasileira, fonte de compostos fenólicos e antocianinas, que são concentrados principalmente na sua casca. A baixa estabilidade desses compostos requer novas técnicas de extração e encapsulação para melhorar e expandir sua aplicabilidade na indústria alimentícia e farmacêutica. Esta tese teve como objetivo encontrar a melhor combinação das variáveis estudadas para melhor extração dos compostos fenólicos da farinha da casca de jabuticaba utilizando extração assistida por ultrassom de alta intensidade e investigar os efeitos dos frutooligossacarídeos (FOS) e da inulina com diferentes graus de polimerização (GP) nas propriedades de sistemas estruturados à base de pectina e seu desempenho como matrizes encapsulantes para o extrato otimizado. Os efeitos da intensidade de ultrassom (1,1, 3,7, 7,3, e 13,0 W/cm²) e da composição do solvente em relação à proporção de água/etanol (0, 25, 50, 75 e 100 g de água/100 g de solvente) foram examinados usando um planejamento experimental fatorial completo (4 × 5), e a máxima recuperação de compostos bioativos ocorreu na intensidade de ultrassom de 3,7 W/cm² e 50 g de água/100 g de solvente, comprovando que a interação entre os parâmetros estudados tem forte influência na eficiência de extração dos grupos de compostos estudados e no seu potencial antioxidante. A identificação e quantificação dos compostos fenólicos presentes no extrato otimizado foram feitas por análise de UPLC/DAD/ESI-MSⁿ e 58 compostos fenólicos foram identificados por tentativa. A atividade antioxidante contra a oxidação lipídica e a bioacessibilidade das antocianinas presentes no extrato foram avaliadas *in vitro* através de um modelo de digestão gastrointestinal considerando uma dieta do tipo ocidental. Ambas as concentrações utilizadas do extrato (1 e 5 mg/mL) foram capazes de inibir a oxidação lipídica, inibindo amplamente a formação dos marcadores de oxidação lipídica analisados (dienos conjugados e 4-hidroxi-2-nonenal). Os ensaios também apresentaram alta bioacessibilidade das antocianinas, principalmente quando a maior concentração foi avaliada, no entanto não foi detectada delfinidina-3-O-glucosídeo bioacessível na menor concentração. A combinação de pectina e inulina com diferentes GPs mostrou-se um promissor sistema de encapsulação dos polifenóis da casca de jabuticaba com potencial uso como ingrediente funcional e estruturante de alimentos. O sistema encapsulante à base de pectina e o sistema encapsulante à base de pectina com adição de inulina com GP ≥ 23 foram os mais eficientes na proteção

do extrato, exibindo os melhores resultados para a estabilidade da capacidade antioxidante.

Palavras-chave: jabuticaba; polifenóis; ultrassom; bioacessibilidade, encapsulação.

ABSTRACT

Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) is a typical Brazilian fruit, source of phenolic compounds and anthocyanins mainly concentrated in the peel. The low stability of these compounds requires new extraction and encapsulation techniques to improve and expand its applicability in the food and pharmaceutical industry. This thesis aimed to find the best combination of the studied variables for better extraction of phenolic compounds from jabuticaba peel powder using high-intensity ultrasound-assisted extraction and investigate the effects of fructooligosaccharides (FOS) and inulin with different degrees of polymerization (DP) on the properties of pectin-based structured systems and their performance as encapsulating matrices for the optimized extract. The effects of ultrasound intensity (1.1, 3.7, 7.3, and 13.0 W/cm²) and solvent composition concerning water/ethanol ratio (0, 25, 50, 75, and 100 g water/100 g) were examined using a full factorial experimental design (4 × 5), and the maximum recovery of bioactive compounds was at an ultrasound intensity of 3.7 W/cm² and 50 g water/100 g, demonstrating that the interaction between the parameters studied has a strong influence on the extraction efficiency of the groups of compounds studied and on their antioxidant potential. The identification and quantification of the phenolic compounds present in the optimized extract were made by UPLC/DAD/ESI-MSⁿ analysis, and 58 phenolic compounds were tentatively identified. The antioxidant capacity against lipid oxidation and the bioaccessibility of the anthocyanins present in the extract were evaluated *in vitro* by a gastrointestinal digestion model concerning a Western-type diet. Both extract concentrations used (1 and 5 mg/mL) were able to inhibit lipid oxidation, largely decreasing the formation of the analyzed lipid oxidation markers (conjugated dienes and 4-hydroxy-2-nonenal). The assays also showed high bioaccessibility of anthocyanins, especially when the highest concentration was evaluated, however, bioaccessible delphinidin-3-O-glucoside was not detected at the lowest concentration. The combination of pectin and inulin with different DP demonstrated to be a promising encapsulating system for jabuticaba peel polyphenols with potential use as functional and structural food ingredient. The pectin-based encapsulating system and the pectin-based encapsulating system with the addition of inulin with DP ≥ 23 were the most efficient in protecting the jabuticaba peel extract, exhibiting the best results for the stability of the antioxidant capacity.

Keywords: jabuticaba; polyphenols; ultrasound, bioaccessibility; encapsulation.

Sumário

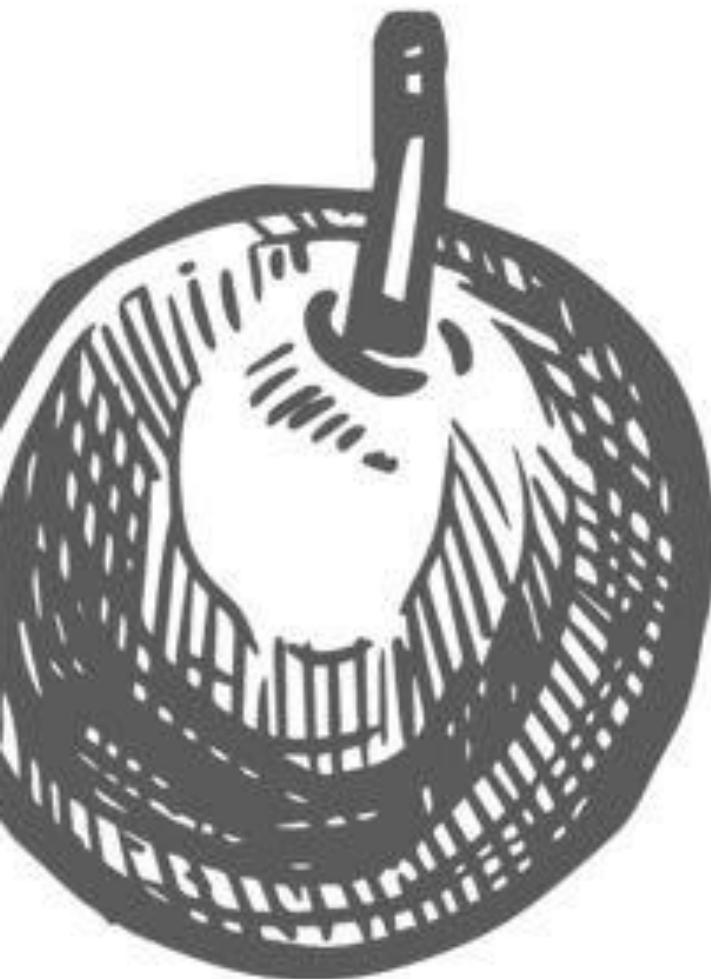
CAPÍTULO I	16
1. Introdução Geral	17
2. Objetivos	20
2.1. Objetivo Geral	20
2.2. Objetivos Específicos	20
3. Estruturação da Tese	21
CAPÍTULO II	23
Anthocyanins: new techniques and challenges in microencapsulation.	24
Highlights.....	24
Graphical Abstract.....	24
Abstract	25
1. Introduction	26
2. What are anthocyanins?.....	27
2.1. How are the anthocyanins absorbed?	29
2.2. Why is it challenging to use anthocyanins?	32
3. Encapsulated delivery systems	34
4. Colloidal carrier systems	36
4.1. Top-down	40
4.1.1. Liposomes	40
4.1.2. Molecular Inclusion	42
4.1.3. Gastroretentive systems	43
4.1.4. Emulsions	43
4.1.5. Complexes and coacervation (biopolymer particles)	45
4.2. Bottom-up: Specialized manufacturing and storage techniques.....	46
4.2.1. Freeze-drying.....	47
4.2.2. Spray-drying	47
4.2.3. Electrospinning and electrospraying techniques.....	49
4.2.4. Supercritical fluid technology	50
4.3. Both.....	52
5. Conclusions.....	53
Declaration of Competing Interest	54
Acknowledgments	54

References	54
CAPÍTULO III	70
High-intensity ultrasound-assisted recovery of anthocyanins from jabuticaba by-products using green solvents: effects of ultrasound intensity and solvent composition on the extraction of phenolic compounds	71
Highlights.....	71
Graphical Abstract.....	71
Abstract	72
1. Introduction	73
2. Material and methods.....	75
2.1. Material	75
2.2. Jabuticaba peel processing	75
2.3. Dried jabuticaba peel extractive optimization by high-intensity ultrasound-assisted extraction	76
2.4. Bioactive compounds content and antioxidant capacity characterization	76
2.5. Confocal laser scanning microscopy (CLSM).....	77
2.6. Color.....	78
2.7. Experimental design and statistical analyses	78
3. Results and Discussion.....	79
3.1. Dried jabuticaba peel extraction by HIUS.....	79
3.2. Temperature.....	85
3.3. Confocal laser scanning microscopy (CLSM).....	86
3.4. Color.....	89
4. Conclusion	94
CRediT authorship contribution statement	95
Declaration of Competing Interest	95
Acknowledgement	96
Supplementary material	97
References	98
CAPÍTULO IV	107
Antioxidant capacity and bioaccessibility in <i>in vitro</i> gastrointestinal digestion of phenolic compounds extracted from jabuticaba peel	108
Abstract	108
1. Introduction	109

2. Materials and Methods	110
2.1. Materials.....	110
2.2. Jabuticaba peel processing.....	110
2.3. Dried jabuticaba peel extraction by high-intensity ultrasound (HIUS).....	111
2.4. Dried jabuticaba peel extraction by a conventional exhaustive method (CM)	
111	
2.5. Hydrolysis of phenolic compounds	112
2.6. Qualitative and quantitative analyses of phenolic compounds and anthocyanins.....	112
2.6.1. Identification of phenolic compounds and anthocyanins by UPLC/DAD/ESI-MS ⁿ	112
2.6.2. Quantification of phenolic compounds and anthocyanins	113
2.7. Simulated static <i>in vitro</i> gastrointestinal digestion	114
2.7.1. Preparation of the solutions used during digestion	114
2.7.2. Preparation of oil-in-water-emulsion	115
2.7.3. <i>In vitro</i> gastrointestinal digestion.....	115
2.7.4. Determination of lipid oxidation products	115
2.7.5. Determination of anthocyanin bioaccessibility	117
2.8. Statistical analysis	117
3. Results and discussion.....	118
3.1. Profile and content of phenolic compounds and anthocyanins of dried jabuticaba peel by UPLC/DAD/ESI-MS ⁿ analyses	118
3.1.1. Hydrolysable tannins	118
3.1.2. Anthocyanins	121
3.1.3. Flavonols	121
3.1.4. Others.....	122
3.1.5. Quantification.....	122
3.1.6. Hydrolysis	128
3.2. Inhibition of lipid oxidation by DJPE-HIUS during static <i>in vitro</i> gastrointestinal digestion	133
3.3. Bioaccessibility of anthocyanins	135
4. Conclusion	137
Acknowledgements	138
Supplementary Material	139

References	143
CAPÍTULO V	148
Inulin/fructooligosaccharides/pectin-based structured systems: Promising encapsulating matrices of polyphenols recovered from jabuticaba peel.....	149
Highlights.....	149
Graphical Abstract.....	149
Abstract	150
1. Introduction	151
2. Materials and Methods	153
2.1. Materials.....	153
2.2. Jabuticaba peel processing	153
2.3. High-intensity ultrasound-assisted extraction of polyphenols from dried jabuticaba peel.....	154
2.4. Production of jabuticaba peel extract-rich encapsulating systems using pectin and fructans	154
2.5. Rheology measurements	155
2.5.1. Flow properties	155
2.5.2. Viscoelastic properties.....	155
2.6. Scanning electron microscopy (SEM)	156
2.7. Color.....	156
2.8. Chemical and bioactivity characterization	156
2.8.1. Total reduced capacity.....	157
2.8.2. Antioxidant capacity	157
2.9. Antioxidant capacity and color stability.....	157
2.9.1. Color stability	158
2.9.2. Antioxidant capacity stability	158
2.10. Encapsulation efficiency.....	158
2.11. Statistical analysis	158
3. Results and Discussion	159
3.1. Rheological behavior and microstructure	159
3.2. Color parameters	164
3.3. Bioactivity characterization and encapsulation efficiency	164
3.4. Antioxidant capacity and color stability	166
3.4.1. Antioxidant capacity stability	167

3.4.2. Color stability	168
4. Conclusions.....	169
CRediT authorship contribution statement	169
Declaration of competing interest.....	169
Acknowledgements	170
References	170
CAPÍTULO VI - Discussão Geral.....	177
CAPÍTULO VII - Conclusão Geral	187
REFERÊNCIAS.....	190
ANEXOS	197
Anexo 1 - Cadastro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado.	198
Anexo 2 - Permissão para reprodução dos artigos.	199



CAPÍTULO I

Introdução Geral, Objetivos e Estruturação da Tese

1. Introdução Geral

A Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) é uma fruta tipicamente brasileira pertencente à família Myrtaceae. Nativa da Mata Atlântica, é cultivada em uma extensa faixa do país, mas é nas áreas do sul e sudeste que está mais amplamente distribuída e cultivada. A fruta cresce no tronco de uma árvore grande e espessa e uma única árvore é capaz de produzir centenas de frutas que apresentam uma polpa branca mucilaginosa e doce coberta com uma casca roxa, quase preta, com características adstringentes e que podem conter até quatro sementes em seu interior (DONADIO, 2000). Em função de suas características sensoriais, apresenta grande potencial de comercialização pois é muito apreciada tanto para o consumo *in natura* como para a fabricação de geleia, xaropes, vinho, bebidas fermentadas, vinagre e licores. Além disso, os frutos também podem ser aproveitados pelas indústrias farmacêutica e nutracêutica devido a seu alto teor de compostos bioativos (NERI-NUMA et al., 2018).

A cadeia de processamento da jabuticaba gera uma grande quantidade de subprodutos que, na maioria das vezes, são desperdiçados, podendo representar até 50% do peso do fruto entre casca e sementes (MORALES et al., 2016). Como estudos recentes têm demonstrado que a casca das espécies de jabuticaba são uma fonte rica de compostos fenólicos (tais como antocianinas, flavonoides, taninos, ácido elágico, ácido gálico, queracetina e outros) (ALBUQUERQUE et al., 2020; NEVES et al., 2018; PLAZA et al., 2016; QUATRIN et al., 2019), as indústrias vem estudando alternativas para utilizar esse subproduto a fim de reduzir o impacto ambiental causado pela industrialização do fruto (DE ALMEIDA et al., 2015), assim como agregar valor nutritivo e comercial ao seu produto final (DA SILVA et al., 2017).

Compostos fenólicos são metabólitos secundários amplamente distribuídos em frutas e vegetais que fazem parte da dieta humana (DEL RIO et al., 2013). Eles são divididos em classes, dependendo da sua estrutura, e subcategorizados dentro de cada classe de acordo com o número e posição dos grupos hidroxila e a presença de outros substituintes tendo, pelo menos, um anel aromático com um ou mais grupos hidroxila ligados. São classificados como flavonoides e não-flavonoides, sendo a classe dos flavonoides a mais importante em termos nutricionais (CROZIER et al., 2006). As antocianinas, um subgrupo de flavonoides solúveis em água, podem ser relacionadas com as atividades antioxidante, antimicrobiana, antiproliferativa, anti-

inflamatória (ALBUQUERQUE et al., 2020) e antimutagênicas (LEITE-LEGATTI et al., 2012) exibidas pela casca da jabuticaba, além de outros benefícios à saúde (BATISTA et al., 2014; DA SILVA et al., 2017; DE SÁ et al., 2014; LENQUISTE et al., 2015; PLAZA; KARIUKI; TURNER, 2014).

Devido às suas cores atraentes e às suas propriedades químicas, as antocianinas são frequentemente utilizadas como aditivo alimentar (E 163), principalmente pelo interesse crescente das indústrias por novas fontes de pigmentos e aditivos naturais para substituir os sintéticos (TARONE; CAZARIN; MAROSTICA JUNIOR, 2020). No entanto, o seu uso é prejudicado pela instabilidade dessas moléculas à condições adversas de processamento e armazenamento (DE MOURA et al., 2018). A estabilidade da cor e das propriedades das antocianinas é afetada pelo pH, solvente, temperatura, concentração e estrutura, oxigênio, luz, enzimas e presença de outros componentes coexistentes (como copigmentos, íons metálicos e outros), e esses fatores podem limitar a sua utilização (SANTOS-BUELGA; GONZÁLEZ-PARAMÁS, 2018). Por esse motivo, torna-se necessária a busca por novas tecnologias de extração que propiciem a mínima degradação dos compostos extraídos e novos meios de encapsulá-los para sua maior proteção e estabilidade.

A extração assistida por ultrassom de alta intensidade é uma técnica não convencional emergente rápida e simples amplamente utilizada para a extração de compostos fenólicos (AL-DHABI; PONMURUGAN; MARAN JEGANATHAN, 2017) e que vem se mostrando muito eficiente na extração de antocianinas (PINELA et al., 2019; WANG et al., 2016). O mecanismo de sonicação é atribuído à cavitação na proliferação de ondas acústicas, onde efeitos físicos, químicos e mecânicos são gerados a partir da formação e subsequente rompimento de microbolhas resultando no colapso da matriz do material e no aumento da taxa de recuperação de compostos fenólicos da matriz para o solvente (CHEMAT et al., 2017). Considerada uma tecnologia verde e limpa, permite a redução no tempo de extração, melhoria da seletividade, rendimento mais elevado dos extratos e manipulação simplificada, além de apresentar baixo custo e redução no consumo de solvente (BAGHDIKIAN et al., 2016; CHEMAT et al., 2017).

A encapsulação é uma tecnologia promissora para estabilizar extratos de compostos fenólicos (BETZ; KULOZIK, 2011). É um processo pelo qual as partículas do material a ser protegido são envolvidas em um material de revestimento gerando i) micro/nano-cápsulas quando o material a ser protegido é recoberto com uma

película fina do material de revestimento ou ii) matrizes encapsulantes quando o material a ser protegido está disperso no material de revestimento. A substância encapsulada a ser protegida é referida como o material de 'recheio', enquanto o material de revestimento protetor é chamado de material de 'parede' (ANAL; SINGH, 2007). O uso de sistemas à base de hidrocoloides como matriz encapsulante de compostos fenólicos e antocianinas vem ganhando espaço na literatura recente (GUO; GIUSTI; KALETUNÇ, 2018; JAKOBÉK; MATIĆ, 2019; TSAI; KITAMURA; KOKAWA, 2017), e alguns estudos demonstraram que as pectinas (BUCHWEITZ et al., 2013; CHUNG et al., 2015; MAIER et al., 2009), FOS e inulinas (ARAUJO-DÍAZ et al., 2017; BERNARDES et al., 2019; LACERDA et al., 2016) quando usadas como materiais de parede nesses sistemas são eficientes para estabilizar esses compostos, podendo retardar sua degradação e melhorar outras propriedades físico-químicas.

Considerando que a casca da jabuticaba é um subproduto pouco utilizado da indústria e rica em compostos fenólicos (principalmente antocianinas) que possuem propriedades antioxidantes benéficas à saúde, mas que são muito instáveis ao processamento, armazenamento e digestão, este trabalho buscou alternativas emergentes para extrair, verificar a bioacessibilidade na digestão *in vitro* e encapsular esses compostos a fim de tornar mais viável sua utilização como aditivo alimentar em indústrias de alimentos e nutracêuticas.

2. Objetivos

2.1. Objetivo Geral

Obter extrato rico em compostos fenólicos (principalmente antocianinas) da farinha da casca de jabuticabas, determinar a bioacessibilidade das antocianinas presentes no extrato e encapsulá-lo.

2.2. Objetivos Específicos

- Estabelecer a melhor combinação das variáveis estudadas para melhor extração dos compostos fenólicos da farinha da casca de jabuticaba utilizando o método de extração assistida por ultrassom de alta intensidade através de um planejamento experimental com base em propriedades físico-químicas dos extratos e na microscopia confocal de varredura a laser da farinha da casca de jabuticabas antes e após o processo de extração;
- Caracterizar e quantificar os compostos fenólicos presentes no extrato da farinha da casca de jabuticabas obtido a partir da melhor condição de extração encontrada, bem como avaliar sua bioacessibilidade *in vitro* e capacidade antioxidante na digestão gastrointestinal *in vitro*;
- Avaliar os efeitos de FOS e inulinas com diferentes graus de polimerização nas propriedades reológicas, físicas e microestruturais de sistemas estruturados à base de pectina e seu desempenho como sistemas encapsulantes de polifenóis extraídos da farinha da casca de jabuticabas.

3. Estruturação da Tese

A pesquisa que deu origem à essa tese foi desenvolvida em etapas que estão aqui apresentadas na forma de capítulos. O capítulo I é composto por uma breve introdução geral para contextualizar o leitor a respeito dos principais tópicos deste estudo, os seus objetivos e a sua estruturação. O capítulo II apresenta um artigo de revisão cujo objetivo foi fornecer uma visão geral da estrutura, absorção e proteção das antocianinas e mostrar os principais métodos convencionais e emergentes de encapsulação utilizados para protegê-las e seus prós e contras. O capítulo III corresponde a um artigo original que apresenta os resultados referentes à obtenção e otimização de um extrato rico em compostos fenólicos e antocianinas obtido a partir da farinha da casca de jabuticabas utilizando o método de extração assistida por ultrassom de alta intensidade. O capítulo IV corresponde a um artigo original que caracteriza e quantifica os compostos fenólicos presentes no extrato otimizado da farinha da casca de jabuticabas, obtido no capítulo anterior, e avalia sua bioacessibilidade e atividade antioxidante na digestão gastrointestinal *in vitro*. O capítulo V corresponde a um artigo original que avalia os efeitos de FOS e inulinas com diferentes graus de polimerização nas propriedades reológicas, físicas e microestruturais de sistemas estruturados à base de pectina e seu desempenho como sistemas encapsulantes do extrato otimizado da farinha da casca de jabuticabas. O capítulo VI apresenta uma discussão geral de todos os resultados experimentais apresentados, destacando os dados mais relevantes. E por fim, o capítulo VII apresenta as conclusões gerais de toda a pesquisa de uma forma sucinta. A **Figura 1** apresenta o fluxograma das principais etapas da tese, as análises desenvolvidas e os principais resultados obtidos em cada uma delas.

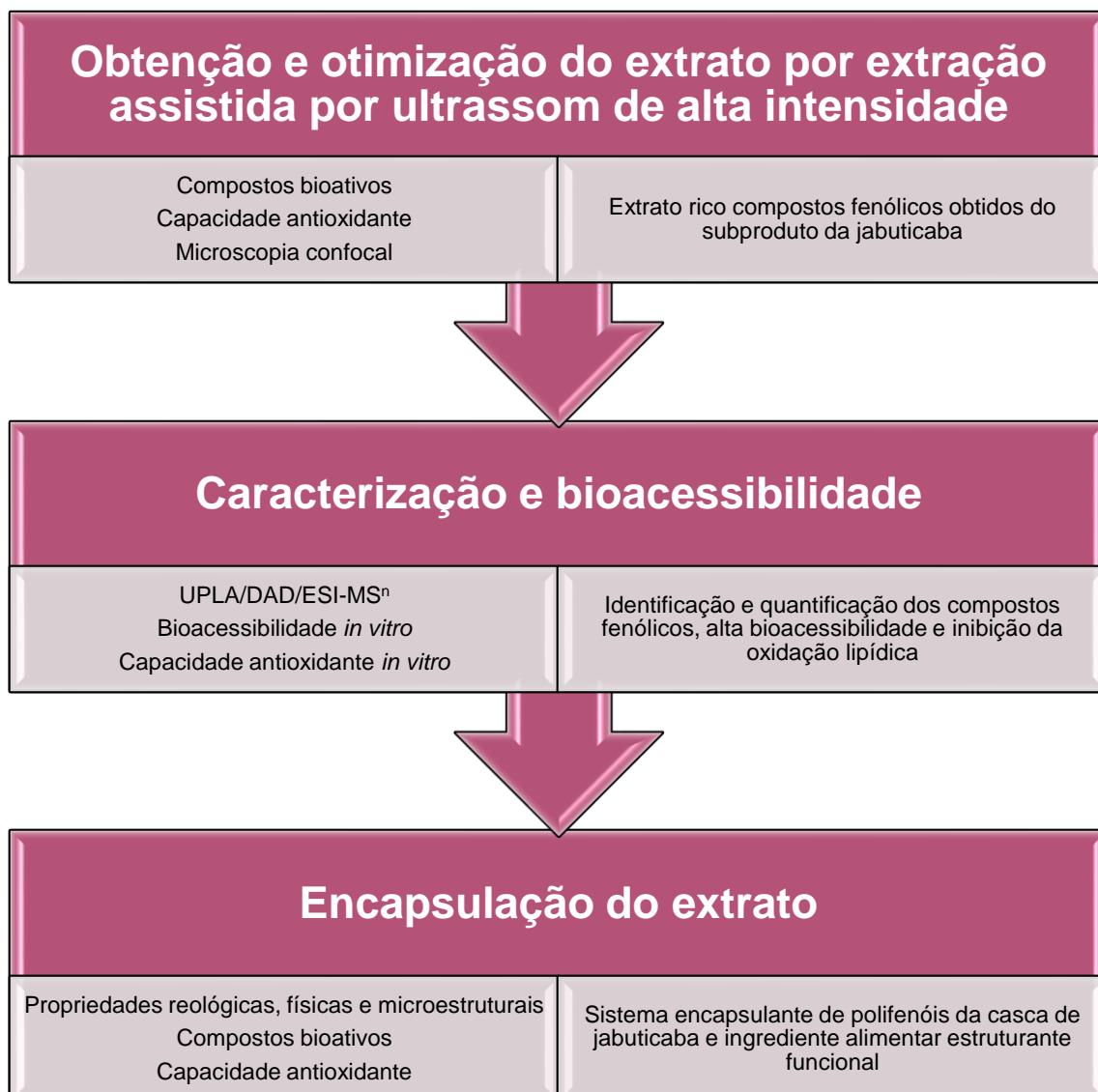


Figura 1. Fluxograma das principais etapas da tese, análises desenvolvidas e principais resultados obtidos.



CAPÍTULO II

Anthocyanins: new techniques and challenges in microencapsulation

Published in Food Research International, v.133, July 2020, 109092

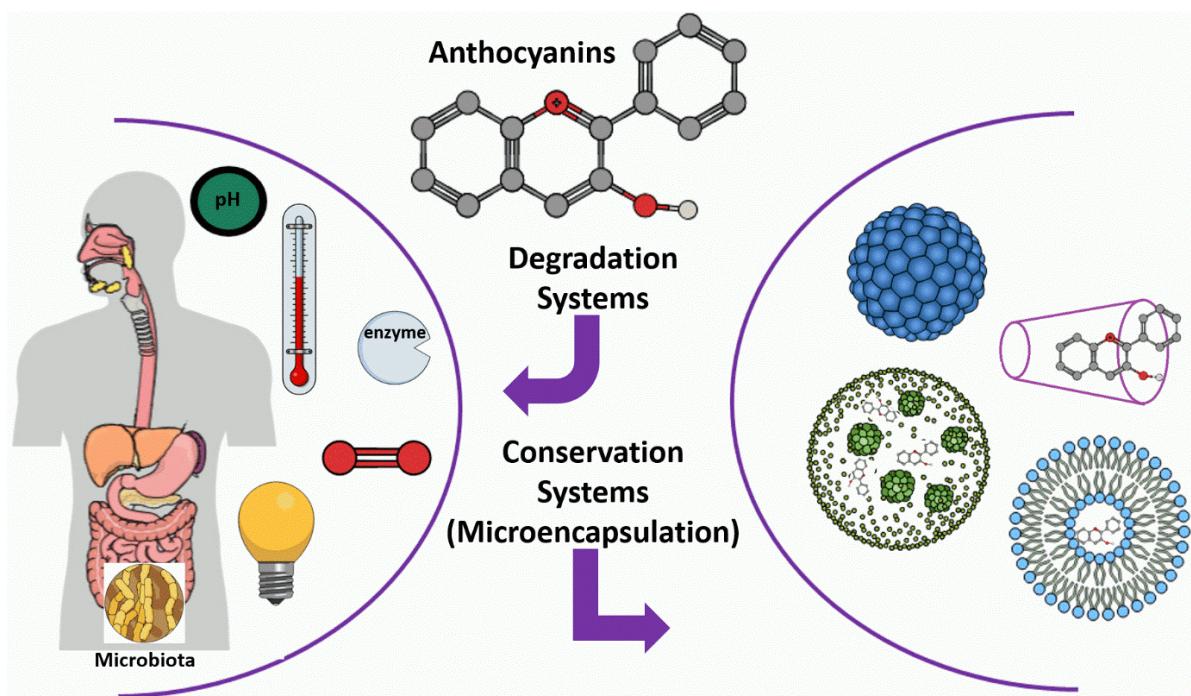
Anthocyanins: new techniques and challenges in microencapsulation.

Adriana Gadioli Tarone; Cinthia Baú Betim Cazarin; Mario Roberto Marostica Junior
School of Food Engineering, University of Campinas – UNICAMP, 13083-862 Campinas, SP, Brazil

Highlights

- Anthocyanins are bioactive compounds, and they have antioxidant properties.
- Anthocyanins are not stable in some environment condition.
- Microencapsulation processes can provide stability for anthocyanins.
- Anthocyanin microencapsulation processes are a challenge for the food industry.

Graphical Abstract



Abstract

Anthocyanins are a bioactive compound belonging to the flavonoid class that is present in human nutrition through plant-based foods. Due to their antioxidant properties, several health benefits related to their consumption are reported in the literature. The stability of the color and the properties of anthocyanins is strongly affected by pH, solvent, temperature, and other environmental conditions. In addition, the insufficient residence time of anthocyanins in the upper digestive tract causes a partial absorption, which needs to be improved. These facts have led researchers to investigate new forms of processing that provide minimal degradation. Microencapsulation is a promising possibility to stabilize anthocyanin extracts and allow their addition to food products in a more stable form. The microcapsules can still provide a prolonged gastrointestinal retention time caused by the improvement of the bioadhesive properties in the mucus covering the intestinal epithelium. Although there are efficient and emerging techniques, anthocyanins microencapsulation is still a challenge for the food industry. The purpose of this work is to provide an overview of anthocyanins structure, absorption and protection, and to show the main conventional and emerging microencapsulation methods and their pros and cons.

Keywords: anthocyanins; antioxidant properties; bioaccessibility; bioavailability; microencapsulation; top-down; bottom-up.

1. Introduction

Anthocyanins are part of the flavonoid family and they are regarded as a bioactive compound mostly due to their antioxidant properties. They are a large group of water-soluble phytopigments and possess a wide diversity of colors like pink, red, orange, purple, and blue hues, depending on the environmental pH. They can be found in most flowers and fruits, but are also present in all types of vascular plants and on any plant tissue, being widely distributed in the human diet through plant-based foods (Santos-Buelga & González-Paramás, 2018). The anthocyanins are synthesized as secondary metabolites and their natural edible sources include colored fruits such as all types of red and black berries, as well as many dark-colored vegetables, such as red onion, red radish, black bean, eggplant, purple corn, red cabbage, purple sweet potato, and others (He & Giusti, 2010).

During the last decades, an increasing number of studies have investigated the diverse protective and promoter effects in human health elicited by anthocyanins present in several fruits and vegetables (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Ghosh & Konishi, 2007; Tsuda, 2012). Plant-based foods rich in anthocyanins have a pharmacological relevance and therapeutic application due to their antioxidant properties, as the literature reports many health benefits related to anthocyanins consumption (Carvalho et al., 2016). Besides, recent studies have observed the relationship between dietary intake of anthocyanins, the protection against neurological diseases, and control of decline in brain and cognitive functions related to age (Subash et al., 2014), reduction of heart diseases risk; enhancement of vision and brain functions, and they exert an anti-inflammatory role related to chronic diseases, like obesity and diabetes by modulation of the microbiota, being that anti-inflammatory agents also can act as anticancer agents (Morais, de Rosso, Estadella, & Pisani, 2016; Riaz, Zia-Ul-Haq, & Saad, 2016; Tsuda, 2012). The food industry has also investigated the anthocyanins potential to act as natural dyes, since the concerns about potential adverse effects of synthetic dyes has increased in society (Lao & Giusti, 2018). They represent an important suitable alternative to these synthetic dyes and have been recognized as food colorants by several countries, with the code E-163.

These recent studies have renewed the interest in anthocyanins and their potential benefits in human health. Besides, novel applications of anthocyanin

pigments as colorants, or supposed bioactives, have arisen to be exploited by the food, pharmaceutical, and cosmetic industries (Santos-Buelga & González-Paramás, 2018). Based on evidence in the literature, anthocyanins intake is hugely interesting; however, some food processing conditions, as well as their chemical instability, have been proven as laborious to improve their bioavailability (de Moura, Berling, Germer, Alvim, & Hubinger, 2018). In this sense, alternatives such as encapsulation, allow the minimization of nutritional and sensory losses, and increase the half-life of the active substances (Bicudo, Ribani, & Beta, 2014). Therefore, the purpose of this review is to discuss traditional and emerging encapsulation methods used to protect the anthocyanins against damaging conditions (environmental, gastrointestinal, etc.), and increase their stability and bioavailability.

2. What are anthocyanins?

Chemically, anthocyanins can be defined as heterosides of an aglycone unit (anthocyanidins) linked to glycosides. They are found in nature as glycosylated polyhydroxy and/or polymethoxy derivatives from the flavylium ion or 2- phenyl benzopyrylium, for which the molecules can have their weights range from 400 to 1200 (medium-size biomolecules). Their structures are composed of two aromatic rings (A and B) linked by three carbons in an oxygenated heterocycle (C), namely a chromane ring with a second aromatic ring (B) attached in position 2 (Hosseinian, Li, & Beta, 2008; Prior & Wu, 2006), as shown in **Fig. 1**. According to these authors, this chromane ring is associated with the aromatic properties of the anthocyanins.

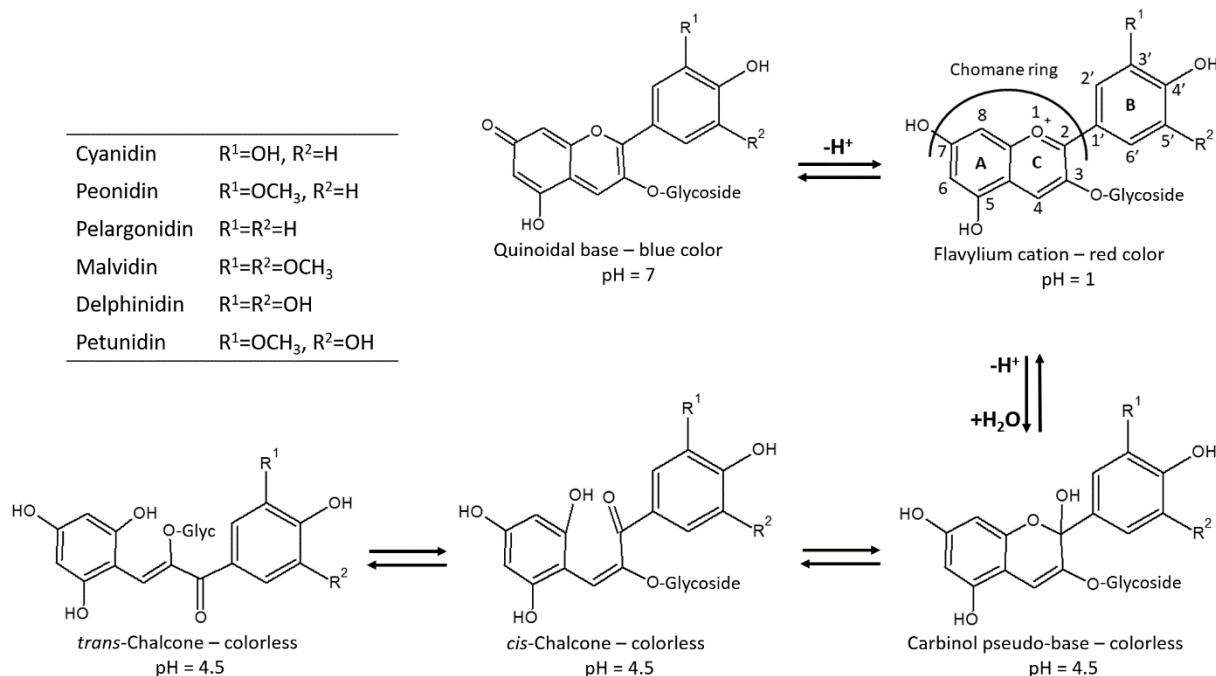


Fig 1. Anthocyanin skeleton showing the aromatic rings (A and B) and the oxygenated heterocycle (C). Replacing the radicals R¹ and R² with those of the table, it is possible to see the six most common different types of anthocyanins (Based on Castañeda-Ovando et al., 2009; Hosseinian et al., 2008).

Anthocyanidins are found linked to one or various sugars, which can be acylated with different organic acids. The presence of hydroxyl groups and sugar(s) on the rings are responsible for the solubility of these compounds in water, ethanol, and in minor part, in methanol (Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004). There are approximately 30 different anthocyanidins identified in nature to which glycosides can be attached, and they differ in the degree and patterns of hydroxylation and methoxylation on the different positions of their rings (Andersen & Jordheim, 2006). More than 700 anthocyanins from natural sources have had their complete structures described, and more than 200 have been tentatively identified, with this number increasing continually (Santos-Buelga & González-Paramás, 2018). These many different groups of anthocyanins vary in the number, position, and type of sugar attached, the number and nature of aromatic or aliphatic acids attached to the sugar, and the number of hydroxyl groups (Kong, Chia, Goh, Chia, & Brouillard, 2003).

Despite this large number of anthocyanins, only six types of them are commonly found in nature. The most common and the most important aglycones

anthocyanidins for human nutrition are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin, whose structures can vary in substitution at positions 3' and 5' (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009), as shown in **Fig. 1**. These forms of anthocyanins conjugate with sugars and organic acids to generate a variety of anthocyanins of differing colors. Their colors can vary from the blue end of the UV/Visible spectrum, when the B ring possesses more hydroxyl groups, to the red end of the UV/Visible spectrum when the B ring possesses more methoxyl groups (He & Giusti, 2010). The anthocyanins' chemical properties are influenced by their chemical structures, which determine some physicochemical properties like stability, color, aqueous equilibrium, and the effect of the combination of anthocyanins and copigments (Bueno et al., 2012; Prior & Wu, 2006).

2.1. How are the anthocyanins absorbed?

Although researchers have shown that anthocyanins have their major site of absorption in the small intestine, some authors have been suggesting their initial absorption starts in the upper gastrointestinal tract through a relative contribution of the stomach in the process of absorption (Celli, Brooks, & Ghanem, 2016; Felgines et al., 2007; Passamonti, Vrhovsek, Vanzo, & Mattivi, 2003; Talavéra et al., 2005) and, if their residence time in these organs is not enough, the undigested portion of anthocyanins will probably be degraded in the intestine due to its high pH (Celli, Brooks, et al., 2016). Despite the beneficial properties of anthocyanins, their effectiveness at preventing or treating a range of diseases depends on their bioaccessibility and bioavailability. These are the major issues regarding their biological effects and remain unclear due to the lack of data available on this matter (Faria et al., 2009). Bioaccessibility is defined as the fraction of a compound that is released from the food matrix and that is available for intestinal absorption (Chang, Sciences, & Jalil, 2018). The bioavailability of a compound is related to its digestive stability, food matrix release, and efficiency of its transepithelial passage (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010), and its definition is the amount of a nutrient that is digested, absorbed and metabolized through normal pathways.

Not just physicochemical properties are influenced and determined by the chemical structures of the anthocyanins, but they also affect their biological properties.

The presence, number, and type of attached groups (hydroxyl groups, sugar moieties, and acylated groups) influence the polarity, size and spatial conformations of anthocyanin, impacting their bioaccessibility and bioavailability (Bueno et al., 2012). However, the main issue is the unfavorable environment present in the gastrointestinal tract, e.g. the wide range and fluctuation in pH, the presence of various enzymes and mucosal barriers, etc., that makes anthocyanins show even lower bioaccessibility, membrane permeability, and bioavailability. So, the biological effects of anthocyanins depend on their bioavailability, which is dependent on its digestive stability, release from the matrix (bioaccessibility), degradation in the oral phase, transepithelial passage efficiency (absorption), distribution in the plasma, and delivery to target tissues. **Fig. 2** shows further details about the absorption of anthocyanins in the gastrointestinal tract and its obstacles.

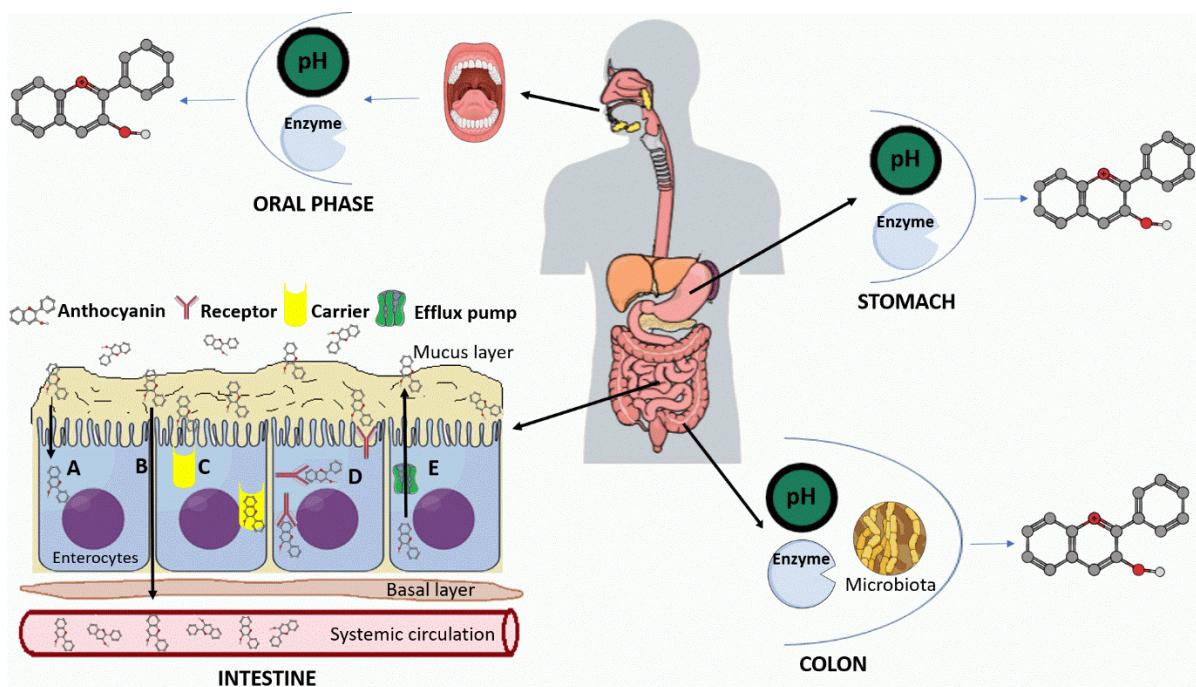


Figure 2. Schematic representation of anthocyanins degradation and absorption in different regions of the human gastrointestinal tract. Oral pH is 7.4, and it contains many saliva amylases. Stomach pH is 1–3, and has many pepsin proteases, some lipases, and amylases. Intestinal pH is 6–7. Anthocyanins can undergo four transport mechanisms in the cell layer, including (A) Passive diffusion: transcellular diffusion, (B) Passive diffusion: paracellular diffusion, (C) Carrier-mediated transport, (D) Receptor-mediated transport and (E) Efflux pump mechanism. The colon pH is 7–8. Microbiota can ferment the non-absorbed anthocyanins.

The absorption of anthocyanins is mainly affected by the physical and physiological barrier of the intestinal epithelium, which is made up of a mucus barrier and cell layer (Chai et al., 2018). Anthocyanins must permeate through the mucus barrier and arrive at the enterocyte surface in the cell layer for further absorption, and this occurs via electrostatic attractions (Andersen & Jordheim, 2006; Peixoto et al., 2016). When the anthocyanins bind to the enterocytes (the most abundant cells in the small intestine), they have microvilli on the lumen side and absorb them via active transport or passive diffusion, the latter being likely to constitute the significant absorptive pathway for anthocyanins (Hu, Liu, Zhang, & Zeng, 2017). Passive diffusion is promoted by osmotic pressure and occurs through either paracellular or transcellular routes. In the paracellular diffusion, anthocyanins are transported through the junctions between intestinal epithelium cells. In the transcellular diffusion, anthocyanins are transferred through the cellular membrane, merging into the membrane. (Hu et al., 2017; Velikov & Pelan, 2008). There are three other possible transport mechanisms for anthocyanins beyond the passive diffusion (**Fig. 2**):

- receptor-mediated transport, wherein anthocyanins specifically bind to the cell-surface receptor and are internalized by cells (Chai et al., 2018);
- carrier-mediated transport which allows anthocyanins to enter or be expelled from cells due to an osmotic pressure gradient utilizing the cellular protein transporters (Chai et al., 2018);
- efflux pump mechanism, where anthocyanins can be pumped out from enterocytes by poly-glycoprotein (P-gp) efflux pumps in the intestinal epithelium, leading to limited bioavailability (Chai et al., 2018).

After absorption into the small intestine, anthocyanins may be subjected to enterocytes biotransformation and then transported to the hepatocytes, resulting in a series of water-soluble conjugate metabolites rapidly liberated to the circulatory system for further distribution to organs and excretion in urine (Rafaela et al., 2018). Thus, the anthocyanins not absorbed in the proximal intestine, reach the colon and undergo degradation, mainly by the local microbiota, into phenolic acids for subsequent absorption (Morais et al., 2016).

Compared to the amount of anthocyanin consumed, the amount absorbed is minimal, suggesting a very low bioavailability. Around 1% of the initial dose is found

in the human plasma, but many studies have reported their metabolites (like protocatechuic acid and the glucuronited and methylated metabolites) and colorless carbinol and chalcone (Riaz et al., 2016) in the same material. Moreover, Kurilich, Clevidence, Britz, Simon, and Novotny (2005) reported that anthocyanins consumption in large doses significantly reduced their recovery in the human plasma, suggesting saturation of the absorption mechanisms. These facts indicate an underestimation of the anthocyanins bioavailability in the results from the last decades' studies.

2.2. Why is it challenging to use anthocyanins?

The recent food safety issues and consumers' demands have led the food industry to improve the efforts to replace synthetic additives and pigments with additives and pigments from natural sources. However, the applications of anthocyanins as a food additive are seriously limited due to their low stability, especially during the processing and storage conditions (Santos-Buelga & González-Paramás, 2018). The color and stability properties of anthocyanins are strongly affected by pH, solvent, temperature, concentration and structure, oxygen, light, enzymes, and other compounds that can interact with the anthocyanin molecules (He & Giusti, 2010; Prior & Wu, 2006; Santos-Buelga & González-Paramás, 2018).

Sun, Bai, Zhang, Liao, and Hu (2011) concluded that three pathways are involved in the thermal degradation of anthocyanins: (1) hydrolysis of the glycosidic bond forming anthocyanidin, its aglycon form (very unstable), (2) hydrolytic opening of the pyrilium ring followed by the hydrolysis of the glycosidic bond to form carbinol pseudo base and (3) the carbinol pseudo is transformed to chalcone, and then to the coumarin glucoside, both colorless. Furtado, Figueiredo, Chaves das Neves, and Pina (1993) found the same final products for photochemical and thermal degradation but through a different kinetic pathway involving the excitation of the flavylium cation. Markakis (1982) says that, like other polyphenols, polyphenol oxidase (PPO), anthocyanase, and glucosidase can degrade anthocyanins enzymatically through indirect oxidative mechanism or by hydrolyzing their glycosidic bonds to yield much more unstable anthocyanidins. In both cases, spontaneous degradation and decolorization occurs. According to Bordignon, Francescatto, Nienow, Calvete, and Reginatto (2009), three main equilibria occur when raising the pH of an acid solution containing an anthocyanin. In the first reaction, the acid-base protonation balance of

the flavylium cation occurs very fast. Then, a pseudo base carbinol is formed and, finally, a tautomeric equilibrium is slowly established, with the formation of a chalcone pseudo base. These reactions lead to a structural configuration of anthocyanins in which, as the pH increases, decreases the number of conjugated double bonds and this characterizes the loss of coloration due to the pyrylium ring (ring C) cleavage. Santos-Buelga and González-Paramás (2018) showed that degradation of anthocyanins can be induced by the ascorbic acid in the presence of oxygen through the cleavage of the pyrylium ring, and by light exposure through the photo-oxidative mechanism, both regardless of the pH of the medium. Moreover, studies suggest that some intramolecular associations between anthocyanins and copigments are able to cause changes in color and to stabilize their colorless forms when the necessary conditions for this occur, as in the presence of a disaccharide residue in position 3 in the pyrylium ring (Brouillard & Dangles, 1994; Figueiredo et al., 1996).

The most stable anthocyanidin is pelargonidin, followed by malvidin, peonidin, petunidin, cyanidin, and delphinidin. Nevertheless, the six most common anthocyanidins have their stability strongly affected by the pattern of substitution in the B-ring, and this stability can be improved with the methoxyl groups or worsened with the increase in the number of hydroxyls. They are very unstable and easily degraded in slightly acidic to neutral mediums, like what occurs in foods and beverages, but both their glycosylation and acylation can make anthocyanins more stable than their parent aglycones (Iacobucci & Sweeny, 1983; Santos-Buelga & González-Paramás, 2018). Allied to these factors, the anthocyanins can be degraded inside the body before they arrive at their absorption site due to their instability in the gastrointestinal adverse environment (González-Barrio, Borges, Mullen, & Crozier, 2010).

These factors can limit the use of anthocyanins because of their high instability and easy susceptibility to degradation when exposed to them. The low stability of these compounds during their extraction, manipulation, storage and consumption, influences the entire production chain. In this way, researchers over the years have been investigating new processes that provide minimal degradation of the extracted compounds and ensure that they are delivered intact to the absorption site. The techniques and methodologies used in the extraction, purification, and concentration processes are important measures to obtain extracts rich in natural compounds that can be used in the liquid or encapsulated form, to be applied not only as food supplements or nutraceuticals and functional food additives, but also in the

pharmaceutical industry or in cosmetic products (Carvalho et al., 2016). Therefore, the challenge is to protect such promising molecules from deterioration and increase their bioavailability (Ahmad, Ashraf, Gani, & Gani, 2018). Given the above reasons, the introduction of anthocyanins into foods has become a significant challenge. In fact, the main challenge for the food industry constitutes the search for improved processing methods to better control anthocyanin losses and/or to address anthocyanin reactions in the right direction to obtain more stable and desirable products (Santos-Buelga, Mateus, & De Freitas, 2014).

3. Encapsulated delivery systems

Many food processing and storage factors, as well as interactions with components found in food matrices, can lead to degradation and cause changes in color and stability of anthocyanins. Nevertheless, some technological alternatives have been used to decrease their nutritional and sensory losses, and to increase their stability during product development, storage and consumption (Bicudo et al., 2014). Many encapsulated delivery systems have been studied in order to protect the bioactive compounds from adverse environmental conditions, to mask their taste (Aditya, Espinosa, & Norton, 2017), to increase their shelf life (Kuck, Wesolowski, & Noreña, 2017), and to improve their permeability through the intestinal mucus and epithelium, protecting these compounds from degradation in the gastrointestinal tract (Chai et al., 2018).

In recent years, several new encapsulation strategies have been developed through colloidal carrier systems with different physico-chemical properties (Velikov & Pelan, 2008). Moreover, several new specialized manufacturing and storage techniques also have been used, like freeze-drying, spray drying, (Aditya et al., 2017), supercritical carbon dioxide (SC-CO₂) technology (Zhao, Temelli, & Chen, 2017) and others. These systems can vary in size, structure, shape, surface characteristics, stability and carrier materials, and can be used to target delivery to specific sites by changing the load release kinetics (Lundquist & Artursson, 2016). Thus, intelligent delivery systems become increasingly necessary in the food industry to make possible the most affective application of bioactive compounds in food matrices, in order to improve their bioaccessibility and bioavailability (Chai et al., 2018).

The encapsulation techniques are considered as a promising protective method; they involve the packing of the core material of a smaller size inside the wall matrix (Ahmad et al., 2018). The encapsulation technology is defined as a technique in which substances in the solid, liquid, or gaseous state (encapsulated agent or active core) are packaged with an encapsulating agent (wall or shell material). An encapsulated material can be engineered to release active ingredients gradually through specific triggers (like fracture by heat, solvation, diffusion, and pressure), and they can also be engineered to open in specific areas of the body (Anal & Singh, 2007). Capsules are small vesicles or particulates that may range from sub-micron to several millimeters in size. The size of the dispersed or powdered particle formed can be classified as a macro (> 5000 mm), micro (1.0–5000 mm) or nano (< 1.0 mm) (Jafari, Assadpoor, He, & Bhandari, 2008). Many morphologies can be produced for encapsulation, but three major morphologies are more commonly used: mononuclear capsules, which have a single core enveloped by a wall material; a polynuclear core, which has two or more cores enveloped by a wall material; and aggregates, which have many cores embedded in a matrix (Schrooyen, Van der Meer, & De Kruif, 2001). These structures are shown in **Fig. 3**. The specific formation of these structures depends on the choice of the core and wall materials, as well as the process technologies that are chosen for their production (Fang & Bhandari, 2010).

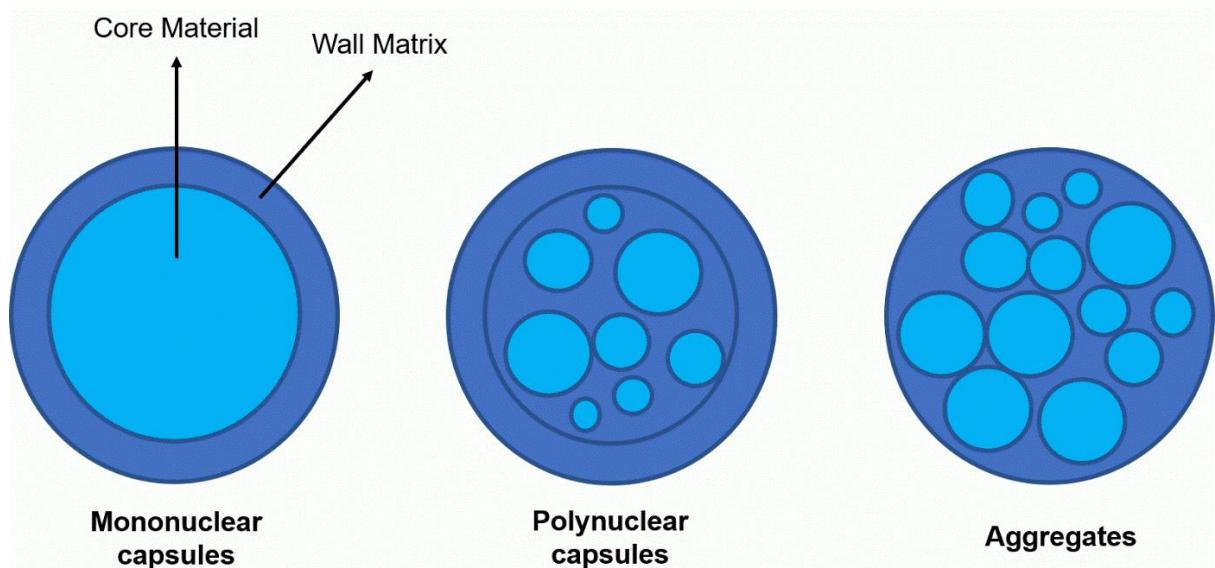


Fig. 3. Three major morphologies produced by encapsulation (Based on Fang & Bhandari, 2010; McClements, 2015).

Some parameters are used to verify the quality of encapsulation, and their characteristics depend on the encapsulation process used. Encapsulation efficiency is one of the most important quality parameters of encapsulation techniques because it determines the wall material's ability to hold the core material inside the microcapsule. This parameter permits us to determine if the encapsulation method and the wall material used is able to encapsulate the core material efficiently (Ahmad et al., 2018). The particle size is another quality parameter to be observed and its reduction, promoted by encapsulation, may improve its delivery properties, solubility, and bioavailability due to the generation of a higher surface area per unit volume, improving their biological activity (Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2013). The small sizes, in the micro and nanoscale, can provide a prolonged gastrointestinal retention time caused by the improvement of the bioadhesive properties in the mucus covering the intestinal epithelium (Chai et al., 2018). Particles made with biopolymers can also use the ζ -Potential (or zeta potential) as a quality parameter. This parameter is a surface property that measures the effective electric potential difference generated by the surface charge density of the biopolymers used and depends on the pH of the medium. The ζ -Potential measurement can be used to verify the stability of aggregation and to control the charged bioactive molecule's retention and release, among other functions (Joye & McClements, 2014; McClements, 2017).

Although several types of encapsulating agents can be used in the encapsulation processes, some characteristics should be considered observed before making a choice, including their affinity with the core material, ability to form films, biodegradability, resistance to the gastrointestinal tract, viscosity, solids content, hygroscopicity, and cost, as well as the compatibility with the encapsulation technique employed (P. I. Silva, Stringheta, Teófilo, & de Oliveira, 2013). These agents can be made of gums, proteins, sugars, natural and modified polysaccharides, lipids, and synthetic polymers (Gibbs, Kermasha, Alli, & Mulligan, 1999).

4. Colloidal carrier systems

An intelligent approach for the stabilization of anthocyanins is the introduction of colloidal carrier systems. Liposomes, cyclodextrin, polymeric particles, and emulsions, are examples that could be used as carriers of encapsulated anthocyanins (Fang & Bhandari, 2010; Velikov & Pelan, 2008). Colloidal systems can

be produced using two general approaches which depend on the physicochemical approach involved. In the first, called “top-down”, large particles or droplets are broken down to smaller ones using mechanical energy. The second, called “bottom-up”, currently under rapid development and widely used to make colloidal particles, uses physical or chemical processes to produce them (Madalena, Pereira, Vicente, & Ramos, 2018; Simões et al., 2017; Velikov & Pelan, 2008). In the food industry, several techniques have been developed to produce colloidal carrier systems for anthocyanins using “top-down” and “bottom-up” approaches, or a combination of both strategies. The use of these techniques allows us to develop micro- and nano-systems with distinct properties and characteristics, like controlled release and delivery, and good interaction with food matrices (Simões et al., 2017).

Due to the vast diversity of available techniques that can be successfully employed for encapsulation of anthocyanins intended for food applications, the main advantages, issues, and differences are discussed below, and some recent studies have been shown in **Table 1**.

Table 1. Characteristics of the different techniques for encapsulation of anthocyanins.

Encapsulation Method	Core Material	Wall Material	Results	Reference
Top-down				
Liposomes	hibiscus extract	soybean lecithin	High EE (62.7%) and improved stability	(Gibis, Zeeb, & Weiss, 2014)
Liposome	elderberry extract	soybean lecithin	Stable liposomes, with a uniform structure, small size (205 nm), but with not so good EE (around 25%)	(Bryła, Lewandowicz, & Juzwa, 2015)
Molecular Inclusion	blackberry purees	β -cyclodextrin	Protected from thermal degradation and increased stability and bioaccessibility	(Fernandes et al., 2018)
Gastroretentive systems	haskap berries extract	alginate-based in situ gelling system	Modulated of the release and absorption	(Celli, Brooks, & Ghanem, 2016)
Gastroretentive systems	<i>Clitoria ternatea</i> petal flower extract	alginate-based in situ gelling system	Reduced degradation and increased biological activity after gastrointestinal digestion; EE 84.87%	(Pasukamonset, Kwon, & Adisakwattana, 2016)
Double emulsion	anthocyanins commercial extract	grape seed oil and guar gum	High EE (90.6%) and kinetic stability and protected against degradation	(Paula et al., 2018)
Double emulsion and biopolymer particles	black raspberry ethanolic extract	soybean oil / gelatin and gum Arabic	Advantage in color conservation and improved thermal stability; EE 81.12%	(Shaddel et al., 2018b)
Emulsion	bilberry extract	bilberry seed oil / whey protein isolate	Stable emulsion structure and protected from degradation	(Svanberg et al., 2019)
Emulsion	black carrot concentrates	sunflower oil / whey protein isolate	Protected the color by increasing its stability	(Bilek, Yılmaz, & Özkan, 2017)
Biopolymer particles	purple corn and blueberry extracts	alginate and pectin	Reduced the photodegradation rate and increased the shelf life; EE 65 and 116% respectively	(Guo, Giusti, & Kaletunc, 2018)
Biopolymer particles	blueberry extracts	chitosan	Improved the EE (94.02%) and stability	(Wang, Jung, & Zhao, 2017)
Bottom-up				
Spray-drying	jabuticaba peel extract	chitosan	High EE (89.74%) and improved thermal stability;	(Cabral et al., 2018)
Spray-drying	maqui juice	inulin or sodium alginate	Improved the stability and increased the bioaccessibility; EE 68.6 and 47.3% respectively	(Fredes, Osorio, Parada, & Robert, 2018)
Spray-drying and Freeze-drying	bordo grape skin extract	gum Arabic, partially hydrolyzed guar gum, and polydextrose	degradation in the freeze-dried powder is more sensitive to temperature than in the spray-dried powder	(Kuck, Wesolowski, & Noreña, 2017)
Freeze-drying	jabuticaba pomace extract	maltodextrin, pectin, and soy protein isolate	Protection to monomeric anthocyanin higher than 76% and improved the stability	(Souza, Gurak, & Marczak, 2017)

Freeze-drying	blackberry extract	maltodextrin	High anthocyanin retention and EE (76%)	(Yamashita et al., 2017)
Freeze-drying	sour cherries extract	β -lactoglobulin	protect the anthocyanins from the gastric digestion, facilitating their release into the intestine; EE 64.69%	(Oancea et al., 2017)
Electrospraying	black carrot extract	chitosan and gelatin	Faster and greater released in the food simulant acetic acid medium; EE 76.9%	(Atay et al., 2018)
Electrospinning	sour cherry concentrate	gelatin or gelatin-lactalbumin	Increased the stability and the bioaccessibility; EE 79.2 and 70.2% respectively	(Isik, Altay, & Capanoglu, 2018)
RESS	jabuticaba extract	polyethyleneglycol	more stability to light and temperature than the free extract; EE 79.79%	(Santos, Albarelli, Beppu, & Meireles, 2013)
SAS	blackberry extract	polyvinylpyrrolidone	dry product richer in anthocyanins and with enhanced purity; improved thermal stability	(Machado et al., 2018)
PGSS	elderberry juice concentrate	palm fat	homogeneously colored free-flowing fine powder rich in anthocyanins and color stability	(Bánvölgyi et al., 2016)

Top-down combined with Bottom-up

Spray-drying and Molecular Inclusion	saffron petals extract	β -glucan or β -cyclodextrin	Increased the bioaccessibility in intestinal section; EE 45 and 63.25% respectively	(Ahmad et al., 2018)
Spray-drying and Biopolymer Particles	red grape juice	soy protein or whey protein / maltodextrin	Improved thermal stability and increased the shelf life	(Moser et al., 2017)
Supercritical Carbon Dioxide and Liposome	commercial anthocyanin from bilberry	soy lecithin	Small size (160 nm), high stability and EE (52.2%)	(Zhao et al., 2017)
Double Emulsion and Spray-drying	Anthocyanin powder from the black carrot	glycerol mono-oleate and soy lecithin / maltodextrin and poloxamer 338	Protected against heat degradation from thermal processing; EE 57.2%	(Ravanfar, Comunian, & Abbaspourrad, 2018)
Double Emulsion, Complex Coacervation and Freeze-drying	black raspberry extract	gelatin and Arabic gum	improved thermostability, conservation of color, long residual action of anthocyanins after being encapsulated; EE 81.12% at	(Shaddel et al., 2018a)
Biopolymer Particles and Freeze-drying	elderberry extract	Whey protein and pectin	Higher stability when subjected to thermal treatment; EE 97.13%	(Stănciu et al., 2018)
Freeze-drying, Complex Coacervation and Molecular Inclusion	blueberry extract	Maltodextrin, β -Cyclodextrin, Whey protein isolate and Gum Arabic	Protect anthocyanins against degradation during heating; EE 82%	(Tao et al., 2017)

EE: Encapsulation Efficiency

4.1. Top-down

Top-down approaches are considered a lithographic method that generates desired patterns in both micro- and nanoscale by physical manipulation, and it offers larger consistency and controllability on the pattern's size and shapes when compared with bottom-up approaches (Smith, Tejeda-Montes, Poch, & Mata, 2011). The top-down approaches promote the breaking of large structured materials into small structures by employing external mechanical disruption forces, which, in addition to reducing the size, promotes the shaping of the structure (Shishir, Xie, Sun, Zheng, & Chen, 2018). Three types of disruptive forces are used to breakdown the particles in these approaches: shear, impact, and compression. Even though widely used in the food industry to form colloidal carrier systems, they have some limitations, such as high equipment maintenance and running costs, less control over particle size, and difficulties in creating particles with well-defined structural properties (Joye & McClements, 2014). Besides, top-down approaches can be very energy-intensive and expensive, and the relatively lower manufacturing capacity and the limited number of applicable matrices are also a shortcoming of most existing top-down methods (Velikov & Pelan, 2008).

4.1.1. Liposomes

Liposomes are nano/micro-sized colloidal vesicles of a phospholipid bilayer used to encapsulate hydrophilic, hydrophobic, and amphiphilic bioactives, and it is a promising encapsulation technique to protect the anthocyanins. Liposomes or lipid vesicles are aggregates formed from amphiphilic molecules (such as polar lipids) in an aqueous medium organized in bilayer-structures. They are typically spherical and can contain a single (unilamellar vesicles) or multiple (multilamellar vesicles) layer of amphiphilic polymolecular membranes (Taylor, Davidson, Bruce, & Weiss, 2005). For food applications, liposomes are commonly formed using accessible and relatively cheap raw materials. Several kinds of surface-active components of bulk oils can be used (like free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, and polar amphiphilic products of lipid oxidation) and the liposomes are formed when these substances aggregate into associated colloids (Kittipongpittaya, Panya, McClements, & Decker, 2014). Phospholipids extracted from eggs, soy, sunflower, or milk, which are popular ingredients of food products and easily found in commercial lecithin



preparations, can also be used, but they should be used sparingly because they also contain other components, such as triglycerides, sterols, and free fatty acids that can influence the liposome's properties (Koynova & Caffrey, 1998; Mozafari et al., 2008; Taylor et al., 2005). Due to their biocompatibility, amphiphilicity, non-toxicity, and non-immunogenicity, liposomes can provide protection of unstable and active compounds against environmental conditions in their aqueous interior core, or within their bilayer membrane, and they also enhance their absorption and bioavailability allowing them to be released at designated targets (Shehata, Ogawara, Higaki, & Kimura, 2008).

In recent years, liposomes have gained increased interest due to their versatile applications in the biomedical, food, and cosmetic industries (Zhao et al., 2017). They are used as drug delivery vesicles and for medical applications due to their ability to simulate the behavior of natural cell membranes (Taylor et al., 2005). In the food and agricultural industries, liposomal encapsulation technologies have been extensively investigated as delivery systems to entrap and protect functional and unstable components, such as antimicrobials, flavors, antioxidants, and bioactive ingredients (Panya et al., 2010). For bioactive compounds, the higher the layer quantity the smaller the liposome loading capacity, with the unilamellar liposome being the one which gives the greatest loading capacity with a minimum carrier agent use (Shishir et al., 2018).

The liposome becomes attractive to anthocyanins encapsulation because of the capacity to protect and entrap hydrophilic bioactive compounds (Shishir et al., 2018) and the possibility of using a unilamellar liposome provides a satisfactory level of anthocyanins intestinal absorption with a smaller use of carrier agent (Mozafari et al., 2008). Bryła, Lewandowicz, and Juzwa (2015) used liposomes from soybean lecithin to encapsulate anthocyanin-rich elderberry extract. They obtained stable liposomes, with a uniform structure, small size (205 nm), but with poor encapsulation efficiency (25%).

Despite their qualities, liposomes are often rapidly destabilized due to the bilayer membranes are too highly flexible and fragile. Also, unsaturated fatty acids, as parts of the membrane structure, may undergo oxidation processes leading to the formation of hydroperoxides, which further break down into typical fat degradation by-products with rancid olfactory substances (Panya et al., 2010). In this case, the anthocyanins can act with antioxidants, being consumed in this process.

4.1.2. Molecular Inclusion

Molecular inclusion complexes are cyclodextrins based encapsulated systems that can provide a stabilizing environment for anthocyanins. The molecular inclusion complexation consists of the encapsulation of a supra-molecular association of a ligand (core material) into a cavity-bearing substrate (wall matrix) done through hydrogen bonding, van der Waals forces, or an entropy-driven hydrophobic effect (Ezhilarasi et al., 2013). They can form inclusion complexes with cyclodextrins and β -glucan molecules, which may protect anthocyanins from hydration and polymerization reactions (Numata & Shinkai, 2011; Yamada, Komiya, & Akaki, 1980).

A cyclodextrin molecule has a tubular form with an inner diameter of 6 Å in α -cyclodextrin (α -CD), or 7.5 Å in β -cyclodextrin (β -CD). Both cyclodextrins are 7 Å long, and the inner side of the tube is rather basic and hydrophobic (Yamada et al., 1980), but the most used in the food industry is the β -CD (Basílio, Fernandes, De Freitas, Gago, & Pina, 2013). β -CD is a truncated cone-shaped structure formed by seven glucose residues linked by α -(1–4) glycosidic bonds in a cyclic oligosaccharide. This structure is an adaptable hydrophobic tridimensional cavity that gives you its main functional property: the ability to form non-covalent inclusion complexes with a wide range of host molecules through hydrogen bonding, van der Waals interaction, or electrostatic attraction (Fernandes et al., 2018). The molecular inclusion with β -CD improves the thermal stability of anthocyanin-rich extracts (Mourtzinos et al., 2008) and their bioavailability, once β -CD has the ability to protect the bioactive compound from the hazardous conditions found in the gastrointestinal tract (Kosaraju, 2005), allowing them to be liberated in the correct locale. Fernandes et al. (2018) encapsulated blackberry purees through molecular inclusion with β -CD and observed an increase in blackberry anthocyanin's thermal stability and a decrease in the rate of degradation under in vitro gastrointestinal conditions.

Even though cyclodextrin complexes have been reported to have enhanced storage stability, they show a poor solubility and can affect other physicochemical properties, like the color. (Basílio et al., 2013). Furthermore, there are few available biopolymers with suitable molecular-level cavities in the food industry (Joye & McClements, 2014).

4.1.3. Gastroretentive systems

Gastroretentive systems (GRS) are used to modulate the release and absorption of anthocyanins and could be a promising strategy to increase their retention time in parts of the gastrointestinal tract where they are absorbed (Celli, Brooks, et al., 2016). The GRS was developed because of the failure of conventional systems in gastric retention. Such delivery systems were designed to retain the GRS in the upper gastrointestinal tract for a longer period, to control the anthocyanins release, and this extended contact allows for an increase in their bioavailability (Lopes, Bettencourt, Rossi, Buttini, & Barata, 2016).

GRS aims to project wall materials (or a mixture thereof) with specific properties that increase gastric retention of the encapsulated compound. Longer gastric retention can be achieved by different strategies such as bioadhesive or mucoadhesive systems, expandable systems, high-density systems, floating systems, super porous hydrogels, and magnetic systems (Garg & Gupta, 2008; Lopes et al., 2016). GRS can deliver and maintain anthocyanin glycosides obtained from plant sources in the upper GI tract, where their stability and absorption are favored (Celli, Kalt, & Brooks, 2016). Still hardly used for the food industry, GRS has attracted the attention of many researchers, like Yao et al. (2014), who suggested that gastroretentive delivery systems could prolong the anthocyanin gastric retention and avoid its degradation in the intestine. Celli, Brooks, et al. (2016) increased the stability and retention time of anthocyanins from haskap berries encapsulated by the alginate-based in situ gastroretentive gelling systems in the stomach for more extended periods, compared to conventional systems and meals, which could contribute to modulation of the anthocyanins release and absorption.

This encapsulation process is very new, little-studied, and its disadvantages are not yet well supported. More studies will be necessary to show the real effect in the longer retention of anthocyanins in the stomach and if it is effective on their absorption.

4.1.4. Emulsions

An emulsion consists of at least two immiscible liquids (usually oil and water, but not always), with one of the liquids being dispersed as small spherical droplets in the other. The dispersed or discontinuous phase is compounded by liquid that makes

up the droplets and the dispersing or continuous phase is compounded by the surrounding liquid (McClements, 2010, 2016).

Anthocyanins can be easily incorporated into the dispersed phase of an emulsion to be protected in its continuous phase against external environmental damaging conditions, degradation (mechanical, chemical, enzymatic), to control their release, and to improve their bioavailability in the human gastrointestinal tract (Mao, Roos, Biliaderis, & Miao, 2017). There are many delivery systems for bioactive compounds developed by emulsion technology that can produce a liquid, or powder particles, which depend on the associated technique employed (Okuro, Furtado, Sato, & Cunha, 2015). Two approaches can be used to prepare emulsions: low-energy and high-energy. Most conventional emulsification techniques used in the food industry are based on the high-energy approaches (like dispersing machines, high-pressure homogenizers, microfluidizers, and ultrasonic homogenizers) that produce microsized and nanosized emulsions through a strong shearing stress applied for the breakdown of macroscopic phases (Santana, Perrechil, & Cunha, 2013). The high-energy approach emulsification can produce emulsions with various particle sizes and different degrees of polydispersity and viscosity, depending on their phase composition and the technology used. Nevertheless, is very difficult to control the particle size distribution, which generates more polydisperse emulsions when compared to emulsions produced by a low energy approach (Ushikubo, Oliveira, Michelon, & Cunha, 2014).

After the formation of the emulsions, additional techniques such as gelation, drying, or coating may be employed to improve their stability (Okuro et al., 2015). Various types of emulsion-based delivery systems have been developed in order to improve the stability and control the digestion and release of bioactive components within the gastrointestinal tract, including conventional emulsions, nanoemulsions, multilayer emulsions, solid lipid nanoparticles, double emulsions, and filled hydrogel particles (McClements & Li, 2010). Paula et al. (2018) added a commercial extract of anthocyanins, together with 1.25% guar gum, in the aqueous phase of a double emulsion and observed a high encapsulation efficiency (90.6%), an increase of thermal stability, and the protection of anthocyanin molecules against degradation. The addition of guar gum increased the color stability.

Although the delivery systems of emulsions present good results in protecting anthocyanins, they are thermodynamically unstable systems that tend to

disrupt over time. Hence, the main challenge of emulsion technology is to confer a sufficiently high kinetic stability to the emulsions so that they are not disrupted so easily (McClements, 2010).

4.1.5. Complexes and coacervation (biopolymer particles)

Biopolymeric particles are (cross-linked) particles composed of polymers with a dense matrix which only incorporate a limited amount of liquid (Joye & McClements, 2014). Different biopolymer matrices have been used to encapsulate anthocyanins, and the most used are protein/polysaccharide matrices (Shaddel et al., 2018b; Zhao et al., 2017). When these macromolecules are oppositely charged, supramolecular structures are produced through electrostatic interactions generated between them, which can thus serve as an encapsulating and delivery system for anthocyanins. Because they are not hazardous when used as food additives, these structures are classed as generally recognized as safe (GRAS) materials, which makes them suitable for food applications (Aditya et al., 2017).

The technique of protein/polysaccharide complex formation through electrostatic interactions between oppositely charged macromolecules involves the phase separation of a single, or a mixture of charged, biopolymers and the subsequent deposition of the formed coacervated phase around the active ingredient. Then, liquid-liquid macroscopic phase separation occurs, leading to discrete liquid coacervate droplets coexisting with a dilute phase (Ezhilarasi et al., 2013; Schmitt & Turgeon, 2011). For the complex formation to occur, three principal stages are necessary: the solubilization of biopolymers in a solvent, the mix of biopolymers in the required ratio, and acidification of the medium (Aditya et al., 2017). The acidification stage is very important because it can strongly influence the formed complex size, and this stage can occur in two different ways. Pre-blending acidification is when the acidification is done before the mix of different biopolymers and post-blending acidification is when it is done after it, given that post-blending acidification produces smaller complexes than pre-blending acidification (Bédié, Turgeon, & Makhlouf, 2008).

The growing interest in the complex coacervation technique is since this technique has higher loading capacity, lower temperature operating conditions, and enables greater control in the release rate than other encapsulation techniques. The control of these parameters in the protein/polysaccharide interactions through complex

coacervation can improve their functional properties without promoting chemical or enzymatic modifications, which makes them excellent encapsulated delivery systems for anthocyanins (Shaddel et al., 2018b; Yan & Zhang, 2014). Guo, Giusti, and Kaletunc (2018) used alginate-pectin hydrogel particles to encapsulate anthocyanins extracted from blueberry and purple corn. They observed a higher encapsulation efficiency for blueberry (116%) than for purple corn (65%) anthocyanins. Both reduced the photodegradation rate and increased the anthocyanins retention percentage during storage, so increasing their shelf-life.

Preventing particle agglomeration and the difficulties encountered in controlling their size are the main drawbacks of using this method. Other potential drawbacks are that the particles formed by this method are highly sensitive to pH and ionic strength, so limiting its use in some matrices. Besides that, the particles show limited stability in aqueous matrices, depending on the biopolymer material used (Joye & McClements, 2014).

4.2. Bottom-up: Specialized manufacturing and storage techniques

Bottom-up approaches happen when changes in environmental conditions (e.g. pH, ionic strength, temperature, or concentration), promoted by physical or chemical processes, form particles through self-assembly or self-organization of molecules that are aggregated in liquid, solid, or gas phases (Joye & McClements, 2014; Velikov & Pelan, 2008). These approaches allow better control over particle formation and properties, facilitating the determination of parameters such as particle size, size distribution, morphology, and physical state. Besides that, they demand less energy and have a lower risk of sample contamination when compared with top-down approaches (Thorat & Dalvi, 2012). Although these approaches are accessible and productive, they are technologically more complex and most of them still have scale-up restrictions and high-costs. The challenge for their implementation is on the process choice, which depends on the specific properties of the material to be encapsulated, the available apparatus, the demand of production, and the cost (Chan & Kwok, 2011; Fu et al., 2018; Velikov & Pelan, 2008).

4.2.1. Freeze-drying

Freeze-drying or lyophilization is also known as one of the best methods to dry anthocyanins (Idham, Muhamad, & Sarmidi, 2012). This drying technique promotes the dehydration of the frozen mixtures of core and wall materials through sublimation under vacuum and low temperatures. The result is products that have their chemical structure maintained, besides presenting a well-reduced risk of undesirable changes (Ezhilarasi et al., 2013). As this process uses low temperatures, it is appropriate for encapsulation of heat-sensitive materials such as anthocyanins (Souza, Gurak, & Marczak, 2017).

The freeze-drying method generates superior-quality products, which are easily reconstituted, does not affect their sensory properties, maintains the biofunctionality, and confers a longer shelf-life for bioactive compounds (Ezhilarasi et al., 2013). For the anthocyanins, this method is used to improve thermal and color stability throughout various wall materials (Garavand, Rahaee, Vahedikia, & Jafari, 2019). Souza et al. (2017) used maltodextrin, pectin, and soy protein isolate as wall materials to encapsulate anthocyanins-rich extract from jabuticaba pomace obtained by freeze-drying, and the authors observed that the carriers were able to protect monomeric anthocyanins higher than 76%, improving their stability. Oancea et al. (2018) encapsulated sour cherry skin's anthocyanins extract in whey proteins isolated by freeze-drying, with an encapsulation efficiency of 70.30. They observed that the wall material was able to protect the anthocyanins from the gastric digestion, facilitating their release into the intestine.

Nevertheless, this technique is expensive due to the vacuum technology employed and requires a longer dehydration time than other encapsulation techniques (Díaz-Sánchez, Santos-López, Kerstupp, Villagómez-Ibarra, & Scheinvar, 2006; Simões et al., 2017). Furthermore, the freeze-drying produced particles generally have a high porosity, which significantly affects the stability of the encapsulated component and their encapsulation and retention efficiency (Ezhilarasi et al., 2013).

4.2.2. Spray-drying

Spray-drying is the oldest encapsulation method known and the most used for being a continuous and low-cost process, able to obtain high-quality dry particles through widely available apparatus (Akhavan Mahdavi, Jafari, Assadpoor, & Dehnad,

2016). This encapsulation method has been successfully used for anthocyanin protection (Kuck et al., 2017) as it can produce a powder anthocyanin particle with higher storage stability, easier handling, and minimized transportation compared to other methods already used in the food industry. Besides, it promotes a faster production and a better control over the particle size distribution (Idham et al., 2012).

In this method, the core material is dispersed in the wall material and the mix is pulverized inside a chamber where the dry particles are expanded and their central area is filled by the void resulting from this expansion (Jafari et al., 2008). The selection of wall material is the primary and most important step in this technique where some parameters (such as compatibility with the food product, mechanical strength, appropriate particle size, appropriate thermal or dissolution release, etc.) must be observed for the best protection of the core material depending on its nature, composition, and physicochemical properties (Akhavan Mahdavi, Jafari, Assadpour, & Ghorbani, 2016). For this, many different kinds of encapsulating agents can be used such as polysaccharides, lipids, and proteins (Gibbs et al., 1999). Fredes, Osorio, Parada, and Robert (2018) used inulin and sodium alginate in the encapsulation of maqui juice by spray-drying and obtained an encapsulation efficiency of 68.6 and 47.3, respectively. They observed that the bioaccessibility of anthocyanins in the generated particles was 10% higher than in the maqui juice without encapsulation. Kuck et al. (2017) encapsulated Bordo grape skin extract by spray-drying and freeze-drying, using gum Arabic, partially hydrolyzed guar gum, and polydextrose as the wall materials. The authors observed that the anthocyanins degradation in the freeze-dried powder was more sensitive to temperature than in the spray-dried powder, and there was no difference between either in the release of anthocyanins in the digestion time.

Although rapid and inexpensive, the high temperatures required to evaporate the liquid phase, the large particle size and heterogeneous size distribution observed, as well as the diverse morphology, can degrade the anthocyanins and limit their absorption and bioavailability. Furthermore, a considerable waste of material can occur, with the loss of fine particulates in the exhaust air (Zhao et al., 2017). For this reason, the wall material must be carefully chosen.

4.2.3. Electrospinning and electrospraying techniques

As an alternative to conventional encapsulation techniques, the electrohydrodynamic process has been recently postulated as an advantageous and straightforward method for generating encapsulation structures for a variety of bioactive molecules (Pérez-Masiá, Lagaron, & López-Rubio, 2014), such as anthocyanins (Anu Bhushani & Anandharamakrishnan, 2014; Atay et al., 2018; Jia, Dumont, & Orsat, 2016; Wang, Marcone, Barbut, & Lim, 2013). The main electrohydrodynamic processes are electrospinning and electrospraying and they are facile, cost-effective, and flexible methods that utilize an electrical field to charge jets of polymer solution for the production of micro- or nanofibers, or particles (Anu Bhushani & Anandharamakrishnan, 2014). This technique is especially interesting for the encapsulation and protection of thermosensitive molecules. They are versatile electrohydrodynamic manufacturing methods which can generate encapsulation structures in a one-step process under mild conditions, without the need of employing high temperatures or toxic solvents, limiting inactivation of the bioactive compounds and generally achieving high loading efficiencies (Pisoschi et al., 2018).

Electrospinning and electrospraying are considered similar technologies; however, there are few aspects that differentiate the two electrohydrodynamic processes. In electrospinning, free charges are induced in the polymer solution in the capillary, where due to two major electrostatic forces, electrostatic repulsion and the Coulombic force, the droplet is distorted into a conical shape known as the Taylor cone. As the electrostatic force counteracts the surface tension, a charged polymer jet is released from the Taylor cone. The jet is elongated due to unevenly distributed charges, with quick evaporation of the solvent taking place, and the deposition of a solid and thin fiber on the grounded collector (Quek, Hadi, & Tanambell, 2019). Electrospraying is a process where a liquid is atomized into droplets through a high voltage electrical field, where the particles are deposited in a charged collector. The electrospraying apparatus is the same as for electrospinning, and the main difference between these two techniques is the final product: nanofibers for electrospinning and nanodroplets for electrospraying. The differentiation of the final product form is determined by the intrinsic viscosity of the solution and the biopolymer concentration (Jia et al., 2016). Isik, Altay, and Capanoglu (2018) used the electrospinning technique to encapsulate sour cherry concentrate with gelatin or gelatin-lactalbumin and obtained

an encapsulation efficiency for anthocyanins of 79.2% and 70.2%, respectively. They showed increased stability and bioaccessibility, with a protection of cyanidin-3-glucoside eight times better than the non-encapsulated concentrate. Already, Atay et al. (2018) used the electrospraying technique to encapsulate anthocyanin-rich black carrot extract with a blend solution of chitosan/gelatin and obtained an encapsulation efficiency around 76%. They showed that the particles formed were displayed and released faster in the acetic medium, the blend composition being the main factor that affected the bioactive release, i.e., the material used in the wall composition must be well chosen.

Even though there are advantages of electrohydrodynamic techniques, the fact that they are slow processes and produce low yields, are their main limitations (Anu Bhushani & Anandharamakrishnan, 2014). These limitations restrict their commercial exploitation at a large scale and their scaling up through multi-jet systems may lead to technical difficulties and less efficiency (Quek et al., 2019). Moreover, a successful electrohydrodynamic process depends on the dispersion properties of the wall material, and some materials may not be readily electrospun, so limiting their use (Pérez-Masiá et al., 2014).

4.2.4. Supercritical fluid technology

The development of novel particle formation techniques using supercritical fluids, as an alternative to the disadvantages of conventional encapsulation processes, is an active field of research. The main motivation is the possibility of exploiting their peculiar properties, often described as intermediate between those of a liquid and a gas, and can be easily changed with changes in their pressure and temperature (Temelli, 2018). With the fluids under high pressure, it allows working with low temperatures, high selectivity, low solvent volume, small samples, short extraction times, and automation. For these reasons, supercritical fluids processes have become an attractive alternative to extract and encapsulate bioactive compounds like anthocyanins (B. V. da Silva, Barreira, & Oliveira, 2016). In particular, supercritical carbon dioxide (SC-CO₂) is the most used supercritical fluid for anthocyanins extraction and encapsulation processes in the food industry (Cocero, Martín, Mattea, & Varona, 2009; Machado et al., 2018; Zhao et al., 2017). The SC-CO₂ method uses carbon dioxide at conditions above its critical point (31.1 °C; 7.4 MPa) as the processing way.

SC-CO₂ is an inert, non-toxic, and environmentally friendly solvent with great potential for encapsulation of anthocyanins without thermal degradation and organic solvent residues (Zhao et al., 2017).

The use of supercritical fluid technology can eliminate or significantly reduce the use of solvents. It leaves no residue in the final product (the supercritical fluid is easily removed from the final product by a depressurization) and, when the use of solvents is necessary, their high solubility in supercritical fluids allows the production of solvent-free final products (Martín & Cocco, 2008). However, its main disadvantage is the high investment cost because of the high-pressure variations applied. The encapsulation process by supercritical fluid technology can be classified according to the role that the supercritical fluid plays on it: solvent, anti-solvent and co-solvent, or solute (Varona, Martín, & Cocco, 2016). The main processes for anthocyanins encapsulation with their classifications are described below.

RESS (Rapid Expansion of Supercritical Solutions) is the best-known process for supercritical fluid working as a solvent. In this process, the target compound and the wall material are dissolved in a supercritical fluid at high pressure. When this supercritical fluid is expanded and pressure is reduced, precipitation of the particles occurs. This process is attractive as it does not use organic solvents, but its application is restricted to products that present a reasonable solubility in supercritical carbon dioxide (low polarity compounds) (Cocco et al., 2009). Santos, Albarelli, Beppu, and Meireles (2013) used the RESS technique with SC-CO₂ as the solvent to encapsulate anthocyanin-rich jabuticaba extract with polyethyleneglycol and obtained an encapsulation efficiency of 79.79%. They observed that the particles produced were more stable to light and temperature than the free extract.

SAS (Supercritical Anti Solvent precipitation) is the best-known process for supercritical fluid working as an anti-solvent. In this process, the target compound and the wall material are dissolved in a liquid solvent and are atomized together with the supercritical fluid. Then, the supercritical fluid acts as an antisolvent through the decrease in the solubility of the solute in the mixture, which results in supersaturation and the formation of nano- or microparticles (Varona et al., 2016). This process is attractive due to attaining high loading efficiencies with controlled particle size and distribution. However, its application has an increase in the mechanical complexity of the equipment, use of organic solvents, and has a low production capacity (Martín & Cocco, 2008). Machado et al. (2018) used the SAS technique with SC-CO₂ as the

antisolvent to encapsulate anthocyanin-rich blackberry residue extracts with polyvinylpyrrolidone to obtain a dry product richer in anthocyanins and with enhanced purity, with improved thermal stability.

PGSS (Particles from Gas Saturated Solutions) is the best-known process for supercritical fluid working as a solute. This process consists of dissolving a supercritical fluid into a suspension of the compound of interest in a wall material and, after this, a rapid depressurization of the saturated solution occurs through a nozzle, leading to the formation of solid particles or liquid droplets. This depends on the system due to the intense cooling effect caused by the release of CO₂ (E. K. Silva & Meireles, 2014). The process is attractive due to the reduced consumption of CO₂ and its lower cost, as it operates at lower pressures and larger scales, but it is challenging to monitor the formed particle size (Jung & Perrut, 2001). Bánvölgyi et al. (2016) used the PGSS technique with SC-CO₂ as the solute to encapsulate elderberry juice concentrate with palm fat and obtained a homogeneously colored, free-flowing, fine powder rich in anthocyanins, with a high color stability during prolonged storage.

4.3. Both

Because “top-down” reduces the size of large particles, and “bottom-up” produces micro- or nanoparticles through molecules in solution (Rodríguez-Meizoso & Plaza, 2015), the blending of both processes can produce particles with the characteristics generated by both approaches. Together these two techniques can solve problems that only one approach does not, which makes them complementary. Several researchers have discovered better results with this blend, and this is illustrated below.

Using a single step supercritical carbon dioxide (SC-CO₂) process to obtain liposomes from soybean lecithin, Zhao and Temelli (2017) encapsulated anthocyanin-rich bilberry extract and obtained liposomes even smaller (160 nm), more stable, and with a high encapsulation efficiency (52.2%) compared with the study cited above (Zhao et al., 2017). Ahmad et al. (2018) observed that the inclusion of molecular complexation with β-glucan and β-CD by a spray drying technique, with an encapsulation efficiency of 45% and 63.25%, respectively, improved the stability of anthocyanin-rich saffron extract during passage through simulated GI tract conditions, so increasing the availability of anthocyanins in the intestinal section. Shaddel et al.

(2018a) encapsulated anthocyanin-rich black raspberry extract by a double emulsion system followed by a complex coacervation method through gelatin, gum Arabic, and freeze-drying. They improved the thermostability, color conservation, and obtained a long residual action of anthocyanins after being encapsulated, with the encapsulation efficiency being 81.12% at the selected pH (pH 4). Tao et al. (2017) used various wall materials, (maltodextrin, β -CD, whey protein isolate, and gum Arabic) combining a molecular inclusion and complex coacervation for encapsulation of blueberry anthocyanin extracts through freeze-drying. They observed that encapsulation efficiency values exceeded 82%, and there was high protection of anthocyanins against degradation during heating.

5. Conclusions

Several classical and emerging microencapsulation methods can protect anthocyanins. Each method cited above has advantages for specific applications, and, as all methods have disadvantages too, it is very important to know the desired application before choosing the encapsulation method.

The classical microencapsulation methods have good results but, in general, do not deliver the bioaccessible and/or bioavailable anthocyanins in their absorption sites, and/or require large amounts of reagents, generating waste. The emerging microencapsulation methods are still not economically feasible for the food industries, mainly because they do not have a high yield and they have high costs.

The combination of methods is a promising alternative since it can resolve delivery and environmental questions, and besides, it solves small technical problems like solubility (dry, hydro, or fat-soluble particles), particle size, flow rate, etc. Nevertheless, in the search to combine the best protection, environmental care, high yield, and low costs, further studies are still required.

At every step, the science of the anthocyanin's conservation advances, along with the process cost reduction and increased environmental protection. In this sense, the real challenge in anthocyanins encapsulation is to put these three topics together in one single process.

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.

Acknowledgments

Mario R. Marostica Junior is grateful to CNPQ for financial support (CNPq 301108/2016-1). Adriana G. Tarone thanks CNPQ for the Ph.D. assistantship (140942/2016-5). Authors acknowledge the São Paulo Research Foundation (FAPESP) for the grant (2015/50333-1). This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES).

References

- Aditya, N. P., Espinosa, Y. G., & Norton, I. T. (2017). Encapsulation systems for the delivery of hydrophilic nutraceuticals: Food application. *Biotechnology Advances*, 35(4), 450–457. <https://doi.org/10.1016/j.biotechadv.2017.03.012>
- Ahmad, M., Ashraf, B., Gani, A., & Gani, A. (2018). Microencapsulation of saffron anthocyanins using β glucan and β cyclodextrin: Microcapsule characterization, release behaviour & antioxidant potential during in-vitro digestion. *International Journal of Biological Macromolecules*, 109, 435–442. <https://doi.org/10.1016/j.ijbiomac.2017.11.122>
- Akhavan Mahdavi, S., Jafari, S. M., Assadpoor, E., & Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. *International Journal of Biological Macromolecules*, 85, 379–385. <https://doi.org/10.1016/j.ijbiomac.2016.01.011>
- Akhavan Mahdavi, S., Jafari, S. M., Assadpoor, E., & Ghorbani, M. (2016). Storage stability of encapsulated barberry's anthocyanin and its application in jelly formulation. *Journal of Food Engineering*, 181, 59–66. <https://doi.org/10.1016/j.jfoodeng.2016.03.003>
- Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science and Technology*, 18(5), 240–251. <https://doi.org/10.1016/j.tifs.2007.01.004>
- Andersen, O. M., & Jordheim, M. (2006). The Anthocyanins. In O. M. Andersen & K. R. Markham (Eds.), *Flavonoids: Chemistry, Biochemistry, and Applications* (pp.

- 471–551). Boca Raton: CRC Press. <https://doi.org/10.1002/anie.200685399>
- Anu Bhushani, J., & Anandharamakrishnan, C. (2014). Electrospinning and electrospraying techniques: Potential food based applications. *Trends in Food Science and Technology*, 38(1), 21–33. <https://doi.org/10.1016/j.tifs.2014.03.004>
- Atay, E., Fabra, M. J., Martínez-Sanz, M., Gomez-Mascaraque, L. G., Altan, A., & Lopez-Rubio, A. (2018). Development and characterization of chitosan/gelatin electrosprayed microparticles as food grade delivery vehicles for anthocyanin extracts. *Food Hydrocolloids*, 77, 699–710. <https://doi.org/10.1016/j.foodhyd.2017.11.011>
- Bánvölgyi, S. Z., Vatai, T., Molnár, Z. S., Kiss, I., Knez, Z., Vatai, G. Y., & Škerget, M. (2016). Integrated process to obtain anthocyanin enriched palm-fat particles from elderberry juice. *Acta Alimentaria*, 45(2), 206–214. <https://doi.org/10.1556/AAlim.2015.0009>
- Basílio, N., Fernandes, A., De Freitas, V., Gago, S., & Pina, F. (2013). Effect of β-cyclodextrin on the chemistry of 3',4',7-trihydroxyflavylium. *New Journal of Chemistry*, 37(10), 3166–3173. <https://doi.org/10.1039/c3nj00588g>
- Bédié, G. K., Turgeon, S. L., & Makhlof, J. (2008). Formation of native whey protein isolate-low methoxyl pectin complexes as a matrix for hydro-soluble food ingredient entrapment in acidic foods. *Food Hydrocolloids*, 22(5), 836–844. <https://doi.org/10.1016/j.foodhyd.2007.03.010>
- Bicudo, M. O. P., Ribani, R. H., & Beta, T. (2014). Anthocyanins, Phenolic Acids and Antioxidant Properties of Juçara Fruits (*Euterpe edulis* M.) Along the On-tree Ripening Process. *Plant Foods for Human Nutrition*, 69(2), 142–147. <https://doi.org/10.1007/s11130-014-0406-0>
- Bilek, S. E., Yılmaz, F. M., & Özkan, G. (2017). The effects of industrial production on black carrot concentrate quality and encapsulation of anthocyanins in whey protein hydrogels. *Food and Bioproducts Processing*, 102, 72–80. <https://doi.org/10.1016/j.fbp.2016.12.001>
- Bordignon Jr., C. L., Francescatto, V., Nienow, A. A., Calvete, E., & Reginatto, F. H. (2009). Influência do pH da solução extrativa no teor de antocianinas em frutos de morango. *Ciência e Tecnologia de Alimentos*, 29(1), 183–188. <https://doi.org/10.1590/S0101-20612009000100028>
- Brouillard, R., & Dangles, O. (1994). Anthocyanin molecular interactions: the first step in the formation of new pigments during wine aging? *Food Chemistry*, 51(4), 365–

371. [https://doi.org/10.1016/0308-8146\(94\)90187-2](https://doi.org/10.1016/0308-8146(94)90187-2)

Bryła, A., Lewandowicz, G., & Juzwa, W. (2015). Encapsulation of elderberry extract into phospholipid nanoparticles. *Journal of Food Engineering*, 167, 189–195.
<https://doi.org/10.1016/j.jfoodeng.2015.07.025>

Bueno, J. M., Sáez-Plaza, P., Ramos-Escudero, F., Jiménez, A. M., Fett, R., & Asuero, A. G. (2012). Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part II: Chemical Structure, Color, and Intake of Anthocyanins. *Critical Reviews in Analytical Chemistry*, 42(2), 126–151.
<https://doi.org/10.1080/10408347.2011.632314>

Cabral, B. R. P., de Oliveira, P. M., Gelfuso, G. M., Quintão, T. de S. C., Chaker, J. A., Karnikowski, M. G. de O., & Gris, E. F. (2018). Improving stability of antioxidant compounds from Plinia cauliflora (jabuticaba) fruit peel extract by encapsulation in chitosan microparticles. *Journal of Food Engineering*, 238(March), 195–201.
<https://doi.org/10.1016/j.jfoodeng.2018.06.004>

Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J., & Queipo-Ortuño, M. I. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *Journal of Nutritional Biochemistry*, 24(8), 1415–1422.
<https://doi.org/10.1016/j.jnutbio.2013.05.001>

Carvalho, A. G. da S., Machado, M. T. da C., da Silva, V. M., Sartoratto, A., Rodrigues, R. A. F., & Hubinger, M. D. (2016). Physical properties and morphology of spray dried microparticles containing anthocyanins of jussara (*Euterpe edulis* Martius) extract. *Powder Technology*, 294, 421–428.
<https://doi.org/10.1016/j.powtec.2016.03.007>

Castañeda-Ovando, A., Pacheco-Hernández, M. de L., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. *Food Chemistry*, 113(4), 859–871.
<https://doi.org/10.1016/j.foodchem.2008.09.001>

Celli, G. B., Brooks, M. S. L., & Ghanem, A. (2016). Development and evaluation of a novel alginate-based in situ gelling system to modulate the release of anthocyanins. *Food Hydrocolloids*, 60, 500–508.
<https://doi.org/10.1016/j.foodhyd.2016.04.022>

Celli, G. B., Kalt, W., & Brooks, M. S. L. (2016). Gastroretentive systems – a proposed strategy to modulate anthocyanin release and absorption for the management of diabetes. *Drug Delivery*, 23(6), 1892–1901.

<https://doi.org/10.3109/10717544.2016.1143058>

Chai, J., Jiang, P., Wang, P., Jiang, Y., Li, D., Bao, W., ... Li, Y. (2018). The intelligent delivery systems for bioactive compounds in foods: Physicochemical and physiological conditions, absorption mechanisms, obstacles and responsive strategies. *Trends in Food Science and Technology*, 78(June), 144–154.
<https://doi.org/10.1016/j.tifs.2018.06.003>

Chan, H. K., & Kwok, P. C. L. (2011). Production methods for nanodrug particles using the bottom-up approach. *Advanced Drug Delivery Reviews*.
<https://doi.org/10.1016/j.addr.2011.03.011>

Chang, S. K., Sciences, H., & Jalil, B. (2018). *How Food Structure and Processing Affect the Bioavailability of Nutrients and Antioxidants. Encyclopedia of Food Chemistry*. Elsevier. <https://doi.org/10.1016/B978-0-12-814026-0.21723-X>

Cocero, M. J., Martín, Á., Mattea, F., & Varona, S. (2009). Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications. *Journal of Supercritical Fluids*, 47(3), 546–555.
<https://doi.org/10.1016/j.supflu.2008.08.015>

da Silva, B. V., Barreira, J. C. M., & Oliveira, M. B. P. P. (2016). Natural phytochemicals and probiotics as bioactive ingredients for functional foods: Extraction, biochemistry and protected-delivery technologies. *Trends in Food Science and Technology*, 50, 144–158. <https://doi.org/10.1016/j.tifs.2015.12.007>

de Moura, S. C. S. R., Berling, C. L., Germer, S. P. M. M., Alvim, I. D., & Hubinger, M. D. (2018). Encapsulating anthocyanins from Hibiscus sabdariffa L. calyces by ionic gelation: Pigment stability during storage of microparticles. *Food Chemistry*, 241(August 2017), 317–327. <https://doi.org/10.1016/j.foodchem.2017.08.095>

Díaz-Sánchez, F., Santos-López, E.-M., Kerstupp, S. F., Villagómez-Ibarra, R., & Scheinvar, L. (2006). COLORANT EXTRACTION FROM RED PRICKLY PEAR (OPUNTIA LASIACANTHA) FOR FOOD APPLICATION. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 2, 1330–1337.

Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2004). Anthocyanins in cereals. *Journal of Chromatography A*, 1054(1–2), 129–141.
<https://doi.org/10.1016/j.chroma.2004.08.152>

Ezhilarasi, P. N., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2013). Nanoencapsulation Techniques for Food Bioactive Components: A Review. *Food and Bioprocess Technology*, 6(3), 628–647. [https://doi.org/10.1007/s11947-012-](https://doi.org/10.1007/s11947-012-012-)

0944-0

- Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - A review. *Trends in Food Science and Technology*, 21(10), 510–523. <https://doi.org/10.1016/j.tifs.2010.08.003>
- Faria, A., Pestana, D., Azevedo, J., Martel, F., de Freitas, V., Azevedo, I., ... Calhau, C. (2009). Absorption of anthocyanins through intestinal epithelial cells - Putative involvement of GLUT2. *Molecular Nutrition and Food Research*, 53(11), 1430–1437. <https://doi.org/10.1002/mnfr.200900007>
- Felgines, C., Texier, O., Besson, C., Lyan, B., Lamaison, J. L., & Scalbert, A. (2007). Strawberry pelargonidin glycosides are excreted in urine as intact glycosides and glucuronidated pelargonidin derivatives in rats. *British Journal of Nutrition*, 98(6), 1126–1131. <https://doi.org/10.1017/S0007114507764772>
- Fernandes, A., Rocha, M. A. A., Santos, L. M. N. B. F., Brás, J., Oliveira, J., Mateus, N., & de Freitas, V. (2018). Blackberry anthocyanins: β-Cyclodextrin fortification for thermal and gastrointestinal stabilization. *Food Chemistry*, 245, 426–431. <https://doi.org/10.1016/j.foodchem.2017.10.109>
- Figueiredo, P., Elhabiri, M., Toki, K., Saito, N., Dangles, O., & Brouillard, R. (1996). New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for colour loss? *Phytochemistry*, 41(1), 301–308. [https://doi.org/10.1016/0031-9422\(95\)00530-7](https://doi.org/10.1016/0031-9422(95)00530-7)
- Fredes, C., Osorio, M. J., Parada, J., & Robert, P. (2018). Stability and bioaccessibility of anthocyanins from maqui (*Aristotelia chilensis* [Mol.] Stuntz) juice microparticles. *LWT - Food Science and Technology*, 91(June 2017), 549–556. <https://doi.org/10.1016/j.lwt.2018.01.090>
- Fu, X., Cai, J., Zhang, X., Li, W. Di, Ge, H., & Hu, Y. (2018). Top-down fabrication of shape-controlled, monodisperse nanoparticles for biomedical applications. *Advanced Drug Delivery Reviews*. Elsevier B.V. <https://doi.org/10.1016/j.addr.2018.07.006>
- Furtado, P., Figueiredo, P., Chaves das Neves, H., & Pina, F. (1993). Photochemical and thermal degradation of anthocyanidins. *Journal of Photochemistry and Photobiology, A: Chemistry*, 75(2), 113–118. [https://doi.org/10.1016/1010-6030\(93\)80191-B](https://doi.org/10.1016/1010-6030(93)80191-B)
- Garavand, F., Rahaee, S., Vahedikia, N., & Jafari, S. M. (2019). Different techniques for extraction and micro/nanoencapsulation of saffron bioactive ingredients.

Trends in Food Science and Technology, 89, 26–44.

<https://doi.org/10.1016/j.tifs.2019.05.005>

Garg, R., & Gupta, G. (2008). Progress in Controlled Gastroretentive Delivery Systems. *Pharm Res Tropical Journal of Pharmaceutical Research*, 7(73), 1055–1066. <https://doi.org/10.4314/tjpr.v7i3.14691>

Ghosh, D., & Konishi, T. (2007). Anthocyanins and anthocyanin-rich extracts: Role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition*, 16(2), 200–208. <https://doi.org/10.3390/ijms13022472>

Gibbs, B. F., Kermasha, S., Alli, I., & Mulligan, C. N. (1999). Encapsulation in the food industry: a review. *International Journal of Food Sciences and Nutrition*, 50(3), 213–224. <https://doi.org/10.1080/096374899101256>

Gibis, M., Zeeb, B., & Weiss, J. (2014). Formation, characterization, and stability of encapsulated hibiscus extract in multilayered liposomes. *Food Hydrocolloids*, 38, 28–39. <https://doi.org/10.1016/j.foodhyd.2013.11.014>

González-Barrio, R., Borges, G., Mullen, W., & Crozier, A. (2010). Bioavailability of anthocyanins and ellagitannins following consumption of raspberries by healthy humans and subjects with an ileostomy. *Journal of Agricultural and Food Chemistry*, 58(7), 3933–3939. <https://doi.org/10.1021/jf100315d>

Guo, J., Giusti, M. M., & Kaletunç, G. (2018). Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. *Food Research International*, 107, 414–422. <https://doi.org/10.1016/j.foodres.2018.02.035>

He, J., & Giusti, M. M. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annual Review of Food Science and Technology*, 1(1), 163–187. <https://doi.org/10.1146/annurev.food.080708.100754>

Hosseinian, F. S., Li, W., & Beta, T. (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry*, 109(4), 916–924. <https://doi.org/10.1016/j.foodchem.2007.12.083>

Hu, B., Liu, X., Zhang, C., & Zeng, X. (2017). Food macromolecule based nanodelivery systems for enhancing the bioavailability of polyphenols. *Journal of Food and Drug Analysis*, 25(1), 3–15. <https://doi.org/10.1016/j.jfda.2016.11.004>

Iacobucci, G. A., & Sweeny, J. G. (1983). The chemistry of anthocyanins, anthocyanidins and related flavylium salts. *Tetrahedron*, 39(19), 3005–3038. [https://doi.org/10.1016/S0040-4020\(01\)91542-X](https://doi.org/10.1016/S0040-4020(01)91542-X)

- Idham, Z., Muhamad, I. I., & Sarmidi, M. R. (2012). Degradation kinetics and color stability of spray-dried encapsulated anthocyanins from Hibiscus sabdariffa L. *Journal of Food Process Engineering*, 35(4), 522–542. <https://doi.org/10.1111/j.1745-4530.2010.00605.x>
- Isik, B. S., Altay, F., & Capanoglu, E. (2018). The uniaxial and coaxial encapsulations of sour cherry (*Prunus cerasus* L.) concentrate by electrospinning and their in vitro bioaccessibility. *Food Chemistry*, 265(March 2017), 260–273. <https://doi.org/10.1016/j.foodchem.2018.05.064>
- Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology*, 26(7), 816–835. <https://doi.org/10.1080/07373930802135972>
- Jia, Z., Dumont, M. J., & Orsat, V. (2016). Encapsulation of phenolic compounds present in plants using protein matrices. *Food Bioscience*. <https://doi.org/10.1016/j.fbio.2016.05.007>
- Joye, I. J., & McClements, D. J. (2014). Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. *Current Opinion in Colloid and Interface Science*, 19(5), 417–427. <https://doi.org/10.1016/j.cocis.2014.07.002>
- Jung, J., & Perrut, M. (2001). Particle design using supercritical fluids: Literature and patent survey. *Journal of Supercritical Fluids*, 20(3), 179–219. [https://doi.org/10.1016/S0896-8446\(01\)00064-X](https://doi.org/10.1016/S0896-8446(01)00064-X)
- Kittipongpittaya, K., Panya, A., McClements, D. J., & Decker, E. A. (2014). Impact of free fatty acids and phospholipids on reverse micelles formation and lipid oxidation in bulk oil. *JAOCs, Journal of the American Oil Chemists' Society*, 91(3), 453–462. <https://doi.org/10.1007/s11746-013-2388-8>
- Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, 64(5), 923–933. [https://doi.org/10.1016/S0031-9422\(03\)00438-2](https://doi.org/10.1016/S0031-9422(03)00438-2)
- Kosaraju, S. L. (2005). Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. *Critical Reviews in Food Science and Nutrition*, 45(4), 251–258. <https://doi.org/10.1080/10408690490478091>
- Koynova, R., & Caffrey, M. (1998). Phases and phase transitions of the phosphatidylcholines. *Biochimica et Biophysica Acta*, 1376, 91–145.
- Kuck, L. S., Wesolowski, J. L., & Noreña, C. P. Z. (2017). Effect of temperature and

- relative humidity on stability following simulated gastro-intestinal digestion of microcapsules of Bordo grape skin phenolic extract produced with different carrier agents. *Food Chemistry*, 230, 257–264. <https://doi.org/10.1016/j.foodchem.2017.03.038>
- Kurilich, A. C., Clevidence, B. A., Britz, S. J., Simon, P. W., & Novotny, J. A. (2005). Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. *Journal of Agricultural and Food Chemistry*, 53(16), 6537–6542. <https://doi.org/10.1021/jf050570o>
- Lao, F., & Giusti, M. M. (2018). Extraction of purple corn (*Zea mays L.*) cob pigments and phenolic compounds using food-friendly solvents. *Journal of Cereal Science*, 80, 87–93. <https://doi.org/10.1016/j.jcs.2018.01.001>
- Lopes, C. M., Bettencourt, C., Rossi, A., Buttini, F., & Barata, P. (2016). Overview on gastroretentive drug delivery systems for improving drug bioavailability. *International Journal of Pharmaceutics*, 510(1), 144–158. <https://doi.org/10.1016/j.ijpharm.2016.05.016>
- Lundquist, P., & Artursson, P. (2016). Oral absorption of peptides and nanoparticles across the human intestine: Opportunities, limitations and studies in human tissues. *Advanced Drug Delivery Reviews*, 106, 256–276. <https://doi.org/10.1016/j.addr.2016.07.007>
- Machado, A. P. da F., Rezende, C. A., Rodrigues, R. A., Barbero, G. F., Vieira e Rosa, P. de T., & Martínez, J. (2018). Encapsulation of anthocyanin-rich extract from blackberry residues by spray-drying, freeze-drying and supercritical antisolvent. *Powder Technology*, 340, 553–562. <https://doi.org/10.1016/j.powtec.2018.09.063>
- Machado, A. P. da F., Rueda, M., Barbero, G. F., Martín, Á., Cocero, M. J., & Martínez, J. (2018). Co-precipitation of anthocyanins of the extract obtained from blackberry residues by pressurized antisolvent process. *Journal of Supercritical Fluids*, 137, 81–92. <https://doi.org/10.1016/j.supflu.2018.03.013>
- Madalena, D. A., Pereira, R. N., Vicente, A. A., & Ramos, Ó. L. (2018). New Insights on Bio-Based Micro- and Nanosystems in Food. *Reference Module in Food Science*, 1–7. <https://doi.org/10.1016/B978-0-08-100596-5.21859-3>
- Mao, L., Roos, Y. H., Biliaderis, C. G., & Miao, S. (2017). Food emulsions as delivery systems for flavor compounds: A review. *Critical Reviews in Food Science and Nutrition*, 57(15), 3173–3187. <https://doi.org/10.1080/10408398.2015.1098586>
- Markakis, P. (1982). Chapter 6 - Stability of Anthocyanins in Foods. In P. Markakis

- (Ed.), *Anthocyanins As Food Colors* (pp. 163–180). Academic Press.
<https://doi.org/https://doi.org/10.1016/B978-0-12-472550-8.50010-X>
- Martín, A., & Cocero, M. J. (2008). Micronization processes with supercritical fluids: Fundamentals and mechanisms. *Advanced Drug Delivery Reviews*, 60(3), 339–350. <https://doi.org/10.1016/j.addr.2007.06.019>
- McClements, D. J. (2010). Emulsion Design to Improve the Delivery of Functional Lipophilic Components. *Annual Review of Food Science and Technology*, 1(1), 241–269. <https://doi.org/10.1146/annurev.food.080708.100722>
- McClements, D. J. (2015). Encapsulation, protection, and release of hydrophilic active components: Potential and limitations of colloidal delivery systems. *Advances in Colloid and Interface Science*. <https://doi.org/10.1016/j.cis.2015.02.002>
- McClements, D. J. (2016). *Food Emulsions: Principles, Practice, and Techniques*. (3rd ed.). Boca Raton: CRC Press.
- McClements, D. J. (2017). Designing biopolymer microgels to encapsulate, protect and deliver bioactive components: Physicochemical aspects. *Advances in Colloid and Interface Science*, 240, 31–59. <https://doi.org/10.1016/j.cis.2016.12.005>
- McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science*, 159(2), 213–228. <https://doi.org/10.1016/j.cis.2010.06.010>
- Morais, C. A., de Rosso, V. V., Estadella, D., & Pisani, L. P. (2016). Anthocyanins as inflammatory modulators and the role of the gut microbiota. *Journal of Nutritional Biochemistry*, 33, 1–7. <https://doi.org/10.1016/j.jnutbio.2015.11.008>
- Moser, P., Telis, V. R. N., de Andrade Neves, N., García-Romero, E., Gómez-Alonso, S., & Hermosín-Gutiérrez, I. (2017). Storage stability of phenolic compounds in powdered BRS Violeta grape juice microencapsulated with protein and maltodextrin blends. *Food Chemistry*, 214, 308–318. <https://doi.org/10.1016/j.foodchem.2016.07.081>
- Mourtzinos, I., Makris, D. P., Yannakopoulou, K., Kalogeropoulos, N., Michali, I., & Karathanos, V. T. (2008). Thermal stability of anthocyanin extract of Hibiscus sabdariffa L. in the presence of β-cyclodextrin. *Journal of Agricultural and Food Chemistry*, 56(21), 10303–10310. <https://doi.org/10.1021/jf801389j>
- Mozafari, M. R., Khosravi-Darani, K., Borazan, G. G., Cui, J., Pardakhty, A., & Yurdugul, S. (2008). Encapsulation of food ingredients using nanoliposome

- technology. *International Journal of Food Properties*, 11(4), 833–844. <https://doi.org/10.1080/10942910701648115>
- Numata, M., & Shinkai, S. (2011). “Supramolecular wrapping chemistry” by helix-forming polysaccharides: A powerful strategy for generating diverse polymeric nano-architectures. *Chemical Communications*, 47(7), 1961–1975. <https://doi.org/10.1039/c0cc03133j>
- Oancea, A. M., Aprodu, I., Ghinea, I. O., Barbu, V., Ioniță, E., Bahrim, G., ... Stănciuc, N. (2017). A bottom-up approach for encapsulation of sour cherries anthocyanins by using β -lactoglobulin as matrices. *Journal of Food Engineering*, 210, 83–90. <https://doi.org/10.1016/j.jfoodeng.2017.04.033>
- Oancea, A. M., Hasan, M., Vasile, A. M., Barbu, V., Enachi, E., Bahrim, G., ... Stănciuc, N. (2018). Functional evaluation of microencapsulated anthocyanins from sour cherries skins extract in whey proteins isolate. *Lwt*, 95(April), 129–134. <https://doi.org/10.1016/j.lwt.2018.04.083>
- Okuro, P. K., Furtado, G. F., Sato, A. C. K., & Cunha, R. L. (2015). Structures design for protection and vehiculation of bioactives. *Current Opinion in Food Science*, 5, 67–75. <https://doi.org/10.1016/j.cofs.2015.09.003>
- Panya, A., Laguerre, M., Lecomte, J., Villeneuve, P., Weiss, J., McClements, D. J., & Decker, E. A. (2010). Effects of chitosan and rosmarinate esters on the physical and oxidative stability of liposomes. *Journal of Agricultural and Food Chemistry*, 58(9), 5679–5684. <https://doi.org/10.1021/jf100133b>
- Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2003). The stomach as a site for anthocyanins absorption from food. *FEBS Letters*, 544(1–3), 210–213. [https://doi.org/10.1016/S0014-5793\(03\)00504-0](https://doi.org/10.1016/S0014-5793(03)00504-0)
- Pasukamonset, P., Kwon, O., & Adisakwattana, S. (2016). Alginate-based encapsulation of polyphenols from Clitoria ternatea petal flower extract enhances stability and biological activity under simulated gastrointestinal conditions. *Food Hydrocolloids*, 61, 772–779. <https://doi.org/10.1016/j.foodhyd.2016.06.039>
- Paula, D. de A., Ramos, A. M., de Oliveira, E. B., Martins, E. M. F., de Barros, F. A. R., Vidigal, M. C. T. R., ... da Rocha, C. T. (2018). Increased thermal stability of anthocyanins at pH 4.0 by guar gum in aqueous dispersions and in double emulsions W/O/W. *International Journal of Biological Macromolecules*, 117, 665–672. <https://doi.org/10.1016/j.ijbiomac.2018.05.219>
- Peixoto, F. M., Fernandes, I., Gouvêa, A. C. M. S., Santiago, M. C. P. A., Borguini, R.

- G., Mateus, N., ... Ferreira, I. M. P. L. V. O. (2016). Simulation of in vitro digestion coupled to gastric and intestinal transport models to estimate absorption of anthocyanins from peel powder of jabuticaba, jamelão and jambo fruits. *Journal of Functional Foods*, 24, 373–381. <https://doi.org/10.1016/j.jff.2016.04.021>
- Pérez-Masiá, R., Lagaron, J. M., & López-Rubio, A. (2014). Development and Optimization of Novel Encapsulation Structures of Interest in Functional Foods Through Electrospraying. *Food and Bioprocess Technology*, 7(11), 3236–3245. <https://doi.org/10.1007/s11947-014-1304-z>
- Pisoschi, A. M., Pop, A., Cimpeanu, C., Turcuş, V., Predoi, G., & Iordache, F. (2018). Nanoencapsulation techniques for compounds and products with antioxidant and antimicrobial activity - A critical view. *European Journal of Medicinal Chemistry*, 157, 1326–1345. <https://doi.org/10.1016/j.ejmech.2018.08.076>
- Prior, R. L., & Wu, X. (2006). Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research*, 40(10), 1014, 1028. Retrieved from <http://dx.doi.org/10.1080/10715760600758522>
- Quek, S. Y., Hadi, J., & Tanambell, H. (2019). Application of Electrospinning as Bioactive Delivery System. In *Encyclopedia of Food Chemistry* (pp. 145–149). Elsevier. <https://doi.org/10.1016/b978-0-08-100596-5.22464-5>
- Rafaela, A., Braga, C., Carisa, D., Mendes, L., Mesquita, D. S., Vera, V., ... de Rosso, V. V. (2018). Bioavailability of anthocyanins: Gaps in knowledge, challenges and future research. *Journal of Food Composition and Analysis*, 68(July 2017), 31–40. <https://doi.org/10.1016/j.jfca.2017.07.031>
- Ravanfar, R., Comunian, T. A., & Abbaspourrad, A. (2018). Thermoresponsive, water-dispersible microcapsules with a lipid-polysaccharide shell to protect heat-sensitive colorants. *Food Hydrocolloids*, 81, 419–428. <https://doi.org/10.1016/j.foodhyd.2018.03.030>
- Riaz, M., Zia-Ul-Haq, M., & Saad, B. (2016). SPRINGER BRIEFS IN FOOD, HEALTH, AND NUTRITION *Anthocyanins and Human Health: Biomolecular and therapeutic aspects.* (R. W. Hartel, J. W. Finley, D. Rodriguez-Lazaro, Y. H. Roos, & D. Topping, Eds.). Basel, SWI: Springer Nature. <https://doi.org/10.1007/s10803-015-2603-6>
- Rodríguez-Meizoso, I., & Plaza, M. (2015). Particle Formation of Food Ingredients by Supercritical Fluid Technology. In T. Fornari & R. P. Stateva (Eds.), *High Pressure*

- Fluid Technology for Green Food Processing* (pp. 155–183). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-10611-3_5
- Santana, R. C., Perrechil, F. A., & Cunha, R. L. (2013). High- and Low-Energy Emulsifications for Food Applications: A Focus on Process Parameters. *Food Engineering Reviews*, 5(2), 107–122. <https://doi.org/10.1007/s12393-013-9065-4>
- Santos-Buelga, C., & González-Paramás, A. M. (2018). Anthocyanins. *Reference Module in Food Science*, 1–12. <https://doi.org/10.1016/B978-0-08-100596-5.21609-0>
- Santos-Buelga, C., Mateus, N., & De Freitas, V. (2014). Anthocyanins. Plant pigments and beyond. *Journal of Agricultural and Food Chemistry*, 62(29), 6879–6884. <https://doi.org/10.1021/jf501950s>
- Santos, D. T., Albarelli, J. Q., Beppu, M. M., & Meireles, M. A. A. (2013). Stabilization of anthocyanin extract from jabuticaba skins by encapsulation using supercritical CO₂ as solvent. *Food Research International*, 50(2), 617–624. <https://doi.org/10.1016/j.foodres.2011.04.019>
- Schmitt, C., & Turgeon, S. L. (2011). Protein/polysaccharide complexes and coacervates in food systems. *Advances in Colloid and Interface Science*, 167(1–2), 63–70. <https://doi.org/10.1016/j.cis.2010.10.001>
- Schrooyen, P. M. M., Meer, R. van der, & Kruif, C. G. De. (2001). Microencapsulation: its application in nutrition. *Proceedings of the Nutrition Society*, 60(04), 475–479. <https://doi.org/10.1079/PNS2001112>
- Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B., & Huang, Q. (2018a). Double emulsion followed by complex coacervation as a promising method for protection of black raspberry anthocyanins. *Food Hydrocolloids*, 77, 803–816. <https://doi.org/10.1016/j.foodhyd.2017.11.024>
- Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B., & Huang, Q. (2018b). Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation. *International Journal of Biological Macromolecules*, 107, 1800–1810. <https://doi.org/10.1016/j.ijbiomac.2017.10.044>
- Shehata, T., Ogawara, K. ichi, Higaki, K., & Kimura, T. (2008). Prolongation of residence time of liposome by surface-modification with mixture of hydrophilic polymers. *International Journal of Pharmaceutics*, 359(1–2), 272–279. <https://doi.org/10.1016/j.ijpharm.2008.04.004>

- Shishir, M. R. I., Xie, L., Sun, C., Zheng, X., & Chen, W. (2018). Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. *Trends in Food Science & Technology*, 78(December 2017), 34–60. <https://doi.org/10.1016/j.tifs.2018.05.018>
- Silva, E. K., & Meireles, M. A. A. (2014). Encapsulation of Food Compounds Using Supercritical Technologies: Applications of Supercritical Carbon Dioxide as an Antisolvent. *Food and Public Health*, 4(5), 247–258. <https://doi.org/10.5923/j.fph.20140405.06>
- Silva, P. I., Stringheta, P. C., Teófilo, R. F., de Oliveira, I. R. N. R. N., Teófilo, R. F., & de Oliveira, I. R. N. R. N. (2013). Parameter optimization for spray-drying microencapsulation of jabuticaba (*Myrciaria jabuticaba*) peel extracts using simultaneous analysis of responses. *Journal of Food Engineering*, 117(4), 538–544. <https://doi.org/10.1016/j.jfoodeng.2012.08.039>
- Simões, L. de S., Madalena, D. A., Pinheiro, A. C., Teixeira, J. A., Vicente, A. A., & Ramos, Ó. L. (2017). Micro- and nano bio-based delivery systems for food applications: In vitro behavior. *Advances in Colloid and Interface Science*, 243, 23–45. <https://doi.org/10.1016/j.cis.2017.02.010>
- Smith, K. H., Tejeda-Montes, E., Poch, M., & Mata, A. (2011). Integrating top-down and self-assembly in the fabrication of peptide and protein-based biomedical materials. *Chemical Society Reviews*, 40(9), 4563–4577. <https://doi.org/10.1039/c1cs15064b>
- Souza, A. C. P., Gurak, P. D., & Marczak, L. D. F. (2017). Maltodextrin, pectin and soy protein isolate as carrier agents in the encapsulation of anthocyanins-rich extract from jabuticaba pomace. *Food and Bioproducts Processing*, 102, 186–194. <https://doi.org/10.1016/j.fbp.2016.12.012>
- Stănciuc, N., Oancea, A. M., Aprodu, I., Turturică, M., Barbu, V., Ioniță, E., ... Bahrim, G. (2018). Investigations on binding mechanism of bioactives from elderberry (*Sambucus nigra L.*) by whey proteins for efficient microencapsulation. *Journal of Food Engineering*, 223, 197–207. <https://doi.org/10.1016/j.jfoodeng.2017.10.019>
- Subash, S., Essa, M. M., Al-Adawi, S., Memon, M. A., Manivasagam, T., & Akbar, M. (2014). Neuroprotective effects of berry fruits on neurodegenerative diseases. *Neural Regeneration Research*, 9(16), 1557–1566. <https://doi.org/10.4103/1673-5374.139483>
- Sun, J., Bai, W., Zhang, Y., Liao, X., & Hu, X. (2011). Identification of degradation

- pathways and products of cyanidin-3-sophoroside exposed to pulsed electric field. *Food Chemistry*, 126(3), 1203–1210. <https://doi.org/10.1016/j.foodchem.2010.12.002>
- Svanberg, L., Malmberg, K., Gustinelli, G., Öhgren, C., Persson, I., Brive, L., & Wassén, S. (2019). Effect of anthocyanins on lipid oxidation and microbial spoilage in value-added emulsions with bilberry seed oil, anthocyanins and cold set whey protein hydrogels. *Food Chemistry*, 272(January 2018), 273–278. <https://doi.org/10.1016/j.foodchem.2018.06.064>
- Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry*, 120(2), 599–606. <https://doi.org/10.1016/j.foodchem.2009.10.030>
- Talavéra, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison, J. L., & Rémésy, C. (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *Journal of Agricultural and Food Chemistry*, 53(10), 3902–3908. <https://doi.org/10.1021/jf050145v>
- Tao, Y., Wang, P., Wang, J., Wu, Y., Han, Y., & Zhou, J. (2017). Combining various wall materials for encapsulation of blueberry anthocyanin extracts: Optimization by artificial neural network and genetic algorithm and a comprehensive analysis of anthocyanin powder properties. *Powder Technology*, 311, 77–87. <https://doi.org/10.1016/j.powtec.2017.01.078>
- Taylor, T. M., Davidson, P. M., Bruce, B. D., & Weiss, J. (2005). Liposomal nanocapsules in food science and agriculture. *Critical Reviews in Food Science and Nutrition*, 45(7–8), 587–605. <https://doi.org/10.1080/10408390591001135>
- Temelli, F. (2018). Perspectives on the use of supercritical particle formation technologies for food ingredients. *Journal of Supercritical Fluids*, 134, 244–251. <https://doi.org/10.1016/j.supflu.2017.11.010>
- Thorat, A. A., & Dalvi, S. V. (2012). Liquid antisolvent precipitation and stabilization of nanoparticles of poorly water soluble drugs in aqueous suspensions: Recent developments and future perspective. *Chemical Engineering Journal*, 181–182, 1–34. <https://doi.org/10.1016/j.cej.2011.12.044>
- Tsuda, T. (2012). Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Molecular Nutrition and Food Research*, 56(1), 159–170. <https://doi.org/10.1002/mnfr.201100526>
- Ushikubo, F. Y., Oliveira, D. R. B., Michelon, M., & Cunha, R. L. (2014). Designing

- Food Structure Using Microfluidics. *Food Engineering Reviews*, 7(4), 393–416.
<https://doi.org/10.1007/s12393-014-9100-0>
- Varona, S., Martín, Á., & Cocero, M. J. (2016). Encapsulation of edible active compounds using supercritical fluid. In J. M. Lakkis (Ed.), *Encapsulation and Controlled Release Technologies in Food Systems* (2nd ed., pp. 16–40). West Sussex, UK: Wiley-Blackwell. <https://doi.org/10.1002/9781118946893>
- Velikov, K. P., & Pelan, E. (2008). Colloidal delivery systems for micronutrients and nutraceuticals. *Soft Matter*, 4(10), 1964–1980. <https://doi.org/10.1039/b804863k>
- Wang, S., Marcone, M. F., Barbut, S., & Lim, L. T. (2013). Electrospun soy protein isolate-based fiber fortified with anthocyanin-rich red raspberry (*Rubus strigosus*) extracts. *Food Research International*, 52(2), 467–472.
<https://doi.org/10.1016/j.foodres.2012.12.036>
- Wang, W., Jung, J., & Zhao, Y. (2017). Chitosan-cellulose nanocrystal microencapsulation to improve encapsulation efficiency and stability of entrapped fruit anthocyanins. *Carbohydrate Polymers*, 157, 1246–1253.
<https://doi.org/10.1016/j.carbpol.2016.11.005>
- Yamada, T., Komiya, T., & Akaki, M. (1980). Formation of an inclusion complex of anthocyanin with cyclodextrin. *Agricultural and Biological Chemistry*, 44(6), 1411–1413. <https://doi.org/10.1080/00021369.1980.10864141>
- Yamashita, C., Chung, M. M. S., dos Santos, C., Mayer, C. R. M., Moraes, I. C. F., & Branco, I. G. (2017). Microencapsulation of an anthocyanin-rich blackberry (*Rubus spp.*) by-product extract by freeze-drying. *LWT - Food Science and Technology*, 84, 256–262. <https://doi.org/10.1016/j.lwt.2017.05.063>
- Yan, C., & Zhang, W. (2014). Coacervation Processes. In A. G. Gaonkar, N. Vasisht, A. R. Khare, & R. Sobel (Eds.), *Microencapsulation in the Food Industry* (pp. 125–137). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-404568-2.00012-1>
- Yao, Y., Lin, G., Xie, Y., Ma, P., Li, G., Meng, Q., & Wu, T. (2014). Preformulation studies of myricetin: A natural antioxidant flavonoid. *Pharmazie*, 69(1), 19–26.
<https://doi.org/10.1691/ph.2014.3076>
- Zhao, L., & Temelli, F. (2017). Preparation of anthocyanin-loaded liposomes using an improved supercritical carbon dioxide method. *Innovative Food Science and Emerging Technologies*, 39, 119–128. <https://doi.org/10.1016/j.ifset.2016.11.013>
- Zhao, L., Temelli, F., & Chen, L. (2017). Encapsulation of anthocyanin in liposomes using supercritical carbon dioxide: Effects of anthocyanin and sterol

concentrations. *Journal of Functional Foods*, 34, 159–167.
<https://doi.org/10.1016/j.jff.2017.04.021>



CAPÍTULO III

High-intensity ultrasound-assisted recovery of anthocyanins from jabuticaba by-products using green solvents: effects of nominal power and solvent composition on the extraction of phenolic compounds

Published in Food Research International, v.140, February 2021, 110048

High-intensity ultrasound-assisted recovery of anthocyanins from jabuticaba by-products using green solvents: effects of ultrasound intensity and solvent composition on the extraction of phenolic compounds

Adriana Gadioli Tarone^a; Eric Keven Silva^b; Helena Dias de Freitas Queiroz Barros^a; Cinthia Baú Betim Cazarin^c; Mario Roberto Marostica Junior^a

^a LANUM (Laboratory of Nutrition and Metabolism)/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

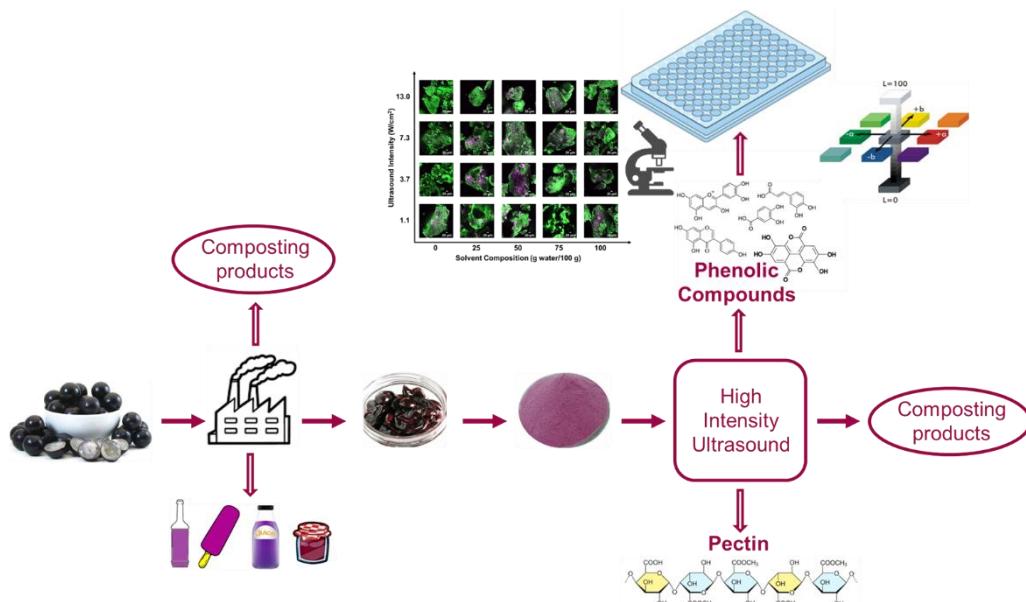
^b LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

^c LAFOP (Laboratory of Protein Source)/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

Highlights

- Jabuticaba peel is an anthocyanins-rich by-product.
- Anthocyanins-rich extracts presented promising antioxidant properties.
- High-intensity ultrasound improved phenolic compounds extraction.
- Ultrasound intensity and solvent composition influenced phenolic compounds recovery.
- CLSM could be used for monitoring the extraction process quality.

Graphical Abstract



Abstract

This study proposes an update for the jabuticaba processing chain to obtain valuable coproducts from jabuticaba peels. High-intensity ultrasound (HIUS) technology was evaluated as a more efficient extraction process to obtain two high added-value coproducts: pectin and an anthocyanins-rich extract. The HIUS-assisted extraction of bioactive compounds like anthocyanins from the jabuticaba peels was evaluated. The effects of ultrasound intensity (1.1, 3.7, 7.3, and 13.0 W/cm²) and solvent composition concerning water/ethanol ratio (0, 25, 50, 75, and 100 g water/100 g) were examined. One-step HIUS processing promoted the best recovery of bioactive compounds at an ultrasound intensity of 3.7 W/cm² and 50 g water/100 g, thus proofing the interaction between ultrasound intensity and the solvent composition has a strong influence on the extraction efficiency of the groups of compounds studied and in the jabuticaba peel antioxidant potential. The confocal laser scanning microscopy confirmed bioactive compounds' exhaustion in the dried jabuticaba peel after the HIUS processing, proving its best recovery. The jabuticaba peel extract exhibited an intense reddish color typical of anthocyanin-rich products at acid pH (4.5). The HIUS technology turned out a promising way to recover these valuable phenolic compounds as a quick, relatively inexpensive, and simple technology that improves the yields and decreases the costs and environmental impacts compared to conventional extraction processes.

Keywords: acoustic cavitation; bioactive compounds; low-frequency ultrasound; confocal microscopy.

1. Introduction

In the last few decades, the search for a healthier diet has increased in the population. Natural foods like fruits and vegetables are increasingly at the consumer table. Brazil is worldwide known for its large biodiversity of native flora and its production of a great variety of edible fruits, which are marketed in their raw or processed form. Fruit consumption is widely associated with health benefits, and berries have been extensively studied in this regard (Albuquerque et al., 2020; Costa, Garcia-Diaz, Jimenez, & Silva, 2013; Gramza-Michałowska, Sidor, & Kulczyński, 2017; Huntley, 2009; Schulz & Chim, 2019; Sidor & Gramza-Michałowska, 2015; Yang & Kortesniemi, 2015).

Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) is a typical Brazilian berry that belongs to the Myrtaceae family. It is a tasty, round fruit with a sweet, white, mucilaginous pulp, and a purple, almost black skin, with an astringent taste (Donadio, 2000). Extremely perishable, jabuticaba has a short commercialization time after harvesting due to its high content of water and sugars and other constituents (Donadio, 2000). For this reason, it is usually transformed into a wide range of products such as jams, liquors, dairy beverages, ice creams, syrups, juice, and others. The jabuticaba processing chain generates a considerable amount of waste by-products, representing up to 50% of the fruit weight between peel and seeds (Morales et al., 2016). The jabuticaba peel consists mostly of insoluble and soluble fibers (pectin) (Neri-Numa, Soriano Sancho, Pereira, & Pastore, 2018). Pectin is widely used in the food and nutraceutical industries for its thickening and gelling properties (De Cindio, Gabriele, & Lupi, 2015). Moreover, many studies show jabuticaba peel as a rich source of phenolic compounds, such as anthocyanins, flavonoids, tannins, ellagic acid, gallic acid, quercetins and others, and they display antioxidant, antimicrobial, antimutagenic and anti-inflammatory activities (Albuquerque et al., 2020; Batista et al., 2014; Lamas et al., 2020; Leite-Legatti et al., 2012; Lenquiste et al., 2015; Plaza et al., 2016). Hence, the jabuticaba peel composition makes this by-product interesting for the food and pharmaceutical industries looking for ways to recover the target compounds (Albuquerque et al., 2020).

Some fruit industries have adopted the biorefineries model to recover high added-value products and harness all potential from their wasted by-products converting them in promising coproducts (Basegmez et al., 2017; Dávila, Rosenberg,

Castro, & Cardona, 2017; González, Moncada, Idarraga, Rosenberg, & Cardona, 2016; Scaglia et al., 2020; Viganó, Zabot, & Martínez, 2017). However, a more productive and sustainable production model is sought to generate value-added products and bioenergy from these jabuticaba by-products (Cherubini, 2010). Emerging technologies such as low frequency and high-power ultrasound have been explored in extraction applications to increase yield while decreasing both the costs and the environmental impact; thus, processes using ultrasound could be more efficient, green, and economically feasible than traditional extraction processes (Dragone et al., 2020; Grigoras, Destandau, Zubrzycki, & Elfakir, 2012).

Considered a clean and green technology, high-intensity ultrasound (HIUS) (16 – 100 kHz and $> 1 \text{ W/cm}^2$) is a fast and simple emerging technique used for phenolic compounds extraction (Al-Dhabi, Ponmurugan, & Maran Jeganathan, 2017). The sonication mechanism is attributed to cavitation on the proliferation of acoustic waves, where physical, chemical, and mechanical effects are generated from the formation and subsequent collapse of microbubbles, increasing the recovery rate of phytochemical compounds from the plant material (Chemat et al., 2017). The main advantages of the HIUS technology are the significant reduction of the extraction time, the improvement of the selectivity and the higher extraction yield, high reproducibility, the simplified handling, low cost, reduced solvent consumption, and has applicability to more diversified samples concerning the other conventional techniques (Baghdikian et al., 2016; Chemat et al., 2017; Tao et al., 2017).

The biorefinery model is already used to obtain new coproducts from the jabuticaba industry. However, the current main process applied to recover phenolic compounds involves high-pressure techniques such as supercritical carbon dioxide technology and pressurized liquid extraction (Santana, Santos, & Meireles, 2019). Both technologies are also considered clean and green. However, their main disadvantages are the high investment and processing cost, greater energy consumption, and longer extraction time (Tabaraki, Heidarizadi, & Benvidi, 2012). Thus, our objective was to evaluate the HIUS technology as a potential replacer for the high-pressure techniques in the production of coproduct rich in anthocyanins and pectin based on a biorefinery model for the jabuticaba industry (**Figure 1**). Thus, in this study the effects of ultrasound intensity (1.1, 3.7, 7.3, and 13.0 W/cm^2) and solvent composition concerning water/ethanol ratio (0, 25, 50, 75, and 100 g water/100 g) on the recovery of phenolic compounds like anthocyanins from jabuticaba peels were examined.

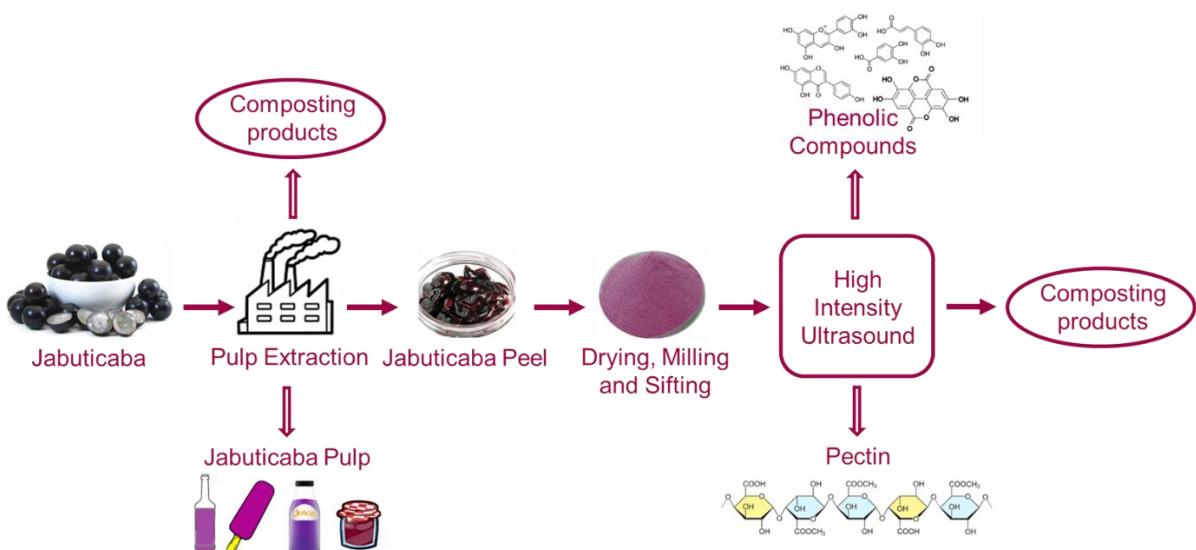


Figure 1. Proposal of biorefinery framework for jabuticaba industry substituting supercritical fluid extraction for high-intensity ultrasound-assisted extraction.

2. Material and methods

2.1. Material

Ethanol absolute, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine); TROLOX (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid); AAPH (2,2'-azobis (2-methylpropionamidine) dihydrochloride), catechin and gallic acid were all obtained from Sigma-Aldrich (São Paulo, Brazil). Fluorescein sodium salt was purchased from Vetec Química Fina (São Paulo, Brazil). Folin-Ciocalteu phenol reagent was purchased from Dinâmica (Brazil). Ultrapure water (resistivity $18.2\text{ M}\Omega\text{ cm}^{-1}$ at 25°C) was obtained with a Millipore OPak 2 (Millipore Corporation, Bedford, MA, USA). The other reagents used were of analytical grade.

2.2. Jabuticaba peel processing

Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg.) fruits were kindly donated by "Indústria e Comércio Lagoa Branca Ltda", located at Boa Vista II Farm, in Casa Branca city (São Paulo, Brazil) under the authorization of the Brazilian Management System of National Genetic Heritage and Traditional Associated Knowledge (#A72354F). The fruits were washed, manually peeled, and the peel was dried in a stove with air circulation (Marconi, Piracicaba, SP, Brazil) at 40°C for 72 h. The dried

peel was transformed into a fine powder by an electrical mill (Marconi, MA 630/1, Piracicaba, SP, Brazil), sifted (mash 20). The dried jabuticaba peel powder was stored at - 20 °C until used.

2.3. Dried jabuticaba peel extractive optimization by high-intensity ultrasound-assisted extraction

The coproducts rich in anthocyanins obtained by HIUS-assisted extraction were obtained using a 13 mm diameter, 19 kHz ultrasonic probe (Unique, Disruptor, Indaiatuba, Brazil) for 3 min. The acoustic powers provided by ultrasound tip to the samples for each nominal power were determined using calorimetric assays according to the methodology described by Mason, Lorimer, Bates & Zhao (1994). Ultrasound intensity (W/cm^2) was calculated using Eq. 1 (Silva & Saldaña, 2020):

$$\text{Ultrasound intensity } \left(\frac{\text{W}}{\text{cm}^2} \right) = \frac{4 \times \text{Acoustic power}}{\pi D^2} \quad (1)$$

where D (cm) is the probe diameter.

The probe contact height with the extracts was kept at 40 mm. The systems were initially at room temperature ($25^\circ\text{C} \pm 1$). An ice bath was used to prevent overheating of the extracts. The temperature of the samples was measured at the outlet of the system. One g of dried jabuticaba peel powder and 25 mL of solvent were used. The effects of ultrasound intensity (W/cm^2) and solvent ratio water/ethanol (g water/100 g) on the bioactive compounds and antioxidant capacity of the extracts were investigated using a full factorial experimental design (4×5) with ultrasound intensity of 1.1 ± 0.1 , 3.7 ± 0.1 , 7.3 ± 0.5 , and $13.0 \pm 1.7 \text{ W}/\text{cm}^2$ and solvent concentration (g water/100 g) of 0, 25, 50, 75 and 100 g water/100 g. Twenty assays were randomly obtained in duplicate. After the HIUS processing, the samples were centrifuged at 10000 rpm for 10 min. The supernatant was collected, and the residue was dried and stored for confocal microscopy analysis.

2.4. Bioactive compounds content and antioxidant capacity characterization

A BioTek Synergy HT Microplate Reader (Winooski, USA) coupled to the data software program Gen5™ 2.0 was used for colorimetric and fluorimetric analyses in 96-well microplates (transparent or dark for ORAC analyses). The methodologies

were adapted to a scale reduction (to analyze in microplate) to save reagents and solvents.

Four classes of bioactive compounds were quantified. The total polyphenolic content measured by reducer capacity was determined by the Folin–Ciocalteu method adapted from Swain and Hillis (Swain & Hillis, 1959). The absorbance was measured at 725 nm. Gallic acid was used in a standard curve. The results were expressed in gallic acid equivalent (mg GAE/L). The monomeric anthocyanins were quantified according to the pH-differential method (Giusti & Wrolstad, 2005) and the results were expressed in mg/L. The content in total flavonoids was determined according to the methodology described by Zhishen, Mengcheng, & Jianming (1999). The absorbance was measured at 510 nm. Catechin was used in a standard curve. The results were expressed in catechin equivalent (mg CE/L). The condensed tannins were measured by the vanillin/HCl reaction method (Herald, Gadgil, Perumal, Bean, & Wilson, 2014). The absorbance was measured at 500 nm. Catechin was used in a standard curve and the results were expressed in catechin equivalent (mg CE/L).

Two methods were used to quantify the antioxidant capacity. The FRAP (Ferric Reducing Antioxidant Power) was determined according to the methodology described by Benzie & Strain (1996). The absorbance was measured at 595 nm. The ORAC (oxygen radical absorbance capacity) was determined according to the methodology described by Ou, Chang, Huang, & Prior (2013) and the absorbance was measured with fluorescent filters at 485 nm for excitation wavelength and 520 nm for emission wavelength. For both methods, Trolox ((\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard and the results were expressed in Trolox equivalent (μ mol TE/L).

2.5. Confocal laser scanning microscopy (CLSM)

The confocal microscopy analyses were performed on the jabuticaba peel powder before and after ultrasound-assisted extractions. They were based on the phenolic compounds' fluorescent properties. After extraction, the powder was analyzed using a Zeiss LSM 780-NLO confocal on an Axio Observer Z.1 microscope (Carl Zeiss AG, Germany) equipped with a 40 \times objective. The images were collected using a laser

wavelength of 514 nm to excite the phenolic compounds' molecules, with the fluorescence emission occurring between 524 nm and 690 nm.

2.6. Color

Color measures were taken in triplicate at room temperature (24 °C) using a spectrophotometer (Hunter Lab - Color Quest II, Reston, USA) in transmittance mode. CIELAB* system (D65, 10° observer) was used, parameters L* a* and b* were registered, and the color rendering of the extracts was obtained. The color measurements were expressed in terms of luminosity L* (L*=0 black and L*=100 white), and of chromaticity defined by a* (+a*=red and -a*=green) and b* (+b*=yellow and -b*=blue). With these parameters, the cylindrical coordinates C* (chroma) and h* (hue angle), which define the intensity and tone of the samples, and color difference (ΔE) between the extracts and a standard solvent (50 g water/100 g) were calculated according to Eqs. 2 (Ferreira et al., 2017), 3 (Ferreira et al., 2017) and 4 (da Silva et al., 2017), respectively.

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

$$h^* = \arctan \left(\frac{b^*}{a^*} \right) \quad (3)$$

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

where Δ means the difference between the extract and the standard solvent.

2.7. Experimental design and statistical analyses

For verifying the effects of ultrasound intensity (W/cm²) and solvent composition (water/ethanol; g water/100 g) on the bioactive compounds, antioxidant capacity and color of the extracts, the experiments were statistically designed and performed according to a full factorial experimental design (4×5) that was completely randomized and conducted in duplicate. A general linear model in MINITAB 16® Statistical Software (Minitab Inc., State College, PA, USA) was used to conduct an analysis of variance (one-way ANOVA) to determine the differences between mean values. Multiple comparisons of mean values were performed by using Tukey's test with a 5% significance (p-value < 0.05 with 95% confidence level).

Analyses of variance also were carried out to compare the other data using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software.

Differences in the mean values obtained were examined by Tukey's test of means at a significance level of 5% (p-value < 0.05 with 95% confidence level).

3. Results and Discussion

3.1. Dried jabuticaba peel extraction by HIUS

The effects of ultrasound intensity and solvent composition on the dried jabuticaba peel extraction were evaluated according to the content of bioactive compounds (total polyphenols, total flavonoids, monomeric anthocyanins, and condensed tannins) and antioxidant capacity (ORAC and FRAP). **Table 1** shows the mean effects of ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the dried jabuticaba peel to these parameters. The content of bioactive compounds and antioxidant capacity were evaluated as a linear effect and as an interaction between ultrasound intensity (X_1) and solvent composition (X_2) function. The results of the analysis of variance (ANOVA) indicated that the linear and interaction contributions were significant, as shown in **Table 1S** (Supplementary Material). The significance of the individual p- values, F-ratios, and R² are also presented in **Table 1S** (Supplementary Material). The model showed high values for R², which indicates that the model could adequately represent the effects of ultrasound intensity and solvent composition on the parameters studied of the coproduct rich in anthocyanins.

Table 1. Influence of ultrasound intensity (W/cm²) and solvent composition (g water/100 g) on the extraction of bioactive compounds from jabuticaba peel and their antioxidant capacity.

Ultrasound Intensity (W/cm ²)	Solvent composition (g water/100 g)	Polyphenol (mg GAE/L)	Anthocyanin (mg/L)	Flavonoids (mg CE/L)	Tannins (mg CE/L)	FRAP (µMol TE/L)	ORAC (µMol TE/L)
1.1	0	282±1	65±5	57±4	1390±347	3351±119	2417±183
	25	2005±104	174±19	263±2	5942±803	20766±1393	5875±503
	50	2924±129	229±18	546±57	7334±789	31487±2144	11882±1256
	75	2471±162	155±7	321±25	8292±198	24969±1370	13650±2114
	100	1349±37	72±6	213±16	2677±393	16429±906	10222±845
3.7	0	425±12	78±3	84±4	1890±404	3840±250	2729±234
	25	2318±98	257±22	351±42	7764±735	25250±2186	16049±11
	50	3391±12	287±12	667±70	11265±2073	43600±3498	14578±1044
	75	2447±348	218±7	553±29	9146±1525	33507±2292	13108±881
	100	1448±182	107±9	344±20	4464±365	20697±1186	11869±1820
7.3	0	420±41	90±3	106±12	2218±339	5246±484	3401±406
	25	2579±125	270±11	430±32	7926±965	28402±1706	10356±830
	50	3028±2	299±15	572±16	9983±2002	40266±1746	12225±547
	75	2825±179	231±23	536±94	6840±817	41083±769	15533±917
	100	1712±46	104±4	292±25	3447±293	19677±906	6720±564
13.0	0	427±21	91±7	103±1	2147±348	5223±231	3197±351
	25	2579±86	244±20	445±46	8034±1118	26866±843	11102±611
	50	3640±142	271±11	607±99	8345±1337	34473±403	17644±1588
	75	3029±286	241±21	536±20	7829±2018	30983±1276	13752±1876
	100	1958 ±5	124±11	349±61	3900±800	21219±968	8020±419

Results are expressed as mean ± standard deviation. GAE = Gallic acid equivalent, TE = Trolox equivalent and CE = Catechin equivalent.

Figure 2 shows the linear effects of ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the content of bioactive compounds and antioxidant capacity values of the extracts. On the other hand, **Figure 3** shows the effects of interaction between ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on these parameters studied. The best results were found employing 3.7 W/cm^2 and 50 g water/100 g.

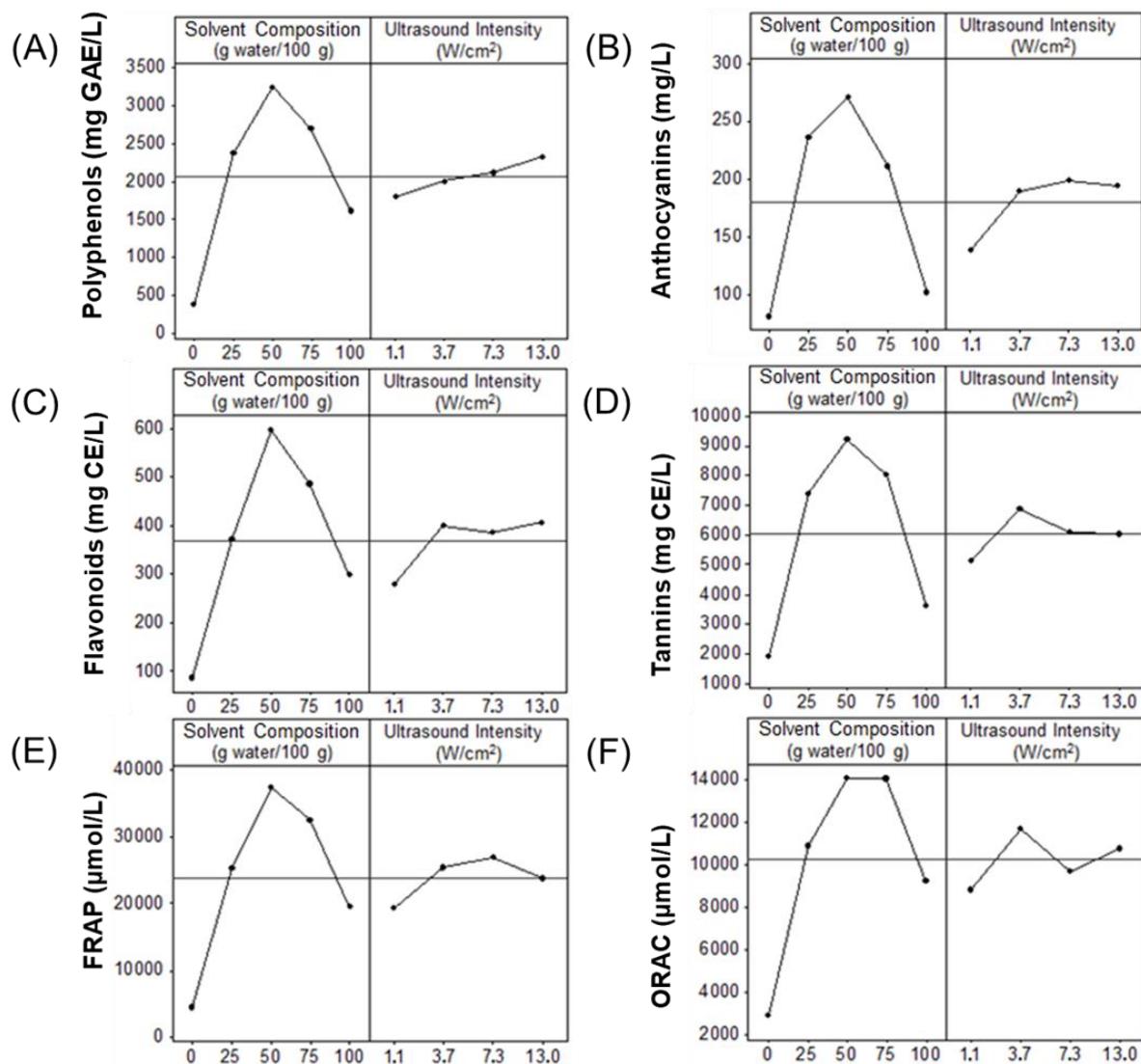


Figure 2. Main effects of ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the content of bioactive compounds (A – Total Polyphenol, B – Monomeric Anthocyanin, C – Total Flavonoids and D – Condensed Tannins) and antioxidant capacity (E – FRAP and F – ORAC) of the anthocyanins-rich extracts.

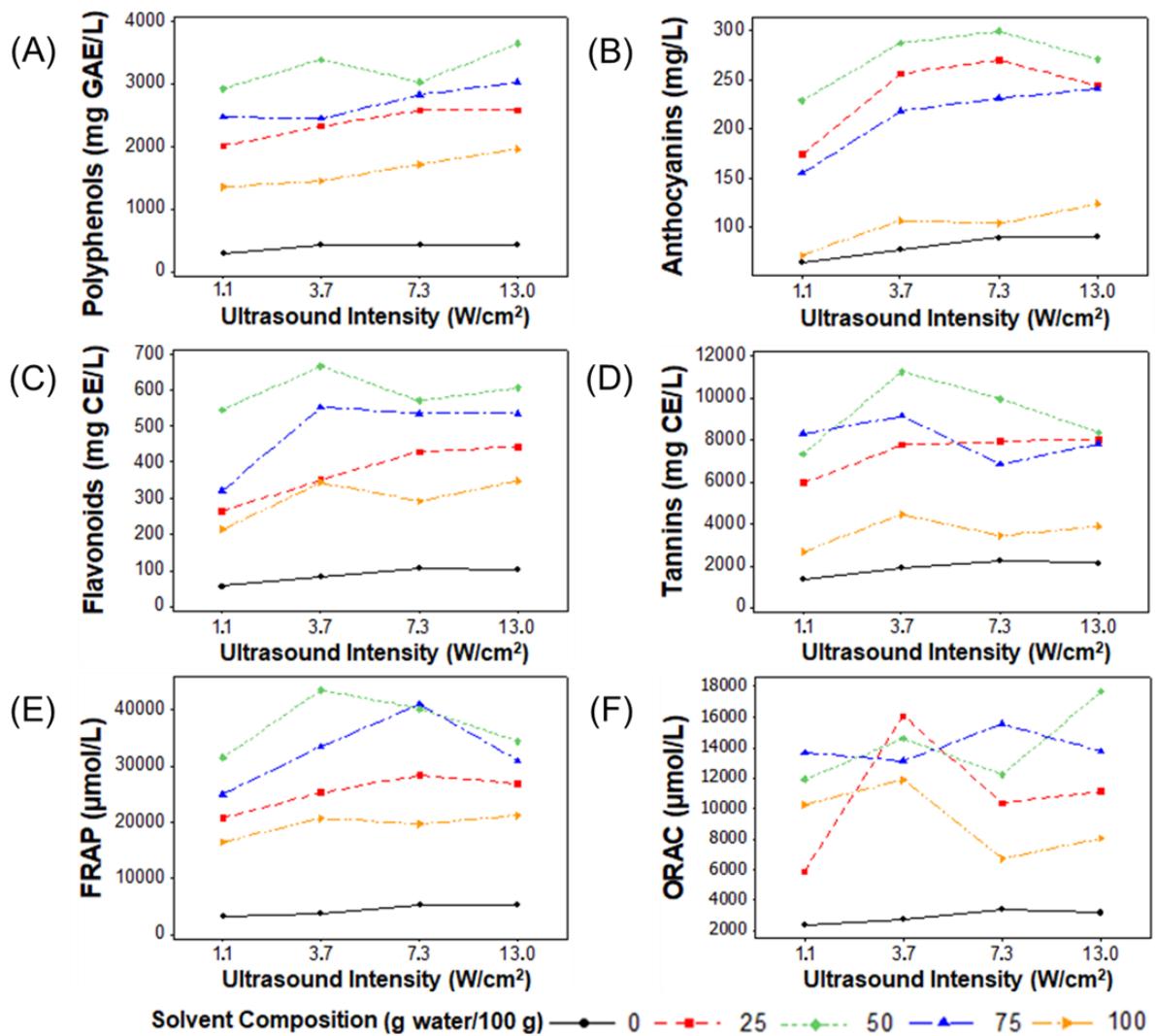


Figure 3. Interaction effects between ultrasound intensity (W/cm²) and solvent composition (g water/100 g) on the content of bioactive compounds (A – Total Polyphenol, B – Monomeric Anthocyanin, C – Total Flavonoids and D – Condensed Tannins) and antioxidant capacity (E – FRAP and F – ORAC) of the anthocyanins-rich extracts.

The ratio of solvents ethanol and water showed more efficiency in extracting phenolic compounds than the use of a mono-component solvent system just with water or ethanol because the mixture reach at the polarity of diverse phenolic compound classes present on the jabuticaba (Azmir et al., 2013; Lao & Giusti, 2018; Tomšík et al., 2016). The ethanol is more effective in extracting flavonoids and tannins than water (Bazykina, Nikolaevskii, Fillipenko, 2002) which has more affinity with anthocyanins (Azmir et al., 2013). Many authors explained that it is possible to improve the solubility of anthocyanins and phenolics using aqueous organic solvent mixtures over a limited

compositional range, with this mixture performing a better extraction of these compounds compared to water and pure organic solvents (Azmir et al., 2013; Delgado-Povedano & Luque de Castro, 2017; Lao & Giusti, 2018; Setford, Jeffery, Grbin, & Muhlack, 2019). This explains why the solvent composition of 50 g water/100 g presented the best results for phenols, anthocyanins, flavonoids, and tannins (**Figure 2**). We observed the similar behavior for FRAP and ORAC (**Figure 2**). The antioxidant capacity to be highly associated with the presence and content of phenolic compounds. Antioxidant capacity changed in the same way as the content of phenolic compounds with the different ratios of water and ethanol, as reported in other studies (Meng et al., 2019; Spigno, Tramelli, & De Faveri, 2007; Zinoviadou et al., 2015). Ghafoor, Hui, & Choi (2011) optimized the ultrasound-assisted extraction of anthocyanins from grape peel using a central composite rotatable design and response surface methodology and found as the best concentration, approximately 50 g water/100 g (water/ethanol) for the maximum total anthocyanins. Wang, Wang, Han, & Han (2016) studied the optimal ultrasound-assisted recovery conditions of polyphenols and anthocyanins from red pears. Likewise, 40 g water/100 g (water/ethanol) was the best solvent ratio for total phenolic compounds and anthocyanins extraction, exhibiting, too, the highest antioxidant capacity (DPPH) at 30°C.

Regarding the ultrasound intensity effects, we did not observe the same behavior for all parameters studied. The major ultrasound intensity (13.0 W/cm²) provided the maximum values for the total phenolic content, which increased with the ultrasound intensity increase. The maximum values for anthocyanin and FRAP were in 7.3 W/cm², with the values for 3.7 W/cm² very close. The ultrasound intensity that gave maximum values for ORAC, tannins, and flavonoids was 3.7 W/cm² (**Figure 2**). The physical, mechanical, and/or chemical effects promoted by ultrasound intensity change several material properties through the acoustical cavitation produced (as a disrupting the physical integrity or acceleration of specific chemical reactions) (Rastogi, 2011). The cavitation microbubbles' formation during the progression of the ultrasonic waves followed by implosions releases large amounts of energy and generates microjets directed towards the solid surface that reach high speeds promoting shear stress. These forces are powerful enough to provoke the cell walls' disruption, increasing the solvent penetration and fast exudation of intracellular components, intensifying the transfer of analyte mass from solid to liquid phase (Chemat, Zill-E-Huma, & Khan, 2011; Rastogi, 2011). This process significantly improves the

extraction of organic compounds from plant material, which increases with the ultrasound intensity increase (Mason, Paniwnyk, & Lorimer, 1996), as observed in this study. The HIUS process intensification by the increase of ultrasound intensity has its main effect on the intensification of the acoustic cavitation phenomenon in the liquid medium. This effect increases the turbulence and temperature in the liquid system, which favors the mass transfer rates. In other words, the diffusion coefficients of the solutes (bioactive compounds), increasing their migration from the plant matrix into the solvent.

On the other hand, the intensification of this phenomenon can promote chemical effects not always desired. The acoustic cavitation increase promotes a high oscillation and the fast collapse of the cavitation bubbles within the solvent, causing major alterations in the microstructure of plant materials by creating greater shear forces that generate critical temperature and pressure. Under these conditions, free radicals could form, which may degrade organic compounds present in the medium (Chemat et al., 2017; Contamine, Wilhelm, Berlan, & Delmas, 1995). Consequently, the extraction rate could be lower for higher ultrasound intensity, as can be seen in **Figure 2 B, C, D, E, and F**. This conclusion leads us to establish 3.7 W/cm² ultrasound intensity as the best one to use for the studied conditions in order to apply the minimum ultrasound intensity required to achieve the best results. Zhao et al. (2006) examined the stability of (all-E)-astaxanthin, one of the carotenoids, under the action of ultrasound in 6 different ultrasound intensities and observed that the degradation of carotenoids increased as ultrasound intensity also increased. Pinela et al. (2019) studied the optimal ultrasound-assisted recovery conditions of anthocyanins from the red calyces of *Hibiscus sabdariffa* by response surface methodology. They combined the effects of three relevant independent variables (time, ultrasound intensity, and ethanol proportion). The authors observed that the ultrasound intensity around 3.7 W/cm² and water proportion around 60 g water/100 g (water/ethanol) obtained the best results, similar to found in this study.

This effect is evident in **Figure 3**. The interaction between both variables tested showed a clear difference in the solvent composition on the parameters studied. The chosen ultrasound intensity was among the best results, although not so clear the differences.

3.2. Temperature

The temperature plays an important role in the extraction of the phenolic compounds (WU et al., 2020). **Figure 4** presents the impact of the ultrasound intensity and solvent composition on the final temperature of the dried jabuticaba peel coproducts right after their HIUS processing.

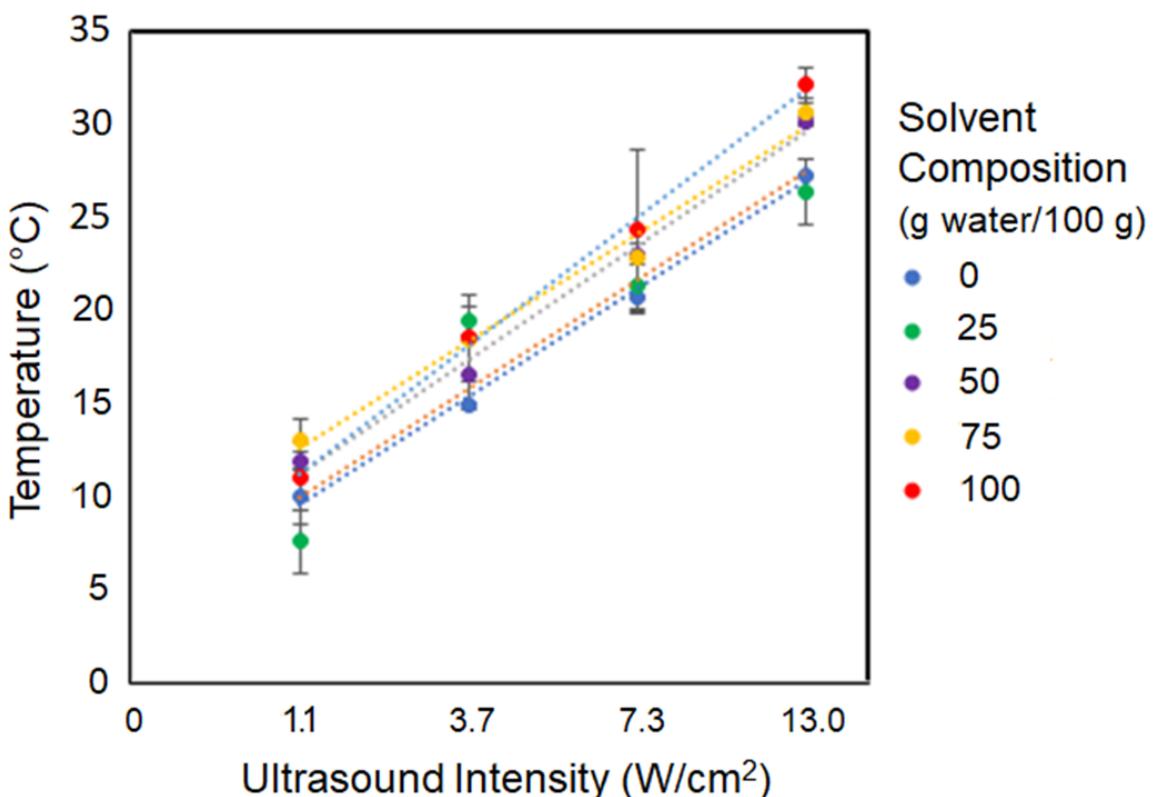


Figure 4. Impact of the ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the final temperature of the anthocyanins-rich extracts right after the high-intensity ultrasound-assisted extraction processing.

The energy released due to cavitation microbubbles results in the generation of pressure, shear, and temperature gradient in the liquid medium through which they propagate (Chemat et al., 2011). This temperature gradient becomes higher with the increase of the ultrasound intensity (Monteiro et al., 2020), which may increase the extraction yield of phenolic compounds by the higher diffusion but may also promote their degradation by oxidation mechanisms (Al-Dhabi et al., 2017; Wang, Cheng, Ma, & Jia, 2020). **Figure 4** shows the increase of the final temperature with the process intensification by increasing ultrasound intensity, which was expected

according to the literature cited above. Monomeric anthocyanins, total flavonoids, condensed tannins, and FRAP were affected by the process intensification. Although the increase of ultrasound intensity increases the diffusion rates into the liquid medium, some bioactive compounds are more sensitive than others to temperature increase. Anthocyanins are very unstable and easily degraded at slightly higher temperatures (He & Giusti, 2010), while tannins and flavonoids are sensitive at high temperatures (Munin & Edwards-Lévy, 2011). Already some classes of polyphenols are very resistant (Al-Dhabi et al., 2017). The higher polyphenol extraction yields were achieved from those treatments more intense, where the higher final temperatures were observed, followed by their antioxidant capacity (ORAC).

3.3. Confocal laser scanning microscopy (CLSM)

Plant tissues cells present an intrinsic property called autofluorescence and their fluorescence emission spectrum may be used to identify secondary metabolites like phenolic acids, flavonoids, chlorophyll, anthocyanins, terpenoids, alkaloids, and others (Talamond, Verdeil, & Conéjero, 2015). Autofluorescence is a photoluminescence process that can be observed when the endogenous atoms or molecules (extracellular matrix and cellular components) are excited by the absorption of electromagnetic radiation in suitable wavelengths. The excited species then relax into the ground state, releasing their excess energy as photons (Skoog, West, Holler, & Crouch, 2008). This property could be used to monitor the extraction process extent of some compounds since any factor able to change the chemical speciation of a molecule (as pH, polarity and concentration of solvent and temperature) may influence their emission of fluorescence and to cause their increase or decrease (García-Plazaola et al., 2015).

Several studies have been demonstrated some polyphenols, tannins, and flavonoids emit in the green region (500-530 nm) of the visible spectrum (Buschmann, Langsdorf, & Lichtenthaler, 2000; Sun et al., 2016; Talamond et al., 2015), and anthocyanins and anthocyanidins emit in the red one (670-700 nm) when they are excited by UV/Vis radiation (Agati, Traversi, & Cerovic, 2008; Chanoca et al., 2015; Moustaka et al., 2018; Roshchina, 2012). Two regions of emission were found in the samples of this study: the emission in the green region (524 nm) and in the red region (690 nm). As cited above, these emissions can characterize groups of polyphenols,

flavonoids and/or tannins in the green region, and a group of anthocyanins in the red one (here showed like magenta due to colorblind people). Most of the experiments showed a more prominent emission than the standard in both regions. Although the solvent has been saturated, this suggests many compounds were exposed and perhaps can be extracted yet. One or more extractions could be done to verify if these compounds are available to transfer to the solvent. **Figures 5 and 6** exhibit the autofluorescence of these two absorbance ranges found in this study for the dried jabuticaba peel before and after HIUS-assisted extraction for the 20 assays performed, respectively.

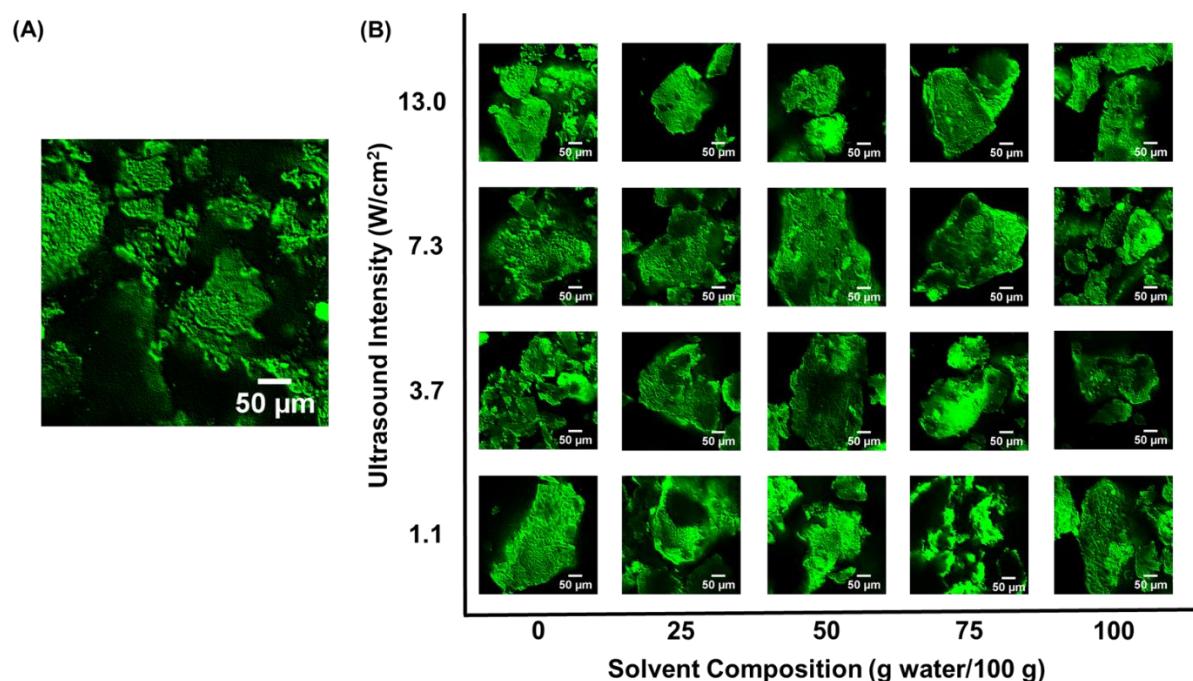


Figure 5. Confocal micrographs of the dried jabuticaba peel before (standard - A) and after high-intensity ultrasound-assisted extraction (B) under UV-radiation. The fluorescence bands in green with $\lambda_{\text{exc.}} = 514 \text{ nm}$ and $\lambda_{\text{em.}} = 524 \text{ nm}$.

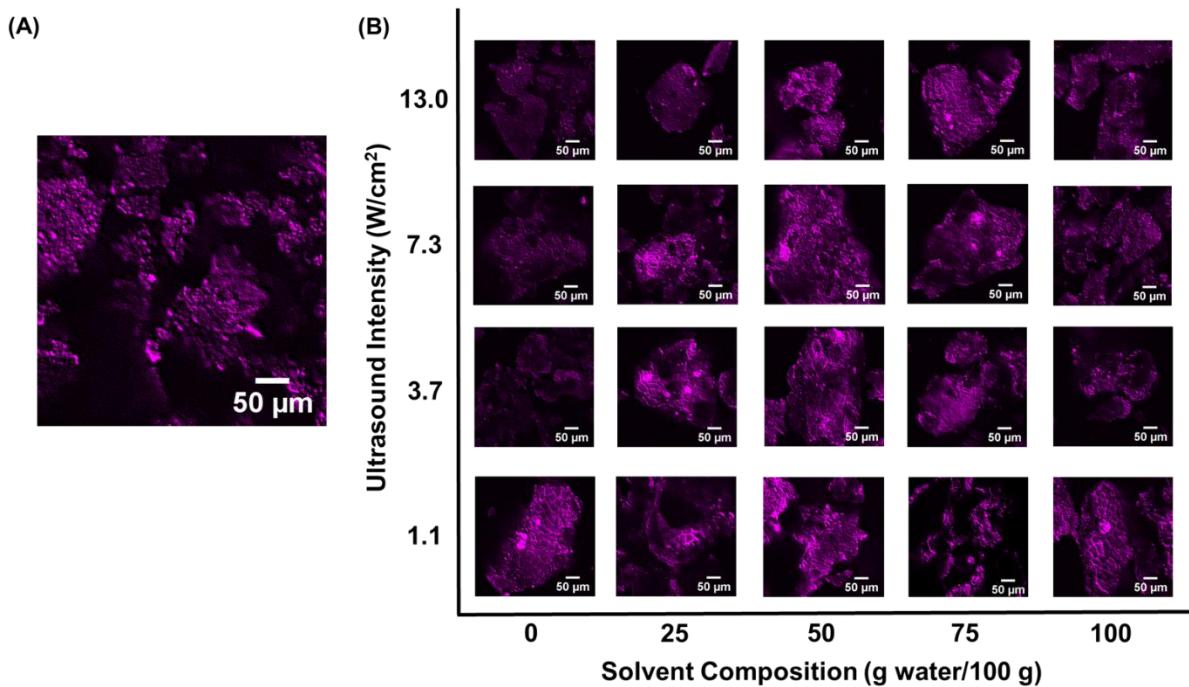


Figure 6. Confocal micrographs of the dried jabuticaba peel before (standard - A) and after high-intensity ultrasound-assisted extraction (B) under UV-radiation. The fluorescence bands in red with $\lambda_{\text{exc.}} = 514 \text{ nm}$ and $\lambda_{\text{em.}} = 690 \text{ nm}$.

We can see in **Figure 5** a minor fluorescence in the condition of 3.7 W/cm^2 and solvent composition of $50 \text{ g water}/100 \text{ g}$. A minor fluorescence means fewer compounds present and, consequently, a better extraction in this condition, corroborating the results found for the contents of total polyphenols, total flavonoids, and condensed tannins. Meanwhile, **Figure 6** shows a different behavior. Solvent mixtures, including the lowest and the highest water contents presented a minor fluorescence, even presenting the lowest results found for the monomeric anthocyanins' contents. This behavior could be associated with some secondary metabolites' fluorescence emission that may change depending on their exposure to the solvent polarity. This phenomenon is known as solvatochromism. It is linked with the conformational state the compound molecule acquires after the exposition to a solvent, which may change their fluorescence emission (for more or less emission) when excited by UV/Vis radiation (García-Plazaola et al., 2015). The solvent effect is closely related to the nature and extent of solute-solvent interactions (Sun, Liang, Zhao, & Fan, 2013).

Anthocyanins can change their conformational state throughout chemical substitutions that include chromophore methoxylation, hydroxylation, glycosylation,

and acylation (Sigurdson, Robbins, Collins, & Giusti, 2018). These structural changes are closely linked with the extraction solvent and its polarity (Lao & Giusti, 2018). They can strongly interfere in their fluorescence emission properties (García-Plazaola et al., 2015). As anthocyanins compose the largest group of water-soluble naturally occurring pigments (He & Giusti, 2010), we can infer in **Figure 6** that the extraction with water contributed to a better fluorescence emission, once micrographs in 100 g water/100 g are stronger than 0 g water/100 g. The ethanol is less polar than water. Solvent mixtures may change their polarity, modifying the interactions between solvent and solute, thus changing the compound's fluorescence emission (Sun et al., 2013).

Consequently, we can infer that the polarity change caused by the solvent mixture contributed to higher emission of the samples in 25, 50, and 75 g water/100 g with an emphasis on 50 g water/100 g. This behavior indicates a better extraction and exposition of anthocyanins in that condition since the fluorescence emission is high in the DJP powder even after the extraction. A minor fluorescence was observed at 3.7 W/cm² using 50 g water/100 g of solvent, which indicates a better anthocyanins extraction corroborating with the results found for the monomeric anthocyanins content.

3.4. Color

Cyanidin, the primary compound of jabuticaba peel, as we could see in this study, was responsible for red colors in the extracts (Lee, Durst, & Wrolstad, 2005). The main color parameters studied were L*, a*, b*, C*, h* and ΔE. **Table 2** shows the mean effects of ultrasound intensity (W/cm²) and solvent composition (g water/100 g) on these parameters. They were evaluated as a linear effect and an interaction between ultrasound intensity (X1) and solvent composition (X2) function. The results of the analysis of variance (ANOVA) indicated that the linear and interaction contributions were significant (p-value < 0.001 for all parameters), and the model showed high values for R² (≥ 0.99), which indicates that the model could adequately describe the effects of the ultrasound intensity and solvent composition on the color parameters studied.

Table 2. Influence of ultrasound intensity (W/cm²) and solvent composition (g water/100 g) on the color parameters of the anthocyanins-rich extracts.

Ultrasound Intensity (W/cm ²)	Solvent Composition (g water/100g)	L*	a*	b*	C*	h°	ΔE
1.1	0	62.68±0.08	21.97±0.18	23.56±0.05	32.21±0.09	0.82±0.01	40.91±0.02
	25	22.97±0.18	49.35±0.15	25.26±0.13	55.43±0.19	0.473±0.001	57.41±0.14
	50	17.15±0.02	46.46±0.01	24.23±0.01	52.39±0.00	0.4808±0.0003	56.33±0.01
	75	17.99±0.01	46.11±0.01	26.21±0.01	53.04±0.02	0.51680±0.00002	56.65±0.02
	100	32.97±0.01	55.25±0.02	34.60±0.01	65.18±0.02	0.5595±0.0001	65.44±0.02
3.7	0	56.70±0.01	27.92±0.04	22.02±0.01	35.55±0.03	0.6678±0.0005	40.42±0.02
	25	17.59±0.05	45.50±0.04	23.77±0.04	51.33±0.06	0.4814±0.0003	55.19±0.04
	50	14.28±0.02	43.16±0.04	20.44±0.07	47.75±0.06	0.442±0.001	53.22±0.07
	75	13.16±0.03	40.68±0.02	19.70±0.04	45.19±0.03	0.4509±0.0005	51.46±0.02
	100	25.57±0.07	51.10±0.11	36.01±0.13	62.51±0.16	0.614±0.001	63.79±0.14
7.3	0	55.76±0.02	27.99±0.03	22.30±0.01	35.79±0.01	0.673±0.001	40.200±0.002
	25	16.95±0.02	44.79±0.08	23.14±0.03	50.41±0.08	0.4769±0.0002	54.56±0.07
	50	14.03±0.01	42.85±0.06	20.09±0.08	47.32±0.09	0.438±0.001	52.94±0.09
	75	10.59±0.21	37.22±0.12	15.86±0.21	40.45±0.19	0.403±0.004	48.72±0.04
	100	19.25±0.25	45.00±0.28	28.67±0.27	53.36±0.38	0.567±0.001	56.55±0.28
13.0	0	53.92±0.04	29.68±0.06	20.41±0.01	36.02±0.05	0.602±0.001	39.59±0.03
	25	15.75±0.02	43.59±0.04	21.94±0.08	48.79±0.07	0.466±0.001	53.55±0.07
	50	13.34±0.02	41.89±0.02	18.88±0.04	45.94±0.03	0.423±0.001	52.03±0.02
	75	5.09±0.11	27.56±0.13	7.39±0.06	28.53±0.14	0.262±0.001	43.31±0.01
	100	10.98±0.09	35.36±0.02	17.07±0.01	39.26±0.03	0.4498±0.0001	47.53±0.03

Figure 7 shows the linear effects of ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the values of the color parameters studied for all the anthocyanins-rich coproducts. The differences in the color rendering of the samples may be better seen in **Figure 8**.

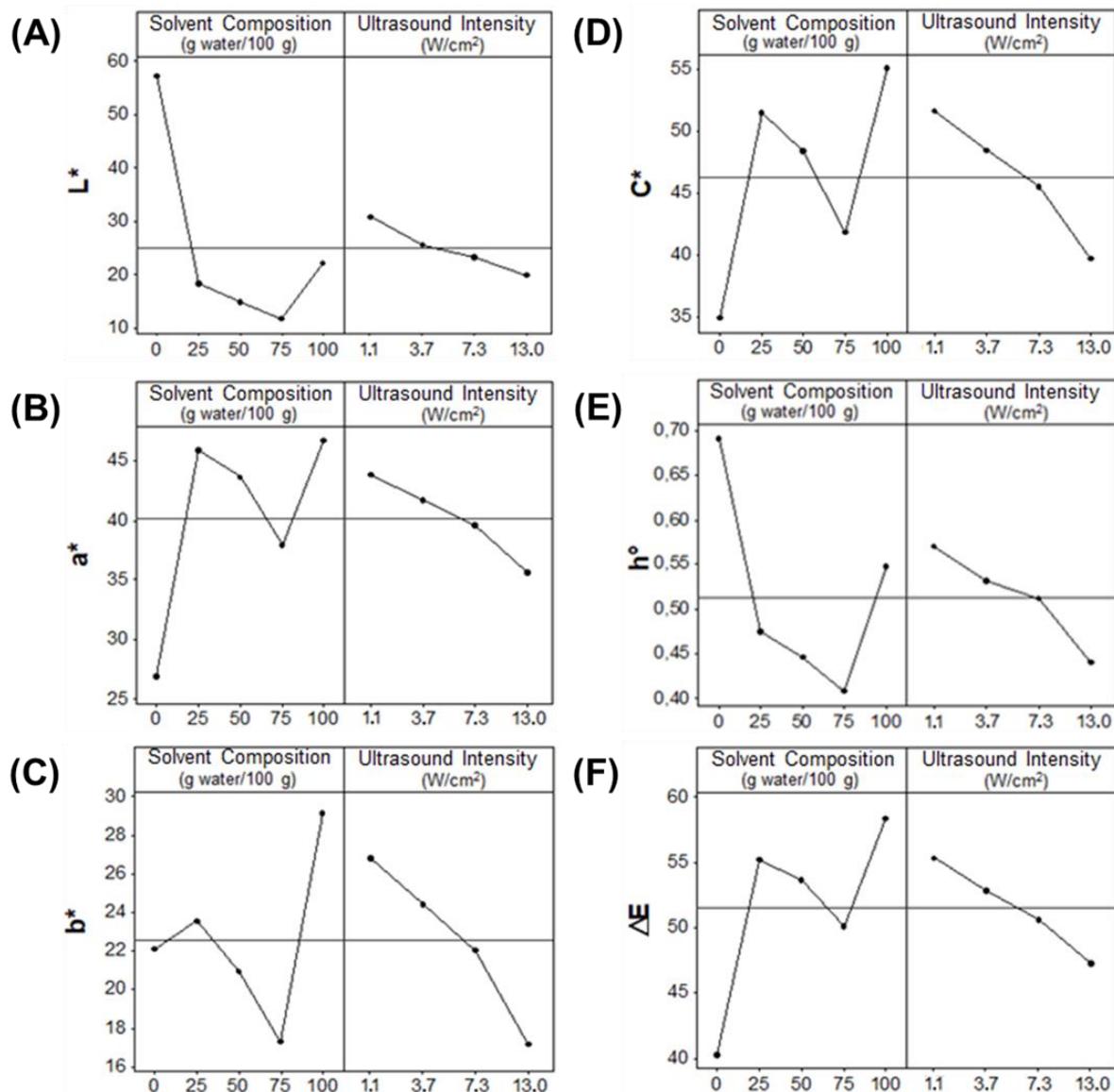


Figure 7. Main effects of ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the color parameters L^* (A), a^* (B), b^* (C), C^* (D), H^* (E) and ΔE (F).

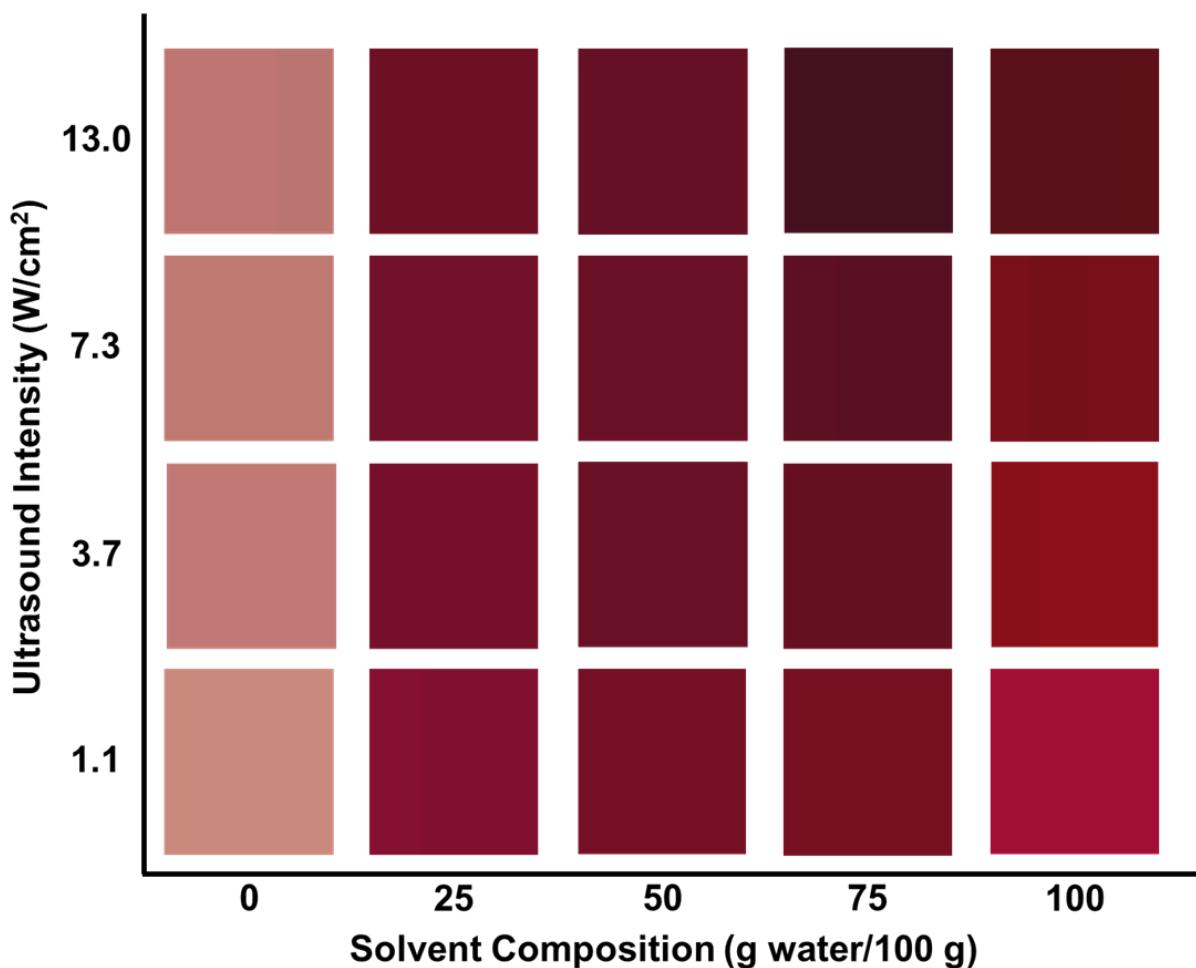


Figure 8. Color rendering of the anthocyanins-rich extracts obtained by the spectrophotometer (Hunter Lab - Color Quest II, Reston, USA) in transmittance mode.

The water concentration increase promoted an L^* and b^* decrease and a^* increase, except for the values of 100 g water/100 g. How L^* is a lightness parameter, the decrease of this parameter with the water concentration increase means that the extracts were becoming darker. With the a^* increase and b^* decrease, a red color was established, which is confirmed by the hue values near 0°, typically found in anthocyanins extracts of red berries in non-basic media (Ferreira et al., 2017; Sigurdson et al., 2018). This inverse behavior between a^* and b^* parameters was correlated by Ferreira et al. (2017) with anthocyanins' high content as also found in this study. A color intensity faded in 0 g water/100 g was observed due to decreases in C^* and increases in L^* values, increasing the hue values. We obtained the opposite behavior with the water concentration increase, increasing the color's intensity, and bringing the hue values closer to 0°. The extracts were closer to intensity brownish color for 100 g water/100 g, according to higher values of a^* , b^* and C^* and hue values

moving away from 0°. These color parameters also were related by these authors as possible degradation products of anthocyanins. The measurement of color difference ΔE shows the extract of 0 g water/100 g of solvent composition is closer to the chosen solvent standard (50 g water/100 g). The ΔE increases with the increase in water concentration. The higher difference is for the extract with 100 g water/100 g, justifying its higher intensity color (higher C^* value).

The process intensification by increasing the ultrasound intensity promoted the decrease of all the parameters. Higher values of these parameters result in more pale and dull hues. The decrease of L^* , a^* , b^* and C^* parameters shows the samples' intensity darkness, which indicates a loss of red color and a minor ΔE in higher ultrasound intensity values. How the content of the bioactive compounds was significantly correlated with the antioxidant capacity and directly related to color parameters (Zhang et al., 2008), in this case with emphasis in the anthocyanins (Tang & Giusti, 2018), this loss of red color corroborates with the results of this study, where we observed a decrease of bioactive compounds and their antioxidant capacity in the higher and smaller ultrasound intensities.

These effects on the color parameters are presented in **Figure 9**, which shows the interaction between ultrasound intensity (W/cm^2) and solvent composition (g water/100 g) on the values of color parameters studied. The impact of the solvent composition on the color parameters studied was observed. The extracts produced using 0 and 100 g water/100 g, in both extremes, had decreased effect of ultrasound intensity on the color parameters.

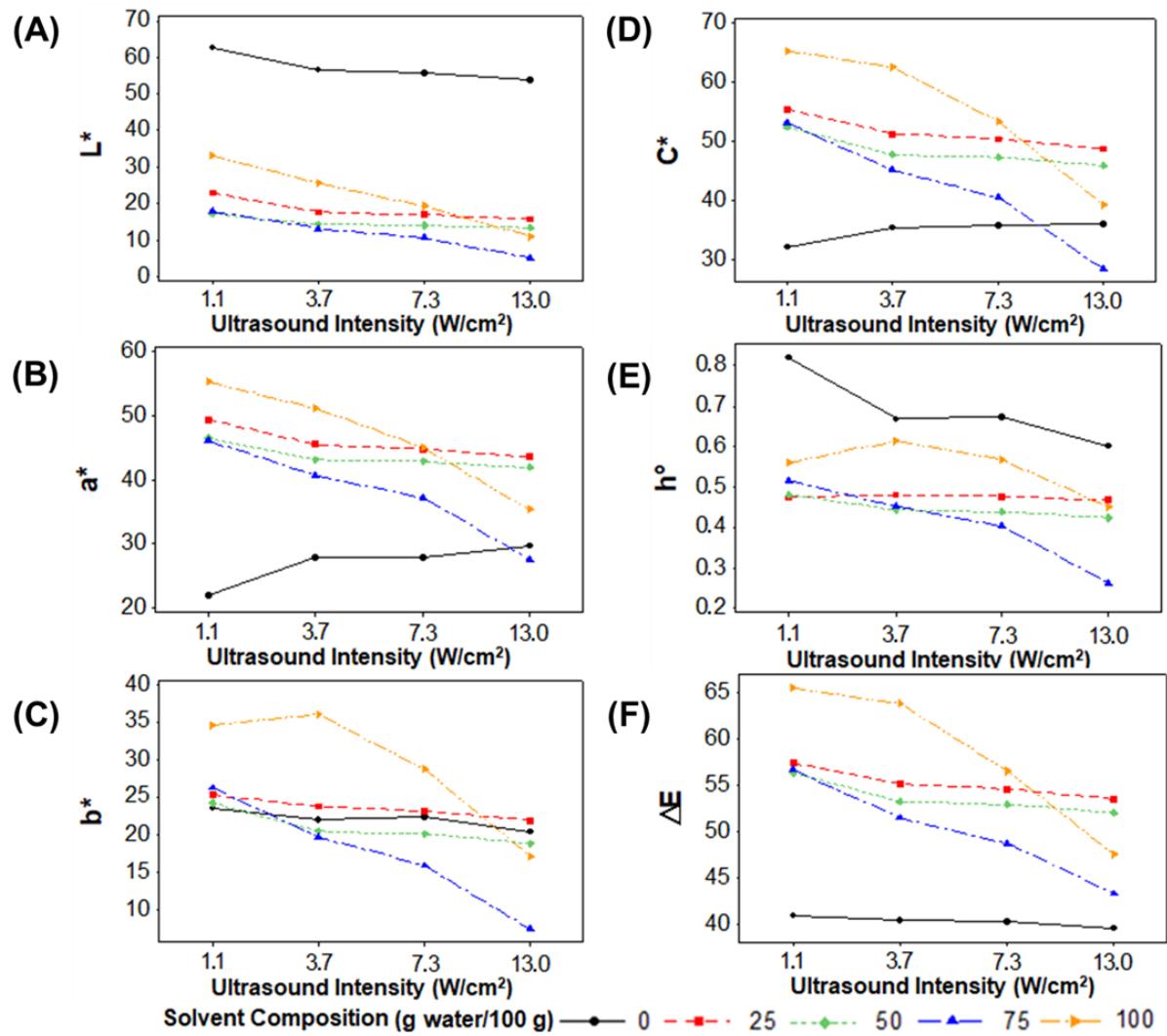


Figure 9. Interaction effects between ultrasound intensity (W/cm²) and solvent composition (g water/100 g) on the color parameters L* (A), a* (B), b* (C), C* (D), H* (E) and ΔE (F).

4. Conclusion

Anthocyanins-rich extracts obtained from the jabuticaba by-product were produced using the HIUS-assisted extraction technique, in addition to obtaining pectin-rich biomass. Our findings were most promising than the results reported by Santos, Albuquerque, & Meireles (2011) and Santos, Veggi, & Meireles (2012) employing high-pressure techniques to recover bioactive compounds from jabuticaba peel. The different conditions of ultrasound intensity and solvent composition (water/ethanol) promoted a strong influence on the bioactive compounds, antioxidant capacity and color parameters. The higher ultrasound intensities presented the lowest values for bioactive compounds and antioxidant capacity. They also presented a brownish color

of extracts, indicating degradation of the compounds and loss of color due to the increase of cavitation effect. For this reason, the best results were achieved for the ultrasound intensity of 3.7 W/cm². The extremes of solvent composition (0 and 100 g water/100 g) presented the lowest values for bioactive compounds and antioxidant capacity, showing the interaction between both solvents in the extraction process is more interesting to extract these compounds and to keep their antioxidant capacity than to use mono-component solvent systems. The best results were obtained using 50 g water/100 g of solvent. The reddish hue and color characteristic of anthocyanins rich extracts were more intense also when there is the interaction between the solvents used. The minor fluorescence in the condition of 3.7 W/cm² and solvent composition of 50 g water/100 g showed a better extraction to the contents of total polyphenols, total flavonoids, and condensed tannins. The mono-component solvent systems were not able to extract anthocyanins, and the condition of 3.7 W/cm² and solvent composition of 50 g water/100 g also showed a better extraction of them. Both coproducts obtained in this study have many applications in the food and nutraceutical industry. The anthocyanins-rich extracts may be applied in chronological diseases treatment and may be used as a preventive treatment to many other disorders. The pectin-rich biomasses may be used as the thickener and gelling agent.

CRediT authorship contribution statement

Adriana Gadioli Tarone: Writing - original draft, Conceptualization, Investigation. Eric Keven Silva: Writing - original draft, Conceptualization, Methodology, Validation. Helena Dias de Freitas Queiroz Barros: Writing - original draft, Investigation, Validation. Cinthia Baú Betim Cazarin: Writing - review & editing, Conceptualization, Validation, Project administration. Mario Roberto Marostica Junior: Writing - review & editing, Conceptualization, Resources, Project administration, 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.

Acknowledgement

Mario R. Marostica Junior are grateful to the National Council for Scientific and Technological Development - CNPQ for his productivity grant (301496/2019-6). Adriana G. Tarone thanks CNPQ (140942/2016-5) for the Ph.D. assistantship. Eric Keven Silva thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (88887.473261/2020-00) for his postdoctoral assistantship at the University of Campinas. Helena D.F.Q. Barros thanks FAPESP (2017/042318) for the Ph.D. assistantship. The authors would like to thank the National Institute of Science and Technology on Photonics Applied to Cell Biology (INFABC) at the University of Campinas for access to equipment and the assistance provided; INFABC is co-funded by FAPESP (2008/57906-3) and CNPq (573913/2008-0). This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; CNPq (403328/2016-0) and FAPESP (2015/50333-1; 2015/13320-9). MRMJ acknowledges Red Iberamericana de Alimentos Autoctonos Subutilizados (ALSUB-CYTED, 118RT0543).

Supplementary material

Table S1. Analysis of variance (ANOVA, $\alpha=0.05$).

Source	Polyphenols (mg GAE/L)		Anthocyanins (mg/L)		Flavonoids (mg CE/L)		Tannins (mg CE/L)		FRAP ($\mu\text{Mol TE/L}$)		ORAC ($\mu\text{Mol TE/L}$)	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Linear effects												
X1 (US. intensity)	49.3	0.000	48.3	0.000	32.5	0.000	5.5	0.007	36.8	0.000	15.6	0.000
X2 (solvent c.)	1025.4	0.000	354.5	0.000	274.3	0.000	80.6	0.000	460.8	0.000	166.9	0.000
Interaction effect												
X1*X2	5.7	0.000	3.2	0.013	3.9	0.004	1.6	0.190	6.7	0.000	11.5	0.000
R ²	0.99		0.98		0.98		0.95		0.99		0.98	

References

- Agati, G., Traversi, M. L., & Cerovic, Z. G. (2008). Chlorophyll Fluorescence Imaging for the Noninvasive Assessment of Anthocyanins ... *Photochemistry and Photobiology*, 84, 1431–1434. <https://doi.org/10.1111/j.1751-1097.2008.00424.x>
- Al-Dhabi, N. A., Ponmurugan, K., & Maran Jegannathan, P. (2017). Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. *Ultrasonics Sonochemistry*, 34, 206–213. <https://doi.org/10.1016/j.ultsonch.2016.05.005>
- Albuquerque, B. R., Pereira, C., Calhelha, R. C., José Alves, M., Abreu, R. M. V., Barros, L., ... Ferreira, I. C. F. R. (2020). Jabuticaba residues (*Myrciaria jaboticaba* (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. *Food Chemistry*, 309, 125735. <https://doi.org/10.1016/j.foodchem.2019.125735>
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436. <https://doi.org/10.1016/j.jfoodeng.2013.01.014>
- Baghdikian, B., Filly, A., Fabiano-Tixier, A. S., Petitcolas, E., Mabrouki, F., Chemat, F., & Ollivier, É. (2016). Extraction by solvent using microwave and ultrasound-assisted techniques followed by HPLC analysis of Harpagoside from *Harpagophytum procumbens* and comparison with conventional solvent extraction methods. *Comptes Rendus Chimie*, 19(6), 692–698. <https://doi.org/10.1016/j.crci.2016.02.020>
- Basegmez, H. I. O., Povilaitis, D., Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Alasalvar, C., & Venskutonis, P. R. (2017). Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO₂, pressurized liquid and enzyme assisted extractions. *Journal of Supercritical Fluids*, 124, 10–19. <https://doi.org/10.1016/j.supflu.2017.01.003>
- Batista, Á. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., Luiz-Ferreira, A., Bogusz, S., ... Maróstica Junior, M. R. (2014). Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods*, 6(1), 450–461. <https://doi.org/10.1016/j.jff.2013.11.011>

- Bazykina, N. I., Nikolaevskii, A. N., Fillipenko, T. a. (2002). Optimization of conditions for the extraction of natural antioxidants from raw plant materials. *Pharmaceutical Chemistry Journal*, 36(2), 46–49.
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Buschmann, C., Langsdorf, G., & Lichtenhaler, H. K. (2000). Imaging of the Blue, Green, and Red Fluorescence Emission of Plants: An Overview. *Photosynthetica*, 38(4), 483–491. <https://doi.org/10.1023/A:1012440903014>
- Chanoca, A., Kovinich, N., Burkel, B., Stecha, S., Bohorquez-Restrepo, A., Ueda, T., ... Otegui, M. S. (2015). Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *Plant Cell*, 27(9), 2545–2599. <https://doi.org/10.1105/tpc.15.00589>
- Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, 34, 540–560. <https://doi.org/10.1016/j.ultsonch.2016.06.035>
- Chemat, F., Zill-E-Huma, & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. In *Ultrasonics Sonochemistry* (Vol. 18, pp. 813–835). <https://doi.org/10.1016/j.ultsonch.2010.11.023>
- Cherubini, F. (2010). The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. *Energy Conversion and Management*, 51(7), 1412–1421. <https://doi.org/10.1016/j.enconman.2010.01.015>
- Contamine, R. F., Wilhelm, A. M., Berlan, J., & Delmas, H. (1995). Power measurement in sonochemistry. *Ultrasonics - Sonochemistry*, 2(1). [https://doi.org/10.1016/1350-4177\(94\)00010-P](https://doi.org/10.1016/1350-4177(94)00010-P)
- Costa, A. G. V., Garcia-Diaz, D. F., Jimenez, P., & Silva, P. I. (2013). Bioactive compounds and health benefits of exotic tropical red–black berries. *Journal of Functional Foods*, 5(2), 539–549. <https://doi.org/10.1016/j.jff.2013.01.029>
- da Silva, J. K., Batista, Â. G., Cazarin, C. B. B., Dionísio, A. P., de Brito, E. S., Marques, A. T. B., & Maróstica Junior, M. R. (2017). Functional tea from a Brazilian berry: Overview of the bioactives compounds. *LWT - Food Science and Technology*, 76,

- 292–298. <https://doi.org/10.1016/j.lwt.2016.06.016>
- Dávila, J. A., Rosenberg, M., Castro, E., & Cardona, C. A. (2017). A model biorefinery for avocado (*Persea americana* mill.) processing. *Bioresource Technology*, 243, 17–29. <https://doi.org/10.1016/j.biortech.2017.06.063>
- De Cindio, B., Gabriele, D., & Lupi, F. R. (2015). Pectin: Properties Determination and Uses. In *Encyclopedia of Food and Health* (pp. 294–300). Elsevier. <https://doi.org/10.1016/B978-0-12-384947-2.00531-6>
- Delgado-Povedano, M. del M., & Luque de Castro, M. D. (2017). Ultrasound-Assisted Extraction of Food Components. *Reference Module in Food Science*, 1–11. <https://doi.org/10.1016/B978-0-08-100596-5.21251-1>
- Donadio, L. (2000). *Jabuticaba (Myrciaria jaboticaba (Vell.) Berg)*. Jaboticabal: FUNEP.
- Dragone, G., Kerssemakers, A. A. J., Driessen, J. L. S. P., Yamakawa, C. K., Brumano, L. P., & Mussatto, S. I. (2020). Innovation and strategic orientations for the development of advanced biorefineries. *Bioresource Technology*. <https://doi.org/10.1016/j.biortech.2020.122847>
- Ferreira, V., Fernandes, F., Carrasco, D., Hernandez, M. G., Pinto-Carnide, O., Arroyo-García, R., ... Castro, I. (2017). Spontaneous variation regarding grape berry skin color: A comprehensive study of berry development by means of biochemical and molecular markers. *Food Research International*, 97(April), 149–161. <https://doi.org/10.1016/j.foodres.2017.03.050>
- García-Plazaola, J. I., Fernández-Marín, B., Duke, S. O., Hernández, A., López-Arbeloa, F., & Becerril, J. M. (2015, July 1). Autofluorescence: Biological functions and technical applications. *Plant Science*. Elsevier Ireland Ltd. <https://doi.org/10.1016/j.plantsci.2015.03.010>
- Ghafoor, K., Hui, T., & Choi, Y. H. (2011). Optimization of ultrasonic-assisted extraction of total anthocyanins from grape peel using response surface methodology. *Journal of Food Biochemistry*, 35(3), 735–746. <https://doi.org/10.1111/j.1745-4514.2010.00413.x>
- Giusti, M. M., & Wrolstad, R. E. (2005). Characterization and Measurement of Anthocyanins by UV-visible Spectroscopy. *Handbook of Food Analytical Chemistry*, 2–2(August 2016), 19–31. <https://doi.org/10.1002/0471709085.ch18>
- González, A. A., Moncada, J., Idarraga, A., Rosenberg, M., & Cardona, C. A. (2016). Potential of the amazonian exotic fruit for biorefineries: The *Theobroma bicolor*

- (Makambo) case. *Industrial Crops and Products*, 86, 58–67. <https://doi.org/10.1016/j.indcrop.2016.02.015>
- Gramza-Michałowska, A., Sidor, A., & Kulczyński, B. (2017, October 1). Berries as a potential anti-influenza factor – A review. *Journal of Functional Foods*. Elsevier Ltd. <https://doi.org/10.1016/j.jff.2017.07.050>
- Grigoras, C. G., Destandau, E., Zubrzycki, S., & Elfakir, C. (2012). Sweet cherries anthocyanins: An environmental friendly extraction and purification method. *Separation and Purification Technology*, 100, 51–58. <https://doi.org/10.1016/j.seppur.2012.08.032>
- He, J., & Giusti, M. M. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annual Review of Food Science and Technology*, 1(1), 163–187. <https://doi.org/10.1146/annurev.food.080708.100754>
- Herald, T. J., Gadgil, P., Perumal, R., Bean, S. R., & Wilson, J. D. (2014). High-throughput micro-plate HCl-vanillin assay for screening tannin content in sorghum grain. *Journal of the Science of Food and Agriculture*, 94(10), 2133–2136. <https://doi.org/10.1002/jsfa.6538>
- Huntley, A. L. (2009, August 20). The health benefits of berry flavonoids for menopausal women: Cardiovascular disease, cancer and cognition. *Maturitas*. <https://doi.org/10.1016/j.maturitas.2009.05.005>
- Lamas, C. A., Kido, L. A., Montico, F., Collares-Buzato, C. B., Maróstica, M. R., & Cagnon, V. H. A. (2020). A jaboticaba extract prevents prostatic damage associated with aging and high-fat diet intake. *Food and Function*, 11(2), 1547–1559. <https://doi.org/10.1039/c9fo02621e>
- Lao, F., & Giusti, M. M. (2018). Extraction of purple corn (*Zea mays* L.) cob pigments and phenolic compounds using food-friendly solvents. *Journal of Cereal Science*, 80, 87–93. <https://doi.org/10.1016/j.jcs.2018.01.001>
- Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, 88(5), 1269–1278. <https://doi.org/10.5555/jaoi.2005.88.5.1269>
- Leite-Legatti, A. V., Batista, A. G., Dragano, N. R. V., Marques, A. C., Malta, L. G., Riccio, M. F., ... Maróstica, M. R. (2012). Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. *Food Research International*, 49(1), 596–603. <https://doi.org/10.1016/j.foodres.2012.07.044>

- Lenquiste, S. A., Marineli, R. da S., Moraes, É. A., Dionísio, A. P., Brito, E. S. de, & Maróstica Junior, M. R. (2015). Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. *Food Research International*, 77, 162–170. <https://doi.org/10.1016/j.foodres.2015.07.023>
- Mason, T. J., Lorimer, J. P., Bates, D. M., & Zhao, Y. (1994). Dosimetry in sonochemistry: The use of aqueous terephthalate ion as a fluorescence monitor. *Ultrasonics - Sonochemistry*, 1(2), 2–6. [https://doi.org/10.1016/1350-4177\(94\)90004-3](https://doi.org/10.1016/1350-4177(94)90004-3).
- Mason, T. J., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry*, 3(3). [https://doi.org/10.1016/S1350-4177\(96\)00034-X](https://doi.org/10.1016/S1350-4177(96)00034-X)
- Meng, L., Zhu, J., Ma, Y., Sun, X., Li, D., Li, L., ... Meng, X. (2019). Composition and antioxidant activity of anthocyanins from Aronia melanocarpa cultivated in Haicheng, Liaoning, China. *Food Bioscience*, 30, 100413. <https://doi.org/10.1016/j.fbio.2019.100413>
- Monteiro, S. H. M. C., Silva, E. K., Guimarães, J. T., Freitas, M. Q., Meireles, M. A. A., & Cruz, A. G. (2020). High-intensity ultrasound energy density: How different modes of application influence the quality parameters of a dairy beverage. *Ultrasonics Sonochemistry*, 63, 104928. <https://doi.org/10.1016/j.ultsonch.2019.104928>
- Morales, P., Barros, L., Dias, M. I., Santos-Buelga, C., Ferreira, I. C. F. R., Ramirez Asquieri, E., & Berrios, J. D. J. (2016). Non-fermented and fermented jabuticaba (*Myrciaria cauliflora* Mart.) pomaces as valuable sources of functional ingredients. *Food Chemistry*, 208, 220–227. <https://doi.org/10.1016/j.foodchem.2016.04.011>
- Moustaka, J., Panteris, E., Adamakis, I. D. S., Tanou, G., Giannakoula, A., Eleftheriou, E. P., & Moustakas, M. (2018). High anthocyanin accumulation in poinsettia leaves is accompanied by thylakoid membrane unstacking, acting as a photoprotective mechanism, to prevent ROS formation. *Environmental and Experimental Botany*, 154, 44–55. <https://doi.org/10.1016/j.envexpbot.2018.01.006>
- Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds: a review. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics3040793>

- Neri-Numa, I. A., Soriano Sancho, R. A., Pereira, A. P. A., & Pastore, G. M. (2018). Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. *Food Research International*, 103, 345–360. <https://doi.org/10.1016/j.foodres.2017.10.053>
- Ou, B., Chang, T., Huang, D., & Prior, R. L. (2013). Determination of total antioxidant capacity by oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescence probe: First action 2012.23. *Journal of AOAC International*, 96(6), 1372–1376. <https://doi.org/10.5740/jaoacint.13-175>
- Pinela, J., Prieto, M. A., Pereira, E., Jabeur, I., Barreiro, M. F., Barros, L., & Ferreira, I. C. F. R. (2019). Optimization of heat- and ultrasound-assisted extraction of anthocyanins from Hibiscus sabdariffa calyces for natural food colorants. *Food Chemistry*, 275(September 2018), 309–321. <https://doi.org/10.1016/j.foodchem.2018.09.118>
- Plaza, M., Batista, Â. G., Cazarin, C. B. B., Sandahl, M., Turner, C., Ostman, E., & Maróstica Junior, M. R. (2016). Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. *Food Chemistry*, 211, 185–197. <https://doi.org/10.1016/j.foodchem.2016.04.142>
- Rastogi, N. K. (2011). Opportunities and challenges in application of ultrasound in food processing. *Critical Reviews in Food Science and Nutrition*, 51(8), 705–722. <https://doi.org/10.1080/10408391003770583>
- Roshchina, V. V. (2012). Vital Autofluorescence: Application to the Study of Plant Living Cells. *International Journal of Spectroscopy*, 2012, 1–14. <https://doi.org/10.1155/2012/124672>
- Santana, Á. L., Santos, D. T., & Meireles, M. A. A. (2019, August 1). Perspectives on small-scale integrated biorefineries using supercritical CO₂ as a green solvent. *Current Opinion in Green and Sustainable Chemistry*. Elsevier B.V. <https://doi.org/10.1016/j.cogsc.2018.11.007>
- Santos, D. T., Albuquerque, C. L. C., & Meireles, M. A. A. (2011). Antioxidant dye and pigment extraction using a homemade pressurized solvent extraction system. *Procedia Food Science*, 1, 1581–1588. <https://doi.org/10.1016/j.profoo.2011.09.234>
- Santos, D. T., Veggi, P. C., & Meireles, M. A. A. (2012). Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba

- skins. *Journal of Food Engineering*, 108(3), 444–452.
<https://doi.org/10.1016/j.jfoodeng.2011.08.022>
- Scaglia, B., D'Incecco, P., Squillace, P., Dell'Orto, M., De Nisi, P., Pellegrino, L., ... Adani, F. (2020). Development of a tomato pomace biorefinery based on a CO₂-supercritical extraction process for the production of a high value lycopene product, bioenergy and digestate. *Journal of Cleaner Production*, 243. <https://doi.org/10.1016/j.jclepro.2019.118650>
- Schulz, M., & Chim, J. F. (2019, October 1). Nutritional and bioactive value of Rubus berries. *Food Bioscience*. Elsevier Ltd. <https://doi.org/10.1016/j.fbio.2019.100438>
- Setford, P. C., Jeffery, D. W., Grbin, P. R., & Muhlack, R. A. (2019). Mathematical modelling of anthocyanin mass transfer to predict extraction in simulated red wine fermentation scenarios. *Food Research International*, 121, 705–713. <https://doi.org/10.1016/j.foodres.2018.12.044>
- Sidor, A., & Gramza-Michałowska, A. (2015, October 1). Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food - a review. *Journal of Functional Foods*. Elsevier Ltd. <https://doi.org/10.1016/j.jff.2014.07.012>
- Sigurdson, G. T., Robbins, R. J., Collins, T. M., & Giusti, M. M. (2018). Impact of location, type, and number of glycosidic substitutions on the color expression of o-dihydroxylated anthocyanidins. *Food Chemistry*, 268, 416–423. <https://doi.org/10.1016/j.foodchem.2018.06.079>
- Silva, E. K., & Saldaña, M. D. A. (2020). High-intensity ultrasound-assisted recovery of cinnamyl alcohol glycosides from *Rhodiola rosea* roots: Effect of probe diameter on the ultrasound energy performance for the extraction of bioactive compounds. *Food and Bioproducts Processing*, 122, 245–253. <https://doi.org/10.1016/j.fbp.2020.05.012>
- Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2008). 27. Espectroscopia de Fluorescência Molecular. In *Fundamentos de Química Analítica* (8th ed., pp. 782–795). São Paulo: Cengage Learning. <https://doi.org/10.1017/CBO9781107415324.004>
- Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering*, 81(1), 200–208. <https://doi.org/10.1016/j.jfoodeng.2006.10.021>
- Sun, L., Chen, W., Meng, Y., Yang, X., Yuan, L., & Guo, Y. (2016). Interactions

- between polyphenols in thinned young apples and porcine pancreatic α -amylase: Inhibition, detailed kinetics and fluorescence quenching. *Food Chemistry*, 208, 51–60. <https://doi.org/10.1016/j.foodchem.2016.03.093>
- Sun, Y., Liang, X., Zhao, Y., & Fan, J. (2013). Solvent effects on the absorption and fluorescence spectra of rhabonticin: Experimental and theoretical studies. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 102, 194–199. <https://doi.org/10.1016/j.saa.2012.10.013>
- Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), 63–68. <https://doi.org/10.1002/jsfa.2740100110>
- Tabaraki, R., Heidarizadi, E., & Benvidi, A. (2012). Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum* L.) peel antioxidants by response surface methodology. *Separation and Purification Technology*, 98, 16–23. <https://doi.org/10.1016/j.seppur.2012.06.038>
- Talamond, P., Verdeil, J. L., & Conéjero, G. (2015). Secondary metabolite localization by autofluorescence in living plant cells. *Molecules*, 20(3), 5024–5037. <https://doi.org/10.3390/molecules20035024>
- Tang, P., & Giusti, M. M. (2018). Black goji as a potential source of natural color in a wide pH range. *Food Chemistry*, 269, 419–426. <https://doi.org/10.1016/j.foodchem.2018.07.034>
- Tao, Y., Wang, Y., Pan, M., Zhong, S., Wu, Y., Yang, R., ... Zhou, J. (2017). Combined ANFIS and numerical methods to simulate ultrasound-assisted extraction of phenolics from chokeberry cultivated in China and analysis of phenolic composition. *Separation and Purification Technology*, 178, 178–188. <https://doi.org/10.1016/j.seppur.2017.01.012>
- Tomšík, A., Pavlić, B., Vladić, J., Ramić, M., Brindza, J., & Vidović, S. (2016). Optimization of ultrasound-assisted extraction of bioactive compounds from wild garlic (*Allium ursinum* L.). *Ultrasonics Sonochemistry*, 29, 502–511. <https://doi.org/10.1016/j.ultsonch.2015.11.005>
- Viganó, J., Zabot, G. L., & Martínez, J. (2017). Supercritical fluid and pressurized liquid extractions of phytonutrients from passion fruit by-products: Economic evaluation of sequential multi-stage and single-stage processes. *Journal of Supercritical Fluids*, 122, 88–98. <https://doi.org/10.1016/j.supflu.2016.12.006>
- Wang, M., Wang, C., Han, R. H., & Han, X. (2016). Novel advances in shotgun

lipidomics for biology and medicine. *Progress in Lipid Research*, 61, 83–108.
<https://doi.org/10.1016/j.plipres.2015.12.002>

Wang, P., Cheng, C., Ma, Y., & Jia, M. (2020). Degradation behavior of polyphenols in model aqueous extraction system based on mechanical and sonochemical effects induced by ultrasound. *Separation and Purification Technology*, 247, 116967.
<https://doi.org/10.1016/j.seppur.2020.116967>

Wu, L., Li, L., Chen, S., Wang, L., & Lin, X. (2020). Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from *Moringa oleifera* L. leaves: Optimization, comparison and antioxidant activity. *Separation and Purification Technology*, 247, 117014.
<https://doi.org/10.1016/j.seppur.2020.117014>

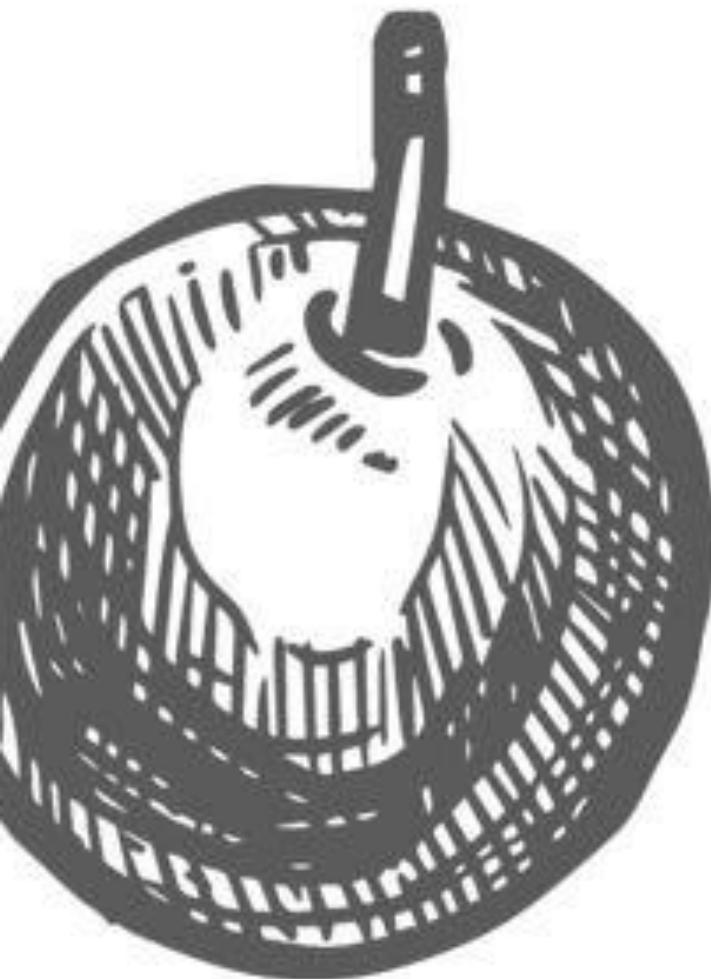
Yang, B., & Kortesniemi, M. (2015, April 1). Clinical evidence on potential health benefits of berries. *Current Opinion in Food Science*. Elsevier Ltd.
<https://doi.org/10.1016/j.cofs.2015.01.002>

Zhang, W., Li, X., Zheng, J., Wang, G., Sun, C., Ferguson, I. B., & Chen, K. (2008). Bioactive components and antioxidant capacity of Chinese bayberry (*Myrica rubra* Sieb. and Zucc.) fruit in relation to fruit maturity and postharvest storage. *European Food Research and Technology*, 227(4), 1091–1097.
<https://doi.org/10.1007/s00217-008-0824-z>

Zhao, L., Zhao, G., Chen, F., Wang, Z., Wu, J., & Hu, X. (2006). Different effects of microwave and ultrasound on the stability of (all-E)-astaxanthin. *Journal of Agricultural and Food Chemistry*, 54(21), 8346–8351.
<https://doi.org/10.1021/jf061876d>

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

Zinoviadou, K. G., Galanakis, C. M., Brnčić, M., Grimi, N., Boussetta, N., Mota, M. J., ... Barba, F. J. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, 77, 743–752. <https://doi.org/10.1016/j.foodres.2015.05.032>



CAPÍTULO IV

Antioxidant capacity and bioaccessibility in *in vitro* gastrointestinal digestion of phenolic compounds extracted from jabuticaba peel

To be submitted to Food Chemistry

Antioxidant capacity and bioaccessibility in *in vitro* gastrointestinal digestion of phenolic compounds extracted from jabuticaba peel

Adriana Gadioli Tarone^a; Pascale Goupy^b; Christian Ginies^b; Mario Roberto Marostica Junior^a; Claire Dufour^b

^a UNICAMP - University of Campinas, School of Food Engineering, LANUM; Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil.

^b INRAE, Avignon University, UMR408 Safety and Quality of Plant Products, F-84000 Avignon, France.

Abstract

High-intensity ultrasound (HIUS) allowed the extraction of hydrolyzable tannins, anthocyanins and flavonols from jabuticaba peel with a similar composition to conventional solvent extraction. HIUS was highly efficient for the extraction of poorly soluble hydrolyzable tannins although affecting the stability of anthocyanins and flavonols. UPLC-DAD-MSⁿ allowed the identification of 44 hydrolyzable tannins as single and mixed hexose derivatives bearing galloyl, HHDP and tergalloyl units. Eleven mixed HHDP-galloylgluconic acids and tergalloyl hexosides were newly discovered. Additionally, acid hydrolysis of both HIUS extract and dried jabuticaba peel conveniently yielded five quantifiable contributors (gallic acid, ellagic acid, gallic acid-C-hexoside, valoneic acid dilactone and sanguisorbic acid dilactone). Largely higher contents in hydrolyzable tannins were also afforded when compared to individual polyphenol analysis by UPLC. Cyanidin-3-O-glucoside and hydrolyzable tannins from the HIUS extract were shown to contribute to the strong inhibition of heme-induced lipoperoxidation during gastric digestion, limiting the formation of lipid-derived conjugated dienes and deleterious 4-hydroxynonenal. Besides that, anthocyanins demonstrating high bioaccessibility.

1. Introduction

Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) is a Brazilian berry that has been widely studied in the last decades for presenting a high potential to reduce the risk of chronic diseases, and its healthy effect comes from the high content and great diversity of phenolic compounds present mainly in its peel (ALBUQUERQUE et al., 2020; BATISTA et al., 2014; DA SILVA et al., 2017; LAMAS et al., 2020; LEITE-LEGATTI et al., 2012; LENQUISTE et al., 2015; PLAZA et al., 2016). As a waste of the jabuticaba processing chain, the peel may represent up to 30% of the fruit weight. The peel is considered as a rich source of phenolic compounds mainly constituted by anthocyanins and hydrolysable tannins such as ellagic and gallic acid derivatives (NEVES et al., 2018; PLAZA et al., 2016; QUATRIN et al., 2019). The ultrasound is a new and emerging technology considered as a green extraction method that is being used to improve the extraction of these compounds and decrease the costs and environmental effects (CHEMAT et al., 2017).

Anthocyanins are the most abundant compounds found in jabuticaba peel and some authors suggest that they are responsible for the healthy effects assigned to the fruit peel (NEVES et al., 2018; PLAZA et al., 2016; QUATRIN et al., 2019). These putative health-promoting effects of anthocyanins are related to their potent antioxidant property. The Western diet widely consumed these days presents high amounts of n-6 polyunsaturated fatty acids (n-6 PUFA), which are responsible for many inflammatory disorders provoked by their oxidation (CHRIST; LAUTERBACH; LATZ, 2019). The lipid peroxides and radical oxygen species generated in the PUFA oxidation are effectively scavenged by anthocyanins, whose antioxidant capacity may finish the chain reaction responsible for the oxidative damage (HE; GIUSTI, 2010). However, the effectiveness of anthocyanins at this process depends on their bioaccessibility (FARIA et al., 2009).

Thus, this study aimed to perform the identification and quantification of the phenolic compounds present in two different extracts of jabuticaba peel as well as to assess the efficiency of high-intensity ultrasound for extraction of dried jabuticaba peel. Moreover, we evaluated the antioxidant capacity of the dried jabuticaba peel extract obtained by high-intensity ultrasound against lipid oxidation and its bioaccessibility in the *in vitro* gastrointestinal digestion of a Western-type diet. This is the first study of the antioxidant capacity of jabuticaba peel taking into consideration both food and gastric digestion conditions.

2. Materials and Methods

2.1. Materials

Kuromanin (Cyanidin 3-O-glucoside), gallic acid, ellagic acid and quercetin 3-O-glucoside (isoquercitrin) were purchased from Extrasynthese (Genay, France). Methanol, acetonitrile, hexane and 2-propanol were HPLC-MS grade from Fisher Scientific (Illkirch, France); formic acid was HPLC-MS grade from Merck (Darmstadt, Germany. Ultrapure water (resistivity $18.2\text{ M}\Omega\text{ cm}^{-1}$ at $25\text{ }^{\circ}\text{C}$) was obtained with a Millipore OPak 2 (Millipore Corporation, Bedford, MA, USA).

Horse heart myoglobin (M1882, type II), pepsin from porcine gastric mucosa (P6887, 2829 U/mg with protocol from Minekus et al. (2014)), pancreatin from porcine (P7545, trypsin activity 2.8 U/mg with protocol from Minekus et al. (2014)), bile extract porcine (B8631) and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). 4-Hydroxy-2-nonenal and 4-hydroxy-2-nonenal-D3 were purchased from Bertin Pharma (Montigny le Bretonneux, France). Commercial sunflower oil (Lot No. A07611) was purchased from Auchan store and stored at $-20\text{ }^{\circ}\text{C}$. According to the manufacturer (Auchan), the commercial sunflower oil contained 50 mg tocopherol/100 g of oil. L- α -phosphatidylcholine from dried egg yolk (P3556) (PL) was purchased from Fluka (Buchs, Switzerland) and its composition analyzed by BOLÉA et al. (2019). They found that commercial PL contained phosphatidylcholine (33%), phosphatidylethanolamine (13%), sphingomyelin (3%), phosphatidylinositol (2%), and lysophosphatidylcholine (2%) along with a neutral fraction containing triacylglycerols (47%). Major fatty acids in oil and PL were quantified by GC/MS after slight modifications of the protocol from BERTON, GENOT and ROPERS (2011): internal standard 19:0 was solubilized in acetone/MeOH (2:1). The oil composition was: 0.2% 18:3n-3, 46% 18:2n-6, 28% 18:1n-9, 7% 18:0, and 10% 16:0.

The other reagents used were of analytical grade.

2.2. Jabuticaba peel processing

The Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg.) was kindly donated by “Indústria e Comércio Lagoa Branca Ltda”, located at Fazenda Boa Vista II, in Casa Branca city (São Paulo, Brazil) under the authorization of the Brazilian Management System of National Genetic Heritage and Traditional Associated Knowledge

(#A72354F). The fruits were washed, manually peeled and the peel was dried in a stove with air circulation (Marconi, Piracicaba, SP, Brazil) at 40 °C for 72 h. The dried peel was transformed in a fine powder by an electrical mill (Marconi, MA 630/1, Piracicaba, SP, Brazil), sifted (mesh 20) and the dried jabuticaba peel powder (DJP) was stored at - 20 °C until use.

2.3. Dried jabuticaba peel extraction by high-intensity ultrasound (HIUS)

The dried jabuticaba peel extract was obtained using a 13 mm diameter, 19 kHz ultrasonic probe (Unique, Desruptor, 800 W, Indaiatuba, Brazil) for 3 min. The probe contact height with the extract was kept at 40 mm and an ice bath was used to prevent overheating of the extract. One gram of dried jabuticaba peel and 25 mL extraction solvent (50% ethanol absolute in ultra-pure water, w/w) at 320 W of nominal power were used. The extraction was made in triplicate and the solvent was removed in a rotavap at 37 °C under vacuum condition. The dry residue was reconstituted in the same volume of ultra-pure water and it was freeze-dried and stored at - 20 °C until use.

2.4. Dried jabuticaba peel extraction by a conventional exhaustive method (CM)

A dried jabuticaba peel extract obtained by a conventional exhaustive method (CM) was prepared. In a 2 mL microtube with conical bottom, balls of zirconium (microtube bottom) were placed with 100 mg (extract used for phenolic compound analyses) or 10 mg (extract used for anthocyanin analyses) of DJP. A volume of 1.5 mL of extraction solvent (70% ethanol absolute in ultra-pure water, v/v, containing 1% of formic acid) was added and shaken in a 1600 MiniG® SPEX SamplePrep (New Jersey, USA) at 1200 rpm for 45 s. Then, the microtubes were centrifugated at 13000 rpm and 4 °C for 5 min. The supernatant was collected, and the residue was extracted again twice with the same volume of extraction solvent under the same conditions. Supernatants were combined, the solvent was removed in a rotavap at 37 °C under vacuum, and the dry residue was reconstituted in 1 mL of a solution containing 98 vol. of 1% formic acid in ultra-pure water (v/v) and 2 vol. of 1% formic acid in acetonitrile (v/v) just before UPLC/DAD/ESI-MS analysis.

2.5. Hydrolysis of phenolic compounds

The hydrolysis of the phenolic extracts was made according to GARCÍA-VILLALBA et al. (2015) with some modifications. Dried HIUS extract (100 mg) or dried jabuticaba peel (100 mg) were put into glass tubes with 3.34 mL of ultra-pure water and 1.66 mL of 37% HCl. The tubes were vortexed for 1 min and incubated at 90 °C for 24 h. After cooling the tubes to room temperature (24 °C), the pH was adjusted to 2.5 with 5 M NaOH and the volume adjusted to 6 mL with ultra-pure water in a graduated syringe. Then, the samples were centrifuged for 5 min at 13000 rpm. The supernatant was recovered and filtered through a Minisart RC4 filter before injection onto UPLC/DAD/ESI-MSⁿ. The pellets that were left were washed twice with 1 mL of DMSO/MeOH (50:50, v/v) by vortexing for 2 min and centrifuged as above. The supernatants were recovered, mixed and filtered through a Minisart RC4 filter before injection onto UPLC/DAD/ESI-MSⁿ. All samples were prepared in triplicate.

2.6. Qualitative and quantitative analyses of phenolic compounds and anthocyanins

Just before UPLC/DAD/ESI-MSⁿ analysis, the dry extracts DJP-HIUS and DJP-CM were treated as follows. For the analyses of phenolic compounds, freshly reconstituted solutions of HIUS extract were prepared in a solution containing 98 vol. of 1% formic acid in ultra-pure water (v/v) and 2 vol. of 1% formic acid in acetonitrile (v/v) at a concentration of 100 mg/mL and freshly produced CM extract was directly used at a concentration of 100 mg/mL. For the analyses of anthocyanins, freshly reconstituted solutions of HIUS extract were prepared in the same solution as above at a concentration of 10 mg/mL and freshly produced CM extract was directly used at a concentration of 10 mg/mL; then, both prepared extract solutions were diluted using 100 µL and 900 µL of a solution containing 98 vol. of 1% formic acid in ultra-pure water (v/v) and 2 vol. of 1% formic acid in acetonitrile (v/v). .

2.6.1. Identification of phenolic compounds and anthocyanins by UPLC/DAD/ESI-MSⁿ

Separation and identification of phenolic compounds were monitored by Ultra Performance Liquid Chromatography (UPLC) using an ACQUITY UPLC® system

(Waters Corp., Milford, MA, USA) coupled simultaneously to both an UV-Vis diode array detector (190–800 nm) and a Bruker Daltonics HCT Ultra Ion Trap MS equipped with an electrospray ion source (UPLC DAD/ESI-MSⁿ). Compass™ software (Bruker Daltonics, Bremen, Germany) was used for acquisition and data processing. Separation was carried out using a reverse-phase Acquity BEH C18 column (100 mm x 2.1 mm i.d., 1.7 µm; Waters, Milford, MA) at 35 °C. A binary solvent system was used with solvent A (1% formic acid in ultra-pure water, v/v) and solvent B (1% formic acid in acetonitrile, v/v) at a flow rate of 0.45 mL/min and with the following elution gradient: 0-1 min, isocratic 2% B; 1-10 min, linear 2-18% B; 10-13 min, linear 18-25% B; 13-15 min, linear 25-50% B; 15-18 min, linear 50-100% B; 18-20 min, linear 100-2% B; 20-23 min, isocratic 2% B. The volume of extract injected was 5 µL. Mass detection was conducted in both negative (for phenolic compounds) and positive (for anthocyanins) electrospray ionization modes from *m/z* 100 to 1200. MS conditions in the negative and positive ion mode were as follows: capillary voltage of ± 2 kV, nitrogen flow rate at 8 L/min; desolvation temperature at 365 °C and nebulization pressure at 50 psi. All samples were injected in triplicate from independently prepared solutions. Software DataAnalysis 4.3 (Bruker Daltonics, Bremen, Germany, 2014) was used to analyze the data.

2.6.2. Quantification of phenolic compounds and anthocyanins

The quantification was performed by UPLC/DAD/ESI-MSⁿ with the conditions described in 2.6.1. Cyanidin-3-O-glucoside (Kuromanin), quercetin-3-O-glucoside (isoquercitrin), gallic acid and ellagic acid were used for 5, 7, 10 and 10 point-calibrations, respectively. All the standards were prepared in methanol except kuromanin that was prepared in methanol acidified with 1% formic acid (v/v). Gallic acid was quantified with its own standard and gallic acid derivatives were quantified as gallic acid equivalent at 280 nm; ellagic acid was quantified with its own standard and ellagic acid derivatives as ellagic acid equivalent at 370 nm; quercetin derivatives and myricetin derivatives as isoquercitrin equivalent at 370 nm, and cyanidin and delphinidin as cyanidin-3-O-glucoside equivalent at 520 nm. The individual compounds were quantified in mg/g of dried jabuticaba peel (DJP). All samples were injected in triplicate from independently prepared solutions.

2.7. Simulated static *in vitro* gastrointestinal digestion

2.7.1. Preparation of the solutions used during digestion

The composition of simulated digestion fluids, Simulated Gastric Fluid (SGF, gastric phase) and Simulated Intestinal Fluid (SIF, intestinal phase) is given in **Table 1**. The simulated gastric (SGF) and intestinal (SIF) fluids were prepared as described in the supplementary material of the harmonized INFOGEST protocol (MINEKUS et al., 2014), with concentrations of electrolytes based on human *in vivo* data. All the solutions of simulated digestion fluids were prepared in ultrapure water.

Table 1: Concentrations of electrolytes in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

Electrolytes	SGF (pH 5) mmol/L	SIF (pH 6.5) mmol/L
KCl	6.9	6.8
KH ₂ PO ₄	0.9	0.8
NaHCO ₃	25	85
NaCl	47.2	38.4
MgCl ₂ (H ₂ O) ₆	0.1	0.33
NH ₄ HCO ₃	0.5	-

Enzymes were prepared just prior to use. The solution of porcine pepsin was prepared as an 8.08 mg/mL solution in SGF for a final concentration in the gastric phase of 1000 U/mL. The solution of porcine pancreatin was prepared as a 94 mg/mL solution in SIF for a final concentration in the intestinal phase of 100 U trypsin/mL. The solution of porcine bile extract was prepared as a 20.85 mg/mL solution in SIF for a final concentration of 5 mg/mL or 10 mM in bile salts. CaCl₂ 0.1 mM was dissolved in ultrapure water. HCl 0.1M or NaOH 0.25 M is added to adjust the pH during digestion, respectively, 5.0 and 3.0 in gastric phase, and 6.5 in intestinal phase. The lipid oxidation initiator solution was prepared as MbFeIII 200 µM ($\epsilon = 7700 \text{ M}^{-1} \text{ cm}^{-1}$ at 525 nm) in SGF (MIKKELSEN; SKIBSTED, 1995). Antioxidant solutions of dried jabuticaba peel extract (DJPE) were prepared just prior to use in SGF. DJPE-HIUS (1 mg or 5 mg) was dissolved in 1 mL of SGF to yield respectively 14.88 µg (E1) or 74.7 µg (E2) of total anthocyanins with 88% of kuromarin and 12% of delphinidin-3-O-glucoside.

2.7.2. Preparation of oil-in-water-emulsion

The physical state of lipids during digestion (triacylglycerols) was simulated by a 12.5% oil-in-water emulsion stabilized by egg yolk phospholipids (PL) prepared according to Boléa et al. (2019) with minor modifications. In a 60 mL short-necked glass bottle, 100 mg of PL were placed in 35 mL of SGF at pH 5; sunflower oil (5 g) was added. The biphasic mixture was homogenized using a rotor stator homogenizer Polytron at 24 000 rpm for 2 min at room temperature. This solution was sonicated for 8 periods of 30 s with a rest interval of 30 s and an amplitude of 60% on ice (Q700, QSonica, 20 kHz) to obtain a fine emulsion.

2.7.3. *In vitro* gastrointestinal digestion

For the *in vitro* gastrointestinal digestion, 12 mL of the fine emulsion were transferred in a 50 mL round-bottom flask kindly agitated by a magnetic barrel stirrer. For the gastric phase, 1 mL of pepsin, 50 µL of CaCl₂ 0.1 mM, 1 mL of antioxidant solution (E1 = 1 mg HIUS extract/mL of SGF or E5 = 5 mg HIUS extract/mL of SGF) or 1 mL of SGF for the blank were added. If necessary, the pH was adjusted to 5 with 0.1 M HCl or 0.25 M NaOH. The first sample was collected and corresponded to G0. Then, 2.5 mL of MbFelli at 200 µM was added to obtain a final concentration of 30 µM. Bottom flasks were protected by punched parafilm and incubated in an oven at 37 °C under constant magnetic agitation at 280 rpm during 60 min with sampling every 30 min. After 60 min of gastric digestion, the pH was adjusted to 3 with 0.1 M HCl and the digestion was carried on during 60 min. After 2 h of digestion, the volume of the remaining gastric phase was 12.55 mL. Volumes of 6.5 mL of pancreatin, 6 mL of bile, and 75 µL de CaCl₂ at 0.1 mM were then added. The pH was adjusted to 6.5 with 0.25 M NaOH and sampling continued for the next 2 h.

The physicochemical conditions for gastric and intestinal phases of digestion were simulated according to the standardized protocol for *in vitro* static digestion proposed by Minekus et al. (2014) with slight modifications. All the experiments were run at least in triplicate.

2.7.4. Determination of lipid oxidation products

Every 30 min, emulsion samples (200 µL) were diluted in a microtube with 1000 µL of a mixture 2-propanol/hexane (2/3 v/v) before centrifugation (10 min at 13

000 rpm, 4 °C). The upper hexane phase (\pm 600 μ L) was collected and 1200 μ L of hexane was added to the lower phase, vortexed and centrifuged (10 min at 13 000 rpm, 4 °C). The second upper phase was pooled with the first one and evaporated under nitrogen, then 2 mL of 2-propanol was added to obtain the extract S1. S1 was used immediately for measurement of conjugated dienes (CD) and then stored at -20 °C until the determination of 4-hydroxy-2-nonenal (4-HNE).

2.7.4.1. Measurement of lipid-derived conjugated dienes (CD)

After further dilution of the extract S1 (300 μ L) in 2-propanol (1700 μ L), the concentration in conjugated dienes (CD) was determined by measuring the absorbance at 234 nm (HP 8453 diode-array spectrometer; optical path length 1 cm) using the molar absorption coefficient of 27 000 M $^{-1}$ cm $^{-1}$ for conjugated linoleyl hydroperoxides (PRYOR; CASTLE, 1984).

2.7.4.2. Measurement of 4-hydroxy-2-nonenal (4-HNE)

The secondary oxidation product 4-hydroxy-2-nonenal (4-HNE), an aldehyde formed during simulated digestion, was quantified in the samples S1 after derivatization with 2,4-dinitrophenylhydrazine (DNPH) as previously described by Andreoli et al. (2003). Derivatization with DNPH was conducted as follows: 300 μ L of the reagent (50 mg DNPH in 20 mL of acetonitrile and 0.4 mL of formic acid), 40 μ L of internal standard (2 μ L of 4-HNE-D3 in 20 mL of acetonitrile) and 260 μ L of S1 were mixed in a 1.5 mL HPLC vial and incubated for 1 h at room temperature (24 °C) under stirring. Five point-calibrations (1.01 μ M to 0.063 μ M) were run with 260 μ L of 4-HNE diluted in acetonitrile (concentration verified by spectrophotometry using $\varepsilon=16000$ L mol $^{-1}$ cm $^{-1}$ at 221 nm) added with 40 μ L of internal standard 4-HNE-D3. After reaction with DNPH as described above, the separation and quantification of derivatized 4-HNE/4-HNE-D3 were performed by using an HPLC system (Bruker Daltonics, Bremen, Germany) coupled to a Triple Quadrupole mass spectrometer (Bruker, EVOQ) equipped with an APCI source. A reverse-phase Acquity BEH C18 column (100 mm \times 2.4 mm i.d., 1.7 μ m; Waters) was operated at 40 °C. The mobile phase was constituted by a binary solvent system with ultrapure water/formic acid (99.95/0.05, v/v, solvent A) and acetonitrile (solvent B) at the flow rate of 0.4 mL min $^{-1}$. The volume injected was 4 μ L. The elution gradient was as follows: 0-9 min, 50-98% B; 9-10 min, linear 98% B;

10-10.1 min, 98-50% B; 10.1-11 min, isocratic 50% B. MS conditions were: ionization in negative mode, APCI spray current 20 µA; cone temperature 300 °C; cone gas flow 20 L min⁻¹; heated probe temperature 300 °C; probe gas flow 40 L min⁻¹; nebulizer gas flow 50 L min⁻¹; exhaust gas off. Quantification was performed in the multiple reaction monitoring (MRM) mode. Quantification was based on the transition between parent ion at *m/z* 335 and fragment ion at *m/z* 167 for derivatized 4-HNE. 4-HNE and 4-HNE-D3 presented two regioisomers each (retention times 3.2 and 3.5 min), and the sum of the areas was used to calculate the 4-HNE concentration (Equation 1).

$$a \times [4\text{HNE}] + b = \frac{\text{AREA}(4\text{HNE})}{\text{AREA}(4\text{HNED}3)} \quad (1)$$

where [4HNE] is the concentration of 4-HNE and AREA(4HNE) and AREA(4HNE3D) are the areas of the respective regioisomer.

The stability of the adducts between DNPH and 4-HNE was evaluated by injection of the same sample every hour for 6 h. The variation was less than 5% (data not shown).

2.7.5. Determination of anthocyanin bioaccessibility

Anthocyanin bioaccessibility was determined as the content of kuromanin and delphinidin-3-O-glucoside in the aqueous phase of the emulsion during digestion. Every 30 min, emulsion samples (600 µL) were collected and placed with 50 µL of 0.1 M HCl in a microtube. After centrifugation (13 000 rpm for 10 min at 4 °C), the aqueous phase was removed via syringe, filtered (Phenex RC 0.45 µm) and stored at -20 °C until analysis of the anthocyanins as described above.

2.8. Statistical analysis

Analysis of variance (ANOVA) was carried out to compare the data using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software. Tukey's test of means at a significance level of 95% (*p*-value < 0.05) examined differences in the mean values obtained.

3. Results and discussion

3.1. Profile and content of phenolic compounds and anthocyanins of dried jabuticaba peel by UPLC/DAD/ESI-MSⁿ analyses

In order to assess the innovative extraction technique HIUS, dried jabuticaba peel extracts produced from HIUS and a conventional extraction method (CM) were compared through their phenolic and anthocyanins profiles. For both extracts, the same 58 phenolic compounds were tentatively identified (**Table 2**). Maximum absorption wavelength, protonated ($[M + H]^+$ for anthocyanins) and deprotonated ($[M - H]^-$ for the other compounds) molecules and fragmentation pattern in MSⁿ were used in the absence of standards for structure assessment.

3.1.1. Hydrolysable tannins

Hydrolysable tannins are polyesters of a sugar moiety and hydroxylated benzoic acid derivatives (SERRANO et al., 2009). For jabuticaba, gallic acid and ellagic acid were the main contributors.

Gallic acid was not found free but esterified with a hexose unit which could be a glucose moiety according to some authors (PEREIRA et al., 2017). Galloylhexose (**1**) displayed a parent ion at *m/z* 331 with major fragment ions at *m/z* 271 and 169, which are typical of galloylglucose fragmentation (QUATRIN et al., 2019). Di- (**4**, **22**; *m/z* 483,), tri- (**34**; *m/z* 635), tetra- (**44**, **46**; *m/z* 787) and pentagalloylhexose (**50**; *m/z* 939) were recovered as several isomers, as found by Quatrín et al. (2019) in jabuticaba and García-Villalba et al. (2017) and Mena et al. (2012) in pomegranate.

Ellagic acid was found free (**42**; *m/z* 301) and bound to a hexose moiety suggested to be a glucose by several authors (FISCHER; CARLE; KAMMERER, 2011; MENA et al., 2012; MORALES et al., 2016; PEREIRA et al., 2017; PLAZA et al., 2016; QUATRIN et al., 2019). Ellagic acid pentoside (**39**) displayed a parent ion at *m/z* 433 and a major fragment at *m/z* 301 indicating the loss of a pentose unit. The HHDP group (hexahydroxydiphenoyl) related to ellagic acid appeared in five isomers of di-HHDP-hexosides (**6**, **9**, **10**, **17**, **40**). Two of them were above the level of quantification. They displayed a parent ion at *m/z* 783 and major fragments at *m/z* 481 and 301 indicating the loss of a HHDP group except for **40** which gave a dehydrated form first (*m/z* 765). PLAZA et al. (2016), QUATRIN et al. (2019) and WU et al. (2012) reported 2 isomers

in jabuticaba peel, while Silva et al. (2016) isolated pedunculagin from jabuticaba seed. Between 5 to 7 isomers were also reported in pomegranate (GARCÍA-VILLALBA et al., 2017; MENA et al., 2012).

Mixed esters containing both galloyl and HHDP groups were identified. HHDP-galloylhexose isomers (**2**, **5**, **16**, **25**) displayed a parent ion at *m/z* 633 and a major fragment ion at *m/z* 301 indicating the loss of HHDP. HHDP-digalloylhexose isomers (**21**, **29**, **37**) were evidenced as previously described in *Myrciaria* species (FRACASSETTI et al., 2013; KANESHIMA et al., 2013; PLAZA et al., 2016; QUATRIN et al., 2019). They presented a parent ion at *m/z* 785 and fragment ions at *m/z* 633 and 483 resulting from the loss of a galloyl or a HHDP group. Di-HHDP-galloylhexose (**26**, **27**, **36**) displayed a parent ion at *m/z* 935 and two different fragmentation patterns. Isomer **26** fragmented to yield a major fragment at *m/z* 917 indicating the loss of a water molecule while the two others yielded *m/z* 633 indicating the loss of a HHDP group. Isomeric casuarictin, stachyurin, potentillin and casuarinin have been previously tentatively identified in jabuticaba (PEREIRA et al., 2017; PLAZA et al., 2016; QUATRIN et al., 2019), camu-camu (FRACASSETTI et al., 2013) and pomegranate (FISCHER; CARLE; KAMMERER, 2011; GARCÍA-VILLALBA et al., 2015). Casuarinin was formerly identified by NMR but not quantified in *Myrciaria cauliflora* by Pereira et al. (2017). Fast elution and close retention times were observed for **26** and **27** suggesting that these compounds could be casuarinin and casuarictin (PLAZA et al., 2016). Di-HHDP-digalloylhexose (**55**) displayed a parent ion at *m/z* 1087 with a major fragment ion at *m/z* 917 indicating the loss of gallic acid as well as a fragment ion at *m/z* 749 indicating the loss of hexahydroxydiphenic acid. This compound is tentatively reported for the first time in this study for the jabuticaba species. Finally, HHDP-trigalloylhexose (**41**) was characterized by a parent ion at *m/z* 937 and displayed losses of a galloyl group (*m/z* 767), HHDP (*m/z* 635), galloyl + HHDP (*m/z* 465) and formation of ellagic acid (*m/z* 301) (FRACASSETTI et al., 2013; PLAZA et al., 2016).

HHDP-galloylgluconic acid (**3**), HHDP-digalloylgluconic acids (**8**, **12**) and di-HHDP-galloylgluconic acid (**13**, **18**, **19** and **31**) were tentatively reported for the first time in the jabuticaba species although they were previously described in pomegranate (FISCHER; CARLE; KAMMERER, 2011; GARCÍA-VILLALBA et al., 2015; TANAKA et al., 1992). The parent ions at *m/z* 649, 801 and 951 differed by 16 amu from those found for HHDP-galloylhexose (*m/z* 633), HHDP-digalloylhexose (*m/z* 785) and di-HHDP-galloylhexose (*m/z* 935), with major fragment ions at *m/z* 605, 757 and 907

indicating the loss of CO₂ which is in agreement with the expected presence of a carboxylic acid function. HHDP (*m/z* 301) could be observed in MS³ for **3** while the loss of HHDP was observed in the MS² fragmentations of **13**, **18**, **19** and **31** with *m/z* 605 resulting from the major fragment at *m/z* 907. They demonstrated to be in smaller amounts compared to similar hexose derivatives. Additionally, the fragmentation of compound **31**, suggested to be di-HHDP-galloylgluconic acid, totally differs from that reported for DHHDP-HHDP-galloylglucose in pomegranate (CALANI et al., 2013).

Isomeric nonahydroxyterphenic acid dilactones (**14** and **30**) were detected as previously found in jabuticaba (WU et al., 2012), camu-camu (FRACASSETTI et al., 2013) and pomegranate (FRACASSETTI et al., 2013). They presented a parent ion at *m/z* 469 and a major fragment ion at *m/z* 425 (loss of CO₂) and differed remarkably by their retention times. Compound **14** was not further fragmented, and it was thus assigned as tergallic acid dilactone owing to strong C-C bonds between gallic acid units. Additional fragmentation for **30** revealed fragments with *m/z* at 407, 301 and 167, as found for valoneic acid dilactone in pomegranate (GARCÍA-VILLALBA et al., 2015), which possesses one ether linkage between 2 units. Two compounds (**20** and **23**) presented a parent ion at *m/z* 631 and a major fragment ion at *m/z* 451. The loss of 180 amu could suggest the presence of a hexose moiety linked through a C- or an O-glycosidic linkage to a tergalloyl group as found in strawberry (SUN et al., 2014) and jabuticaba (QUATRIN et al., 2019).

Six compounds with a parent ion at *m/z* 933 were identified although only 3 of them could be quantified. They differed in their fragmentation pathways as also observed in pomegranate (GARCÍA-VILLALBA et al., 2015). Fragment ions at *m/z* 451 and 631 were observed for four compounds (**15**, **33**, **38** and **52**) corresponding respectively to the tergalloyl group and the loss of HHDP from the parent ion. This fragmentation pathway could agree with structures of vescalagin, castalagin, α- and β-alnusiiin. The occurrence of these four compounds was reported in jabuticaba by Albuquerque et al. (2020), Quatrin et al. (2019) and Pereira et al. (2017). The latter authors secured the structures by NMR experiment, although no MS was provided. According to these authors, vescalagin was only present in peel of *Myrciaria cauliflora* whereas vescalagin and castalagin were both present in seeds and pulp. The two other compounds (**7**, **11**) displayed common fragment ions at *m/z* 915 (-H₂O), 613 and 569, as observed for vescalagin and castalagin standards (TAVARES et al., 2016).

Standards are clearly required to assess these compounds either as HHDP-tergalloylhexosides or gallagylgalloylhexosides.

Related compounds bearing a tergalloyl or a gallagyl group appeared in trace amounts. $[M-H]^-$ was evidenced at m/z 1085 for HHDP-galloyltergalloylhexose or digalloylgallagylhexose (**51**, **57**) and m/z 1083 for ditergalloylhexose or HHDP-digallagylhexose (**53**, **56**). Both m/z were tentatively reported for the first time in the jabuticaba species although they were previously described in pomegranate (GARCÍA-VILLALBA et al., 2015; MENA et al., 2012). Isomers **51** and **57** displayed m/z at 1085 and 542, corresponding to singly- and doubly charged ions, respectively, and a fragment ion at m/z 633 which could be HHDP-galloylhexose. Isomers **53** and **56** displayed m/z at 1083 and 541 (doubly charged ion) and yielded m/z 631 upon fragmentation as found for tergalloylhexose. It is worth noting that no gallagic acid dilactone could be recovered after acid hydrolysis. Only dilactones of valoneic acid and sanguisorbic acid (tergalloyl derivatives) were quantified after pellet washing suggesting that gallagyl derivatives are absent from jabuticaba.

3.1.2. Anthocyanins

Two main anthocyanins, delphinidin-3-O-glucoside (**24**) and cyanidin-3-O-glucoside (**28**) were identified, as described before in jabuticaba peel and extensively reported in the *Myrciaria* genus (ALBUQUERQUE et al., 2020; NEVES et al., 2018; PLAZA et al., 2016; QUATRIN et al., 2019). Delphinidin-3-O-glucoside (**24**) displayed a parent ion in positive mode at m/z 465 with a major fragment ion at m/z 303 indicating the loss of a hexose moiety, which is typical for this compound fragmentation (MENA et al., 2012; PLAZA et al., 2016). Cyanidin-3-O-glucoside (**28**) (m/z 449 in MS in positive mode; 287 in MS^2) was identified by comparison with the chromatographic and MS characteristics of the standard compound. Two minor anthocyanins, pelargonidin-O-hexoside (**32**, $[M]^+$ at m/z 433 and fragment ion at m/z 271) and peonidin-O-hexoside (**35**, $[M]^+$ at m/z 463 and fragment ion at m/z 301) were also identified, as described before in jabuticaba peel by QUATRIN et al. (2019).

3.1.3. Flavonols

Myricetin deoxyhexose (**43**), quercetin hexoside (**45**, **47**), quercetin pentoside (**48**, **49**), quercetin deoxyhexose (**54**) and quercetin (**58**) were assessed

based on minor fragments at *m/z* 179 and 151 which are typical of the fragmentation of their aglycone A ring (MORALES et al., 2016; WU et al., 2012). It should be noted that a major fragment at *m/z* 301 and maximal absorption at 350-360 nm are not sufficient to discriminate between ellagic acid and quercetin derivatives.

3.1.4. Others

No caffeoylquinic acids and no flavan-3-ols ((epi)catechin, type-B procyanidin dimers, (epi)catechin gallate, gallo(epi)catechin) could be evidenced in jabuticaba peel after a search of their parent ions. Thioacidolysis was additionally conducted on both peel extract and peel powder indicating the absence of flavan-3-ol oligomers in these materials.

3.1.5. Quantification

Quantification was possible for 32 out of the 58 compounds tentatively assigned for the HIUS extract (27 for the CM extract) when UPLC peaks could be assigned to single compounds. **Table 3** presents this quantification.

Gallic acid derivatives are the largest class of compounds found for both extracts. They comprise single and mixed esters of hexose with the galloyl, HHDP, tergalloyl and possibly gallagyl groups. HUIS extract presented the highest amount for gallic acid derivatives (9.2 ± 0.3 mg/g DJP), differing statistically from CM extract (4.9 ± 0.7 mg/g DJP). Plaza et al. (2016), who carried out a pressurized hot water extraction (an emerging technology) on DJP, found amounts of 8.2 mg/g DJP (data in kuromanin equivalent) whereas Quatrin et al. (2019) obtained 7.9 mg/g DJP (conventional extraction and various standards for quantification), similarly to our HIUS extract. Albuquerque et al. (2020) (conventional extraction, standards unknown) found an amount of 3.5 mg/g DJP, closer to CM extract. Anthocyanins, mainly composed by cyanidin-3-O-glucoside and delphinidin-3-O-glucoside, are the second most abundant group. Contents in anthocyanins were found to be significantly lower for HUIS extract (7.8 ± 0.1 mg/g DJP) than for CM extract (10.2 ± 1.2 mg/g DJP) suggesting that HIUS is potentially less adequate for anthocyanin extraction. Plaza et al. (2016) and Albuquerque et al. (2020) found higher amounts of anthocyanin derivatives (32.2 and 24.5 mg/g DJP, respectively), while Alezandro et al. (2013) (Conventional extraction, standards), Quatrin et al. (2019) and Marques-Peixoto et al. (2016) found relatively

lower or similar amounts (3.5, 11.5 and 16.7 mg/g DJP). Flavonol derivatives, represented by myricetin and quercetin derivatives, are minor constituents of the jabuticaba peel. CM extract presented a higher content for this group (0.32 ± 0.02 mg/g DJP) differing statistically from HIUS extract (0.24 ± 0.01 mg/g DJP). Both Quatrin et al. (2019) and PLAZA et al. (2016) found amounts in a similar range (0.6 mg/g DJP) suggesting that both extraction methods used in these studies (conventional and pressurized hot water) are equivalent for flavonol derivative extraction. Only Albuquerque et al. (2020) found 10 times lower contents (0.05 mg/g DJP). Ellagic acid derivatives were quantified in both extracts, showing no significant differences (1.08 ± 0.02 mg/g DJP for HIUS extract and 1.11 ± 0.07 mg/g DJP for CM extract). Our contents are in the same range as contents found by Plaza et al. (2016) (2.0 mg/g DJP, cyanidin-3-O-glucoside used for calibration) and Inada et al. (2015) (1.8 mg/g DJP, ellagic acid for calibration). Lower contents (0.7 mg/g DJP) were found by Quatrin et al. (2019) although in the absence of ellagic acid as standard. Although significant differences were evidenced between classes, our work did not reveal any difference for the sums of the phenolic compounds whatever the extraction method used.

Quantification differences showed for both HIUS and CM extracts can be explained by the different extraction methods applied in this work. The acoustic cavitation produced by ultrasound technology promotes a very efficient cell wall disruption, which increases considerably the transfer of analyte mass from solid to liquid phase and improves significantly the extraction of phenolic compounds in comparison to conventional extraction methods (CHEMAT et al., 2017). This explains the high amounts of gallic acid derivatives found in HIUS extract. However, this process promotes the creation of shear forces that generate critical temperature and pressure, locally generating hydroxyl radicals which cause the degradation of compounds sensitive to these adverse conditions such as anthocyanins and flavonols (WANG et al., 2020). The CM extraction did not present these adverse conditions, explaining the higher contents in anthocyanin and flavonol derivatives.

Table 2. Phenolic compounds identified by UPLC/DAD/ESI-MSⁿ in dried jabuticaba peel extracts obtained by high-intensity ultrasound technology and a conventional method.

N°	RT (min)	λ _{max} (nm)	[M - H] ⁻ / [M] ⁺ (m/z)*	MS ² fragments (m/z)	Tentative identification
1	1.1	277	331	271-169	Galloylhexose ^a
2	1.3	270	633	301-275-249	HHDP-galloylhexose (1) ^{ab}
3	1.6	266	649	605-481-479-425	HHDP-galloylgluconic acid ^c
4	1.6	266	483	331-313-169	Digalloylhexose (1) ^{adj}
5	2.1	264	633	301-275-249	HHDP-galloylhexose (2) ^{ab}
6	2.1	264	783	765-721-507- 481-419-341-301-275	Di-HHDP-hexose (1) ^{abdhj}
7	2.2	282	933	915-871-613-569	HHDP-tergalloylhexose or Gallagylgalloylhexose (1) ^{defg}
8	2.2	282	801	783-757-633	HHDP-digalloylgluconic acid (puniguconin) (1) ^{cd}
9	2.5	260	783	481-301-275	Di-HHDP-hexose (2) ^{abdhj}
10	2.7	260	783	481-301-275	Di-HHDP-hexose (3) ^{abdhj}
11	3.1	260	933	915-631-613-569	HHDP-tergalloylhexose or Gallagylgalloylhexose (2) ^{adefg}
12	3.1	260	801	757-633	HHDP-digalloylgluconic acid (puniguconin) (2) ^{cd}
13	3.1	260	951	907-783-605-481-405-347-301	Di-HHDP-galloylgluconic acid (1)
14	3.5	370	469	425	Tergallic acid dilactone (1)
15	3.6	260	933	631-571-451-425-301	HHDP-tergalloylhexose or Gallagylgalloylhexose (3) ^{adef}
16	3.8	260	633	615-481-301-275	HHDP-galloylhexose (3) ^{ab}
17	4.2	260	783	481-421-301-275	Di-HHDP-hexose (4) ^{abdhj}
18	4.4	260	951	907-783-605-481-425-361-299	Di-HHDP-galloylgluconic acid (2)
19	4.6	260	951	907-783-605-481-425-361-299	Di-HHDP-galloylgluconic acid (3)

20	4.7	260	631	613-571-451-407-351	Valoneic (sanguisorbic) acid dilactone C-hexoside or Tergalloyl-O-hexose (cauliflorin) ^e or Tergalloyl-C-hexose (Castalin) ^a (1)
21	5.0	270	785	633-483-419-331-301-275	HHDP-digalloylhexose (1) ^{abd}
22	5.1	272	483	331-271-169	Digalloylhexose (2) ^{adj}
23	5.1	272	631	613-571-451-407-351	Valoneic (sanguisorbic) acid dilactone C-hexoside or Tergalloyl-O-hexose (cauliflorin) ^e or Tergalloyl-C-hexose (Castalin) ^a (2)
24	5.4	526	465*	303*	Delphinidin-3-O-glucoside ^{ab}
25	5.6	260	633	615-463-301	HHDP-galloylhexose (4) ^{ab hj}
26	5.7	260	935	917-873-855-573	Di-HHDP-galloylhexose (1) (Casuarinin/Stachyurin/Casuarictin/Potentillin) ^{abdefhl}
27	5.9	265	935	917-873-783-659- 633-571-301	Di-HHDP-galloylhexose (2) (Casuarinin/Stachyurin/Casuarictin/Potentillin) ^{abcdeh}
28	6.2	514	449*	287*	Cyanidin-3-O-glucoside (std) ^{ab}
29	6.3	270	785	633-483-419-301-249	HHDP-digalloylhexose (2) ^{abd}
30	6.3	372	469	451-425-407-301-167	Valoneic acid dilactone ^{c fh}
31	6.5	260	951	907-783-605	Di-HHDP-galloylgluconic acid (4)
32	6.9	516	433*	271*	Pelargonidin-O-hexoside ^a
33	7.0	260	933	763-631-481-451-425-301	HHDP-tergalloylhexose or Gallagylgalloylhexose (4) ^{adef}
34	7.2	264	635	617-483-423-271	Trigalloylhexose ^a
35	7.4	524	463*	301*	Peonidin-O-hexoside ^a
36	7.7	260	935	917-765- 633-615-481-451-301	Di-HHDP-galloylhexose (3) (Casuarinin/Stachyurin/Casuarictin/potentillin) ^{abdf}
37	7.9	264	785	633-483-301	HHDP-digalloylhexose (3) ^{abd}
38	8.0	260	933	631-481-451-301	HHDP-tergalloylhexose or Gallagylgalloylhexose (5) ^{adef}
39	8.3	355	433	301-287-273-209	Ellagic acid pentoside ^{abdfh}
40	8.3	-	783	765-613-465-451-419-301	Di-HHDP-hexose (5) ^{abdhj}
41	8.3	-	937	785-767-741-635-465-419-301-275	HHDP-trigalloylhexose ^{b fh}
42	8.6	367	301	229-185	Ellagic acid (std) ^{abcdeh}
43	9.0	350	463	317-237-179-151	Myricetin deoxyhexose ^{ahi}

44	9.0	264	787	635-617-467-447-403	Tetragalloylhexose (1) ^{ad}
45	9.2	350	463	301-273-245-213-179-151	Quercetin hexoside (1) ^{aij}
46	9.3	272	787	617-465-449	Tetragalloylhexose (2) ^a
47	9.4	350	463	301-273-257-229-179-151	Quercetin hexoside (2) ^{aij}
48	9.8	350	433	301-271-257	Quercetin pentoside (1) ^{ai}
49	10.0	350	433	301-271-253-225-179-151-125	Quercetin pentoside (2) ^{ai}
50	10.3	275	939	787-769-617-599-447	Pentagalloylhexose ^{ab}
51	10.3	275	1085	783-633-613-451-301	HHDP-galloyltergalloylhexose or Digalloylgallagylhexose (1) ^{adj}
52	10.5	260	933	631-481- 451-301	HHDP-tergalloylhexose or Gallagylgalloylhexose (6) ^{adef}
53	10.5	260	1083(Nf), 541[M-2H] ²⁻	[541]: 631-451	Ditergalloylhexose or HHDP-digallagylhexose (trace) (1) ^{dj}
54	10.7	350	447	301-255--217-207-179-151	Quercetin deoxyhexose (quercetin-3-rhamnoside ^b) ^{ahi}
55	10.8	260	1087	935- 917-749-451	Di-HHDP-digalloylhexose
56	10.9	260	1083(Nf), 541[M-2H] ²⁻	[541]: 631-451	Ditergalloylhexose or HHDP-digallagylhexose (trace) (2) ^{dj}
57	12.2	260	1085	783-633-613-481-451-301	HHDP-galloyltergalloylhexose or Digalloylgallagylhexose (2) ^{adj}
58	13.3	366	301	179-151	Quercetin ⁱ

*Ionization in negative mode for all compounds except for anthocyanins in positive mode. The main fragment in MS² is given in boldface, the minor fragments are given in normal font and the ultra-minor fragments are given in italics. HHDP: hexahydroxydiphenoyl group. Std: identified with authentic standard. Nf: not fragmented. [M-2H]²⁻: doubly charged ion.

^a (QUATRIN et al., 2019)

^b (PLAZA et al., 2016)

^c (FISCHER; CARLE; KAMMERER, 2011)

^d (GARCÍA-VILLALBA et al., 2015)

^e (PEREIRA et al., 2017)

^f (FRACASSETTI et al., 2013)

^g (TAVARES et al., 2016)

^h (WU et al., 2012)

ⁱ (NEVES et al., 2018)

^j (MENA et al., 2012)

^k (MORALES et al., 2016)

Table 3. Phenolic compounds quantified in dried jabuticaba peel obtained after extraction by high-intensity ultrasound technology and a conventional method.

	Gallic acid derivatives (mg/g DJP)	Ellagic acid (mg/g DJP)	Flavonol derivatives (mg/g DJP)	Anthocyanin derivatives (mg/g DJP)	Sum of phenolic compounds (mg/g DJP)
HIUS extraction	9.23 ± 0.32 (a)	1.08 ± 0.02 (a)	0.24 ± 0.01 (b)	7.81 ± 0.06 (b)	18.36 ± 0.36 (a)
Conventional extraction	4.89 ± 0.65 (b)	1.11 ± 0.07 (a)	0.32 ± 0.02 (a)	10.22 ± 1.17 (a)	16.53 ± 1.19 (a)

Values represented mean ± SD ($n = 3$). Sum of phenolic compounds is obtained from the different columns on the left (UPLC). DJP: dried jabuticaba peel. Different letters indicate a significant difference between both extracts at $p < 0.05$.

3.1.6. Hydrolysis

Acid hydrolysis of dried HIUS extract and dried jabuticaba peel was conducted to reveal the structures of the phenolic acids present as hexosides. As these phenolic compounds may not be soluble in aqueous media, an additional step of pellet washing with a mixture of methanol and DMSO was added as proposed by García-Villalba et al. (2015). The results of the identification and quantification of these compounds are shown in **Table 4** (the chromatographic profiles of the phenolic compounds for HIUS extract before and after hydrolysis, CM extract and DJP after hydrolysis are presented in **Supplementary Material**).

Gallic acid (**1**) (*m/z* 169 in MS; 125 in MS²) was identified by comparison with the chromatographic and MS characteristics of the standard compound. This compound was found after acid hydrolysis of jabuticaba peel by Da Silva (2019) and alkaline and acid hydrolyses of the residue from conventional polyphenol extraction by Inada et al. (2015) and Quatrin et al. (2019). Digalloylhexose (**2**) presented similar MS pattern as compound **4** before hydrolysis (**Table 2**) with parent ions at *m/z* 483 and fragment ions at *m/z* 331, 313 and 169. Its resistance to acid hydrolysis points to C-glycosidic linkages. The dehydrated form of gallic acid C-hexoside (**3**) was identified with parent ions at *m/z* 313 and fragmentation indicating the loss of gallic acid (fragment at *m/z* 169). DiOH-phenylpropionic acid (**4**) was identified with parent ions at *m/z* 181 and fragment ions at *m/z* 137 and 109 as for standard.

No gallagic acid dilactone was found to be formed after 24 h of hydrolysis in contrast to pomegranate peel (GARCÍA-VILLALBA et al., 2015). Compounds **7**, **11**, **15**, **33**, **38** and **52** (**Table 2**) were ambiguously assessed to HHDP-tergalloylhexosides or gallagylgalloylhexosides and amounted to 0.55 mg/g of DJP after conventional extraction and 1.03 mg/g after HIUS extraction. The absence of gallagic acid dilactone is in favor of HHDP-tergalloylhexosides in jabuticaba and thus of compounds such as alnusiin, vescalagin and castalagin as proposed by several authors (PEREIRA et al., 2017; QUATRIN et al., 2019). Therefore, hexosides acylated with the gallagyl group such as pedunculagin III, punicalagin and punicalin could be typical of pomegranate ellagitannins (GARCÍA-VILLALBA et al., 2015). Similarly, **51** and **57** could be HHDP-galloyltergalloylhexosides while **53** and **56** ditergalloylhexosides. Additionally, various valoneic/sanguisorbic acid dilactone C-hexosides or tergalloyl-C-hexosides were formed in small amounts although not quantified in this work (**7**, **9**, **11**, **12** and **13**).

Tergalloyl-C-glucose is expected to be released by castalagin and vescalagin after acid hydrolysis while alnusiin will give valoneic acid dilactone.

Newly formed compounds **14** and **15** were tentatively assigned as valoneic acid dilactone and sanguisorbic acid dilactone, respectively. Sanguisorbic acid dilactone presented a parent ion at *m/z* 469 and fragment ions at *m/z* 425, 301 and 299 as observed from sanguisorbic acid dilactone obtained from hydrolysis of strawberry (MATTILA; KUMPULAINEN, 2002). Additionally, valoneic acid dilactone eluted earlier than sanguisorbic acid dilactone and presented fragment ions at *m/z* 425, 407 and 301 as in pomegranate (GARCÍA-VILLALBA et al., 2015). Contents in valoneic acid dilactone were 3-fold higher than contents in sanguisorbic acid dilactone in both DJP and the HIUS extract.

Ellagic acid (**16**) (*m/z* 301 in MS; 258, 229 and 186 in MS²) was identified by comparison with the chromatographic and MS characteristics of the standard compound as previously. Four ellagic acid derivatives were identified (**5**, **6**, **8**, **10**) with parent ions at *m/z* 783 and fragmentation indicating the loss of ellagic acid ([M-302-H] at *m/z* 481 and fragment at *m/z* 301) rather than HHDP as these compounds absorb between 365 and 375 nm. They could be C-hexosides rather than O-hexosides as resistant to acidic hydrolysis or other structures arising from a rearrangement of the 5 di-HHDP hexosides detected at different retention times in peel (**Table 2**).

Most abundant hydrolysis compounds are in decreasing order: gallic acid C-hexoside (**3**), ellagic acid (**16**), gallic acid (**1**) and valoneic acid dilactone (**14**). Gallic acid was not present in the jabuticaba peel and appeared after acid hydrolysis amounting to 10.7 mg/g in DJP and 6.3 mg/g in the HIUS extract. Da Silva (2019) reported 5.9 mg/g DJP after 24h of acid hydrolysis. The content in ellagic acid was 16.4 mg/g for hydrolyzed DJP and increased from 1.08 to 10.9 mg/g DJP for the HIUS extract upon hydrolysis. These values agree well with those found by Alezandro et al. (2013), Da Silva (2019) and Abe et al. (2012) for hydrolyzed DJP (22.5 - 24.0 mg/g DJP) and the slight difference can be explained by varying ripening stages of the fruits and pedoclimatic conditions (ALEZANDRO et al., 2013). Largely lower contents in gallic acid (0.4 and 0.15 mg/g DJP, respectively) and ellagic acid (8.9 and 0.3 mg/g DJP, respectively) were found by Quatrin et al. (2019) and Inada et al. (2015) after polyphenol extraction and subsequent alkaline and acid hydrolyses of the extraction residue supporting the degradation of the oxidizable trihydroxyphenyl moiety in basic conditions.

Overall, hydrolytic products of ellagitannins amounted to 70.3 mg/g DJP before extraction and 42.4 mg/g DJP after HIUS extraction. The hydrolysis results suggest that the HIUS extraction was not able to extract all the compounds present in the DJP. The amounts of compounds found is markedly higher than that obtained by UPLC analysis of individual compounds for the HIUS extract, which is of 10.3 mg/g DJP (anthocyanins and flavonols not taken into consideration, Table 3). This suggests that acid hydrolysis which relies mainly on the titration of two standards (ellagic acid and gallic acid) is by far more accurate for jabuticaba peel than UPLC in the absence of individual standards. Subsequent washing of the pellet with MeOH/DMSO provided more than one third of the hydrolysis products. It is worth noting that hydrophobic molecules such as ellagic acid and valoneic acid dilactone were mainly recovered using this additional step whereas sanguisorbic acid dilactone was only soluble in this solvent system.

Table 4. Acid hydrolysis followed by UPLC/ESI-MSⁿ of phenolic compounds in dried jabuticaba peel (DJP) and dried jabuticaba peel extract obtained after extraction with high-intensity ultrasound technology (HIUS).

Nº	RT (min)	λ_{max} (nm)	[M - H] ⁻ (m/z)	MS ² fragments (m/z)	Tentative identification	DJP (mg/g DJP)			HIUS (mg/g DJP)		
						Supernatant	Pellet	Sum	Supernatant	Pellet	Sum
1	1.2	271	169	125	Gallic acid (Std) ^{a,b}	10.7 ± 0.3	0.75 ± 0.02	11.4 ± 0.3	6.27 ± 0.73	0.02 ± 0.01	6.29 ± 0.74
2	1.35	281	483	331-313-169	Digalloyl hexose ^{a,b,c}	0.95 ± 0.23	-	0.95 ± 0.23	0.89 ± 0.03	-	0.89 ± 0.03
3	2.2	284	313	295-245-169-125-107	Gallic acid C-hexoside (dehydrated form) ^d	26.9 ± 4.6	3.49 ± 0.09	30.4 ± 3.8	18.6 ± 1.5	0.77 ± 0.34	19.4 ± 1.8
4	2.55	276	181	137-109	DiOH-phenylpropionic acid ^e	5.07 ± 0.72	-	5.07 ± 0.72	0.67 ± 0.18	-	0.67 ± 0.18
5	3.6	374	783	481-451-301-299-271	Ellagic acid derivative (1)	0.12 ± 0.04	-	0.12 ± 0.04	0.13 ± 0.04	-	0.13 ± 0.04
6	3.85	375	783	481-451-301-299-271	Ellagic acid derivative (2)	0.45 ± 0.07	-	0.45 ± 0.07	0.32 ± 0.05	-	0.32 ± 0.05
7	4.4	365	631	451-407-299-271	Valoneic/sanguisorbic acid dilactone C-hexoside or Tergalloyl-C-hexoside (1) ^b	-	-	-	-	-	-
8	4.6	365	783	481-451-301-299-271	Ellagic acid derivative (3)	-	-	-	-	-	-
9	4.65	365	631	587-451-301- 299 -271	Valoneic/sanguisorbic acid dilactone C-hexoside or Tergalloyl-C-hexoside (2) ^b	-	-	-	-	-	-
10	4.7	365	783	767- 483 -453-303-271	Ellagic acid derivative (4)	-	-	-	-	-	-
11	4.75	365	631	451-301- 299 -271	Valoneic/sanguisorbic acid dilactone C-hexoside or Tergalloyl-C-hexoside (3) ^b	-	-	-	-	-	-
12	5	365	631	587-451- 299 -271	Valoneic/sanguisorbic acid dilactone C-hexoside or Tergalloyl-C-hexoside (4) ^b	-	-	-	-	-	-

13	5.1	365	631	587-451-301- 299 -271	Valoneic/sanguisorbic acid dilactone C-hexoside or Tergalloyl-C-hexoside (5) ^b	-	-	-	-	-	-	-
14	5.9	370	469	451- 425 -407-301-167	Valoneic acid dilactone ^{bf}	0.73 ± 0.06	3.44 ± 0.09	4.17 ± 0.09	0.99 ± 0.07	2.02 ± 0.10	3.02 ± 0.17	
15	6.15	365	469	451- 425 -301-299	Sanguisorbic acid dilactone ^{bf}	-	1.39 ± 0.04	1.39 ± 0.04	-	0.85 ± 0.04	0.85 ± 0.04	
16	8.4	367	301	258-229-186	Ellagic acid (Std) ^{abg}	3.52 ± 0.14	12.9 ± 0.5	16.4 ± 0.4	3.62 ± 0.38	7.24 ± 0.61	10.9 ± 1.0	
TOTAL PHENOLIC COMPOUNDS						48.4 ± 4.1	21.9 ± 0.9	70.3 ± 3.1	31.5 ± 1.3	48.4 ± 4.1	21.9 ± 0.9	

The main fragment in MS² is given in boldface, the minor fragments are given in normal font and the ultra-minor fragments are given in italics. Values represented mean ± SD (*n* = 3). Sum of phenolic compounds is obtained from the different columns on the left (UPLC). Std: identified with authentic standard. -: Means below quantification limit or not present.

^a (QUATRIN et al., 2019)

^b (GARCÍA-VILLALBA et al., 2015)

^c (MENA et al., 2012)

^d (FISCHER; CARLE; KAMMERER, 2011)

^e Fragmentation as for standard

^f (MORALES et al., 2016)

^g (NEVES et al., 2018)

3.2. Inhibition of lipid oxidation by DJPE-HIUS during static *in vitro* gastrointestinal digestion

The lipid oxidation initiated by metmyoglobin (MbFeIII) of a 10% oil-in-water emulsion and its inhibition by the HIUS extract prepared at two different initial concentrations ($E1 = 1 \text{ mg/mL}$ and $E5 = 5 \text{ mg/mL}$) were investigated. For this, we made the quantification of lipid-derived conjugated dienes (CDs) as a primary marker of lipid oxidation and 4-hydroxy-2-nonenal (4-HNE) as a secondary lipid oxidation marker. The results report the oxidizability of lipids and are shown in **Figure 1**.

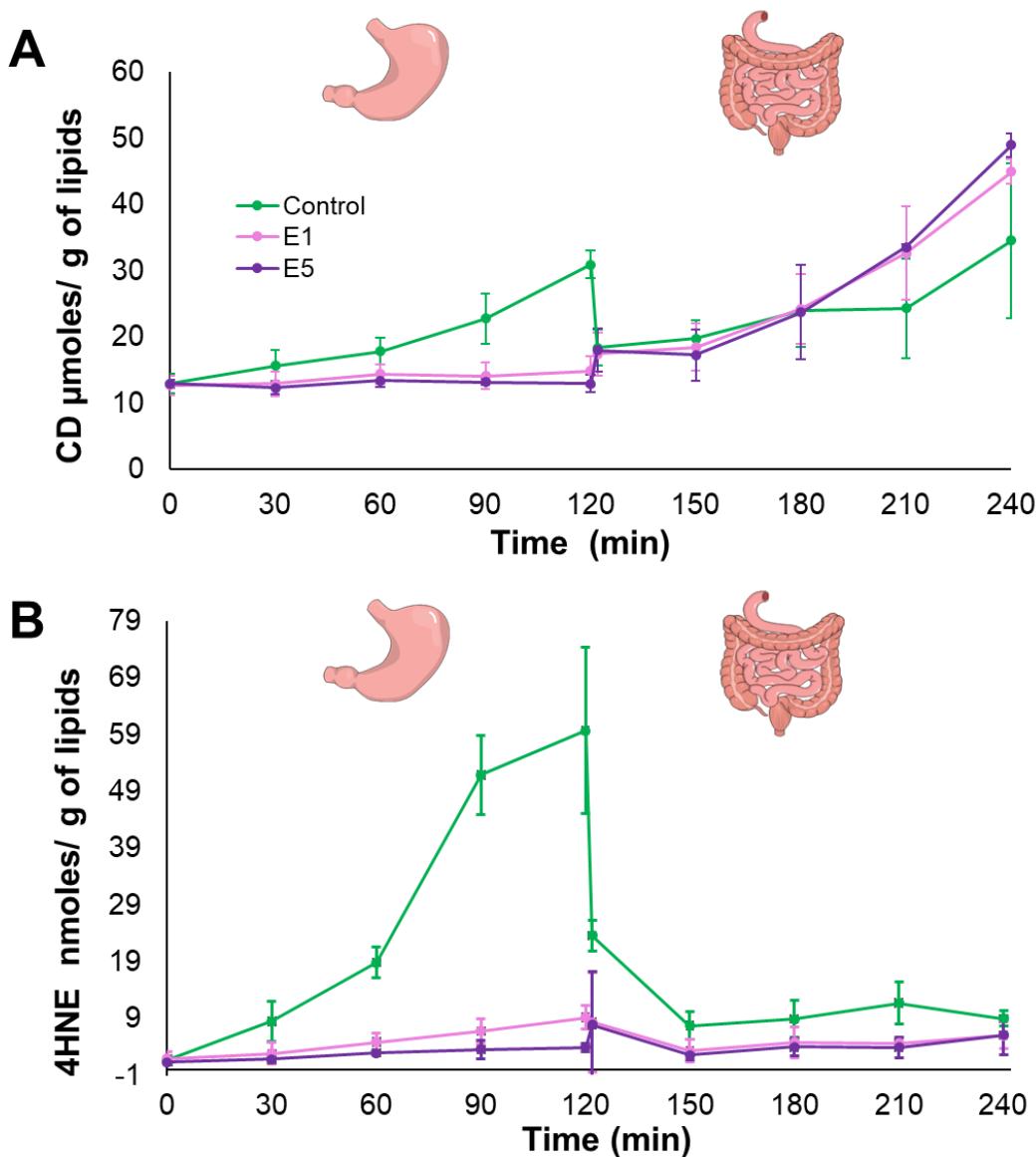


Figure 1. Inhibition of the accumulation of conjugated dienes ($n = 3-5$) (A) and 4-HNE ($n = 3$) (B) by the HIUS extract during static *in vitro* gastrointestinal digestion. $E1 = 1 \text{ mg/mL}$ and $E5 = 5 \text{ mg/mL}$. Data represent mean \pm SD.

During the whole gastric (G) phase (0 to 120 min), lipid oxidation was almost totally inhibited by both extracts E1 and E5. Statistically significant inhibition of CD formation was observed for both extracts from 60, 90 and 120 min when compared to control assay, supporting a full protection of polyunsaturated lipids by E1 and E5 in the G phase. The relatively linear accumulation of CDs is correlated with an increase in 4-HNE, a terminal marker specific of the oxidation of linoleic acid. It is worth noting a lower rate of formation of 4-HNE during the first 30 min in agreement with the multiple reactions required to provide 4-HNE from linoleoyl hydroperoxides. At the end of the G phase, CDs, which are mainly constituted by lipid-derived hydroperoxides arising from the linoleic acid fatty acid chain, reach 29 µmol/g lipids when 4-HNE can be found at the level of 59 nmol/g lipids for the control assay. The low free 4-HNE/CDs ratio of 3 to 1000 observed at this stage can be explained by the formation of various other secondary oxidation products such as short-chain aldehydes, epoxides and alcohols as well by the high reactivity of 4-HNE. Electrophilic 4-HNE reacts rapidly with nucleophilic amino acids although this reaction could be favored at higher pH as found in the intestinal phase (GASC et al., 2007). As observed for CDs, statistically significant inhibition of 4-HNE formation was observed for both extracts throughout the G phase when compared to the control assay. Again, E5 appears to bring total protection of lipid oxidation compared to the slightly weaker protection brought by E1 although there was not statistical difference between them. A higher level in polyphenols, as shown for E5 compared to E1, appears to be correlated to larger protection of lipid oxidation, although there is no significant difference for both CDs and 4-HNE accumulations.

In the intestinal (I) phase, pH is increased to 6,5 and bile salts and pancreatin are added diluting by a factor 2 the digestion medium. The CDs kept accumulating at a similar if not faster rate in this phase. The drop for the CDs level between the final sample in the G phase and the first sample in the I phase can potentially be attributed to a fast degradation of the CDs in intestinal conditions or, more likely, to an incomplete extraction of triglycerides by hexane in micelles formed after the addition of bile salts. In the presence of extracts E1 and E5, lipid oxidation initiates at a similar rate after 30 min in intestinal conditions apparently leading to higher levels of CDs compared to control after 90 min, although without any significant difference at 90 and 120 min. This increase in CDs, which is not supported by the formation of 4-HNE, could be ascribed to the extraction of substances absorbing at

234 nm and released from the extracts during the I phase. As to 4-HNE, it sharply decreased upon the change in digestion conditions for control and both E1 and E5 extracts. A higher pH and the additional presence of proteins upon pancreatin addition may favor 4-HNE binding to peptides and proteins (BOLÉA et al., 2019). For both CD and 4-HNE markers, no statistical differences were found between E1 and E5 in the I phase.

3.3. Bioaccessibility of anthocyanins

The bioaccessibility of anthocyanins from the HIUS extract prepared at two different initial concentrations (E1 = 1 mg/mL and E5 = 5 mg/mL) was evaluated during the *in vitro* gastrointestinal digestion of the 10% oil-in-water emulsion. Other phenolic compounds were individually present at a too low level to be detected. Bioaccessible anthocyanins are defined as the molecules present in the aqueous phase after separation of the two phases of the emulsion used to simulate the physical state of lipids during digestion. The bioaccessibility of cyanidin-3-O-glucoside (COG) for both E1 and E5 is shown in **Figure 2**. Less abundant delphinidin-3-O-glucoside (DOG) could only be recovered from E5.

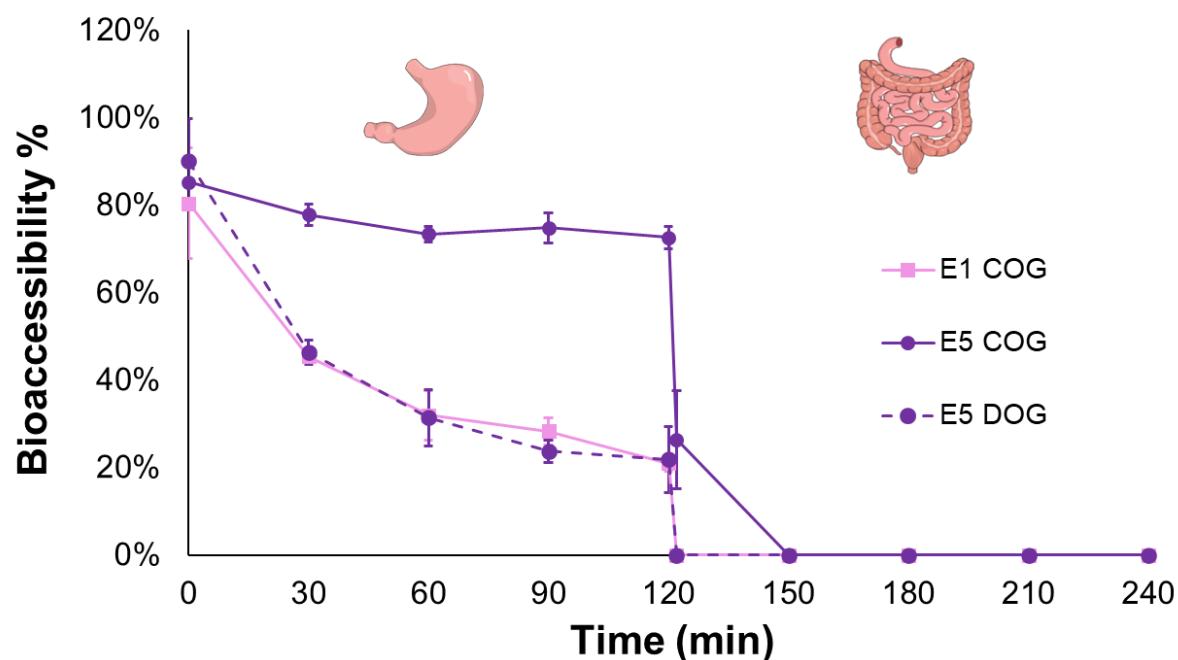


Figure 2. Bioaccessibility of anthocyanins in the DJPE-HIUS throughout the *in vitro* gastrointestinal digestion. E1 = 1 mg/mL ($n = 3$) and E5 = 5 mg/mL ($n = 4$). COG is Cya-3-O-Glc and DOG is Del-3-O-Glc.

COG proved to be largely stable when added as the E5 extract, showing a recovery of 73% at the end of the G phase. When COG present at a 5-fold less level as in E1, it decreased exponentially with only 21% of recovery at the end of the G phase. At the highest concentration of the extract (E5), DOG was also recovered, although at a lower rate (22%) than COG. This low recovery is mainly due to the higher oxidizability of the 1,2,3-trihydroxyphenyl moiety of delphinidin, when compared to that of the 1,2-dihydroxyphenyl nucleus of cyanidin (JANEIRO; BRETT, 2007). Similarly, Quatrin et al. (2019) showed a higher recovery of COG (85%) than DOG (71%) at the end of the G step for a jabuticaba peel powder (digestion conditions from Minekus et al., 2014). Marques-Peixoto et al. (2016) showed an anthocyanin (COG plus DOG) recovery of 13% for the gastric digestion of a jabuticaba peel powder when our overall recovery amounted to 67%. Besides, these authors used the dried jabuticaba peel in the powder form (not in the extract form like in this work) and they started the gastrointestinal digestion assay with the oral phase, not performed in this work. They also used samples in different concentrations, promoting differences in anthocyanins bioaccessibility. Marques-Peixoto et al. (2016) reported a much higher degradation for anthocyanins under gastric conditions probably because this phase is preceded by a long oral phase (10 min), while Quatrin et al. (2020) reported a bioaccessibility closer to that for E5 because their initial sample had a higher concentration in anthocyanins.

COG and DOG present at the end of the gastric phase demonstrated to be completely degraded in a few minutes after the change in digestion conditions, no matter the extract employed. This degradation occurring in the I phase arises from hydration of the flavylium cation at higher pH leading to hemiketal formation, a reaction that is followed by rapid cleavage of the main backbone of anthocyanins. Additionally, deprotonation leads to unstable carbinol pseudo-bases from the flavylium cation (SANTOS-BUELGA; GONZÁLEZ-PARAMÁS, 2018). Marques-Peixoto et al. (2016) reported a decrease in anthocyanin bioaccessibility of only 3% after the I phase, while Inada et al. (2020) (peel and seed powder) reported a decrease of 20-30% for COG and DOG, respectively. The fact that both works found anthocyanins at the end of the I phase is probably because the authors used a simpler model of gastrointestinal digestion, without the addition of the emulsion used to simulate the physical state of lipids during digestion and also without ongoing lipid oxidation. Additionally, they used peel or peel and seed powders which can protect anthocyanins in their matrix for a longer time.

4. Conclusion

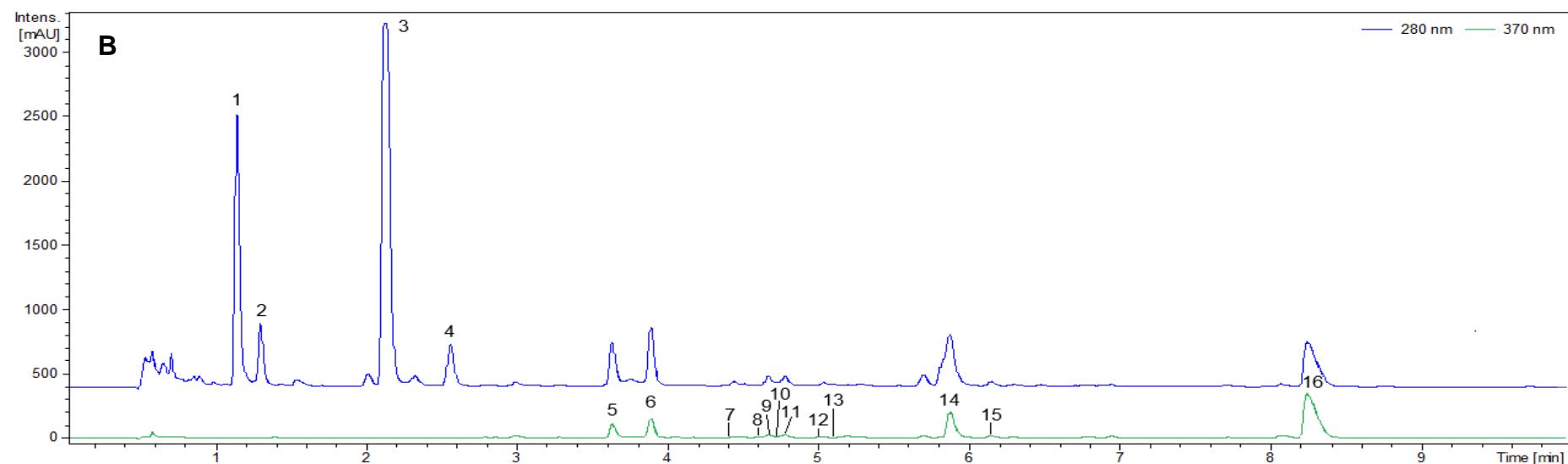
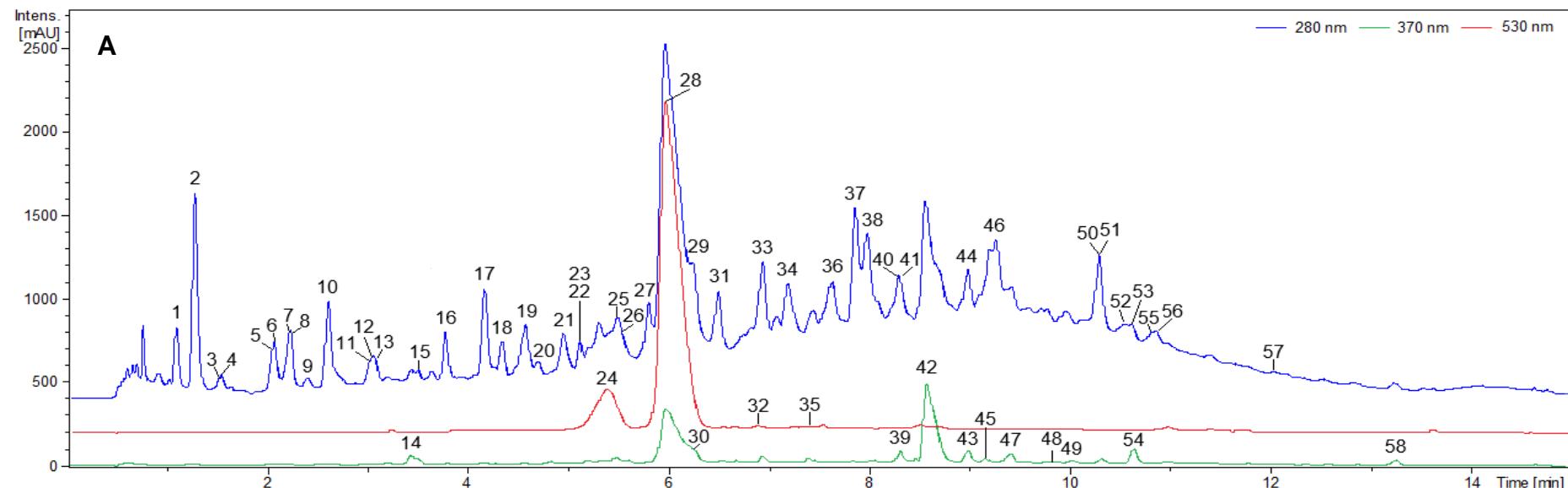
In the comparison of phenolic and anthocyanins profiles of HIUS extract and CM extract, the same 58 phenolic compounds were tentatively identified in both extracts, which 11 were reported for the first time in the jabuticaba species. Both extraction methods exhibited the same efficiency to extract ellagic acid. The HIUS extraction presented greater efficiency to extract gallic acid derivatives while the CM extraction presented greater efficiency to preserve anthocyanin derivatives and flavonol derivatives. Hydrolysis of dried HIUS extract and dried jabuticaba peel was able to reveal the structure of the major phenolic acids covalently bound to hexoses. The main products released after hydrolysis were gallic acid, ellagic acid and valoneic acid dilactone. The highest levels of gallic acid were released in the soluble extract, while ellagic acid and valoneic acid dilactone were released in the insoluble extract, after extraction with DMSO/MeOH (1:1). The high amount of hydrolytic products arising from ellagitannins compared to the sum of phenolic compounds before hydrolysis suggests that acid hydrolysis which relies mainly on the titration of two standards (ellagic acid and gallic acid) is a more relevant technique than UPLC in the absence of individual standards for quantifying phenolic compounds from jabuticaba peel.

The DJPE-HIUS was able to inhibit lipid oxidation at very low concentrations (0.065 and 0.32 mg/mL of gastric digesta for E1 and E5) during the whole gastric phase largely inhibiting the formation of the lipid oxidation markers analyzed (CD and 4-HNE). During the intestinal phase, the accumulation of CD increased, which was not supported by the formation of 4-HNE, suggesting that 4-HNE reacted with proteins and is thus not a relevant marker. Although E5 afforded a larger protection of lipid oxidation than E1, there were no significant differences between E1 and E5 for the results of both lipid oxidation markers CD and 4-HNE suggesting that lower extract concentrations can be used. In opposite to lipid oxidation analyses, the extract concentration had a great influence on the bioaccessibility results. In the gastric phase, the total bioaccessibility of anthocyanins was 21% for E1 and 67% for E5. Only COG was detected in E1 while COG (73%) and DOG (22%) were detected in E5. It shows that the dosage of extract used has a great influence on the content in anthocyanins ready for intestinal absorption or able to play an antioxidant role in the gastrointestinal tract. In the intestinal phase, COG and DOG present at the end of the gastric phase

were completely degraded in a few minutes after the change in digestion conditions for both extracts likely leading to different phenolic structures.

Acknowledgements

Mario R. Marostica Junior is grateful to CNPQ for financial support (CNPq 301108/2016-1). Adriana G. Tarone thanks CNPQ for the Ph.D. assistantship (140942/2016-5). Authors acknowledge the São Paulo Research Foundation (FAPESP) for the grant (2015/50333-1) and INRAE. This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Supplementary Material

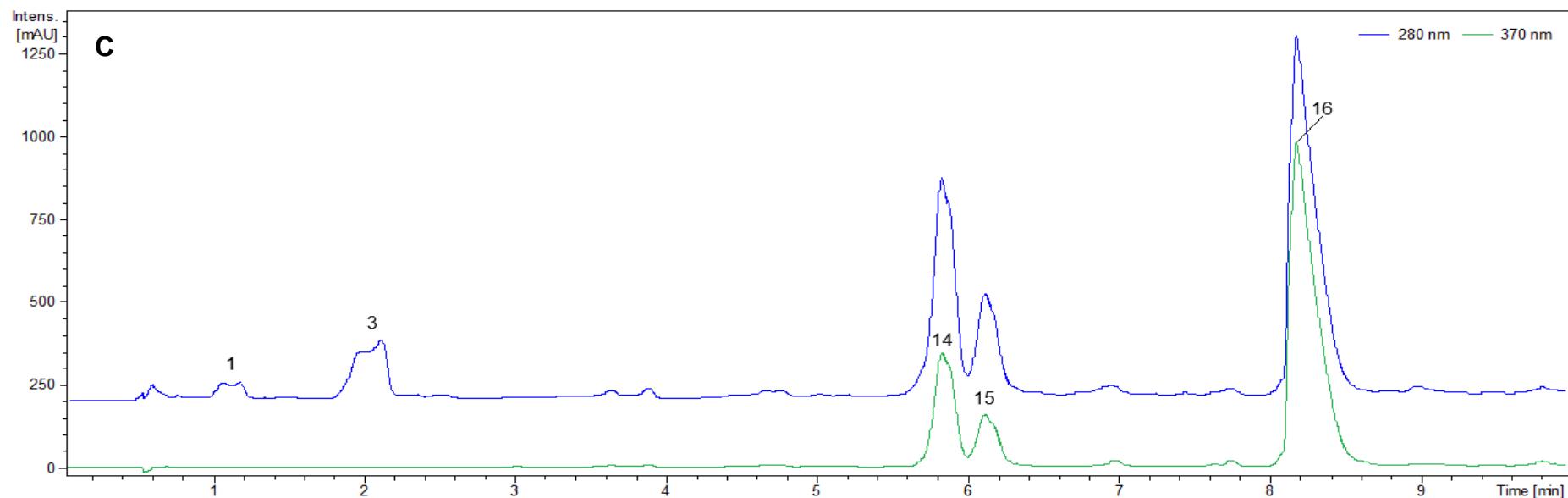


Figure 3. Chromatographic profile of the phenolic compounds for HIUS extract before hydrolysis at 280 nm, 370 nm and 530 nm (A) and after hydrolysis at 280 nm and 370 nm (B – Supernatant and C – Pellet).

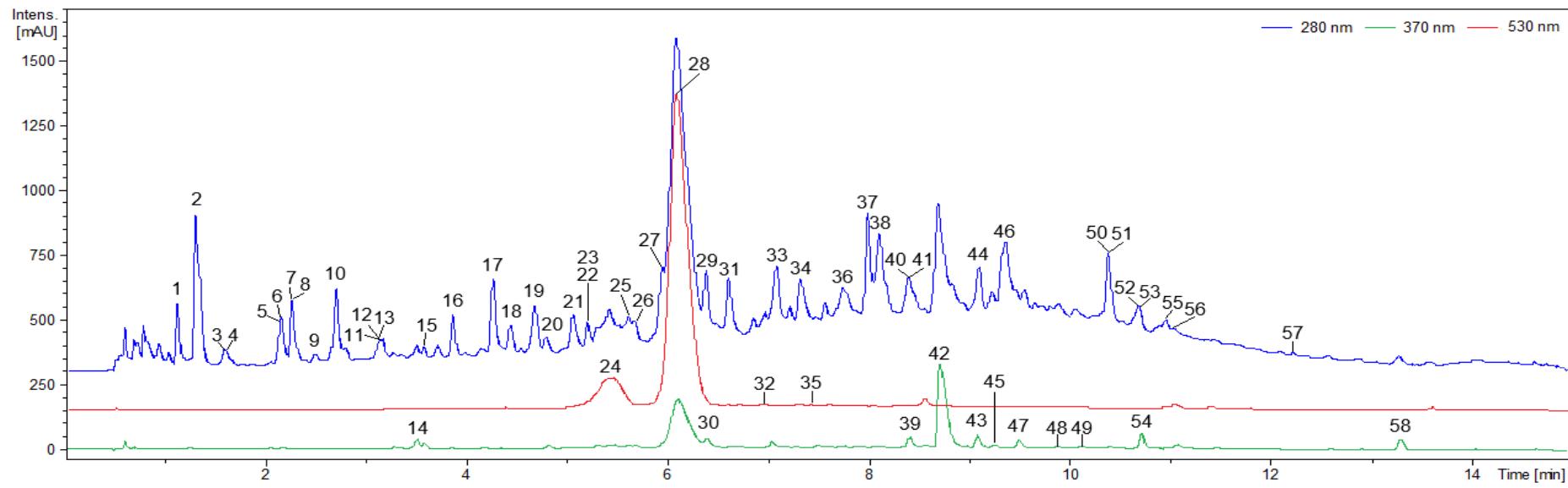


Figure 4. Chromatographic profile of the phenolic compounds for CM extract at 280 nm, 370 nm and 530 nm.

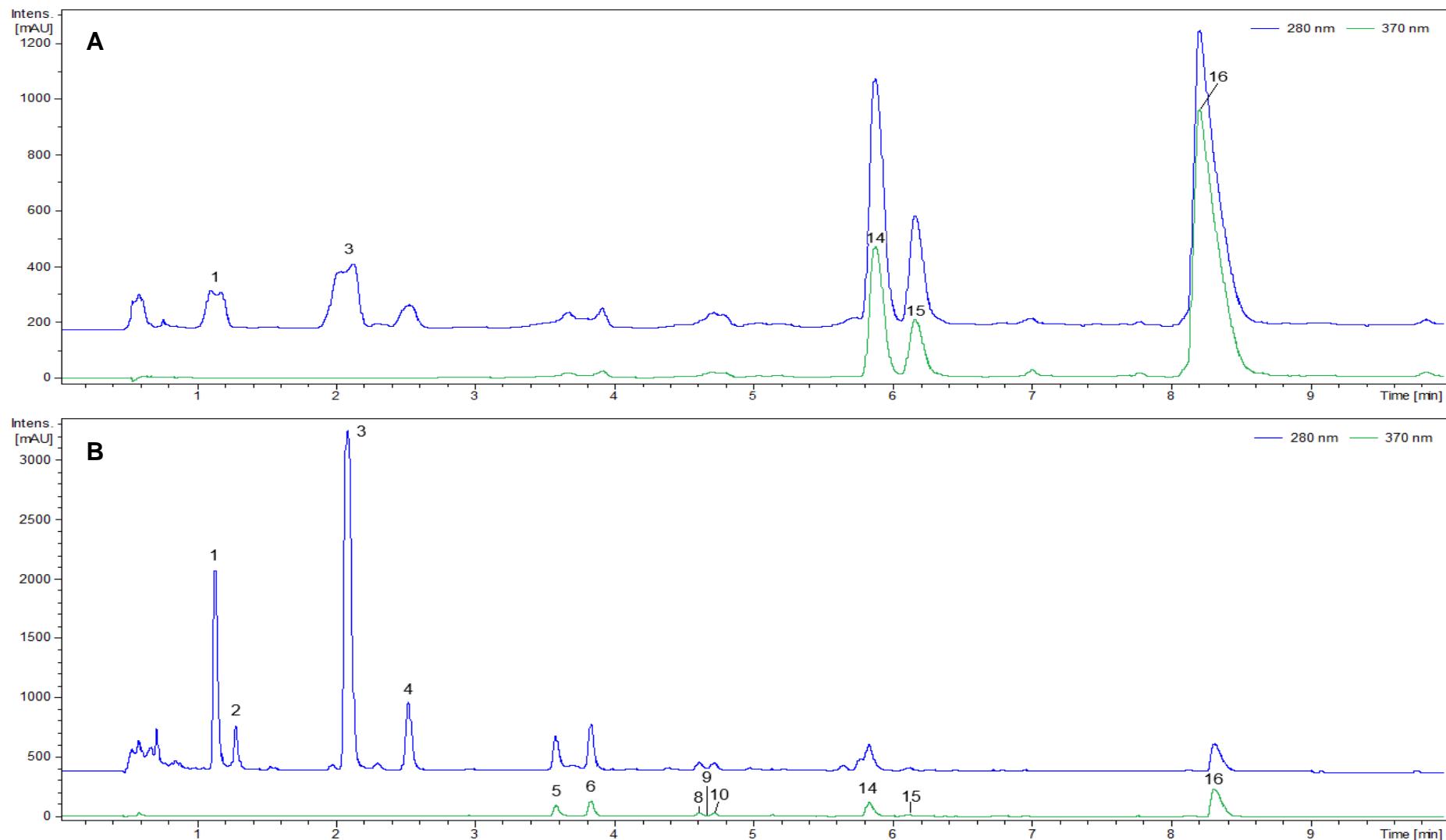


Figure 5. Chromatographic profile of the phenolic compounds for DJP after hydrolysis at 280 nm and 370 nm (A – Supernatant and B – Pellet).

References

- ABE, L. T.; LAJOLO, F. M.; GENOVESE, M. I. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg). **Journal of the Science of Food and Agriculture**, v. 92, n. 8, p. 1679–1687, 2012.
- ALBUQUERQUE, B. R. et al. Jabuticaba residues (*Myrciaria jaboticaba* (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. **Food Chemistry**, v. 309, p. 125735, out. 2020.
- ALEZANDRO, M. R. et al. Comparative study of chemical and phenolic compositions of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. **Food Research International**, v. 54, n. 1, p. 468–477, 2013.
- ANDREOLI, R. et al. Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry. **Rapid Communications in Mass Spectrometry**, v. 17, n. 7, p. 637–645, 2003.
- BATISTA, Â. G. et al. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. **Journal of Functional Foods**, v. 6, n. 1, p. 450–461, 2014.
- BERTON, C.; GENOT, C.; ROPERS, M. H. Quantification of unadsorbed protein and surfactant emulsifiers in oil-in-water emulsions. **Journal of Colloid and Interface Science**, v. 354, n. 2, p. 739–748, 2011.
- BOLÉA, G. et al. Lipid protection by polyphenol-rich apple matrices is modulated by pH and pepsin in in vitro gastric digestion. **Food & Function**, v. 10, p. 3942–3954, 2019.
- CALANI, L. et al. Ultra-HPLC-MSn (poly)phenolic profiling and chemometric analysis of juices from ancient *Punica granatum* L. cultivars: A nontargeted approach. **Journal of Agricultural and Food Chemistry**, v. 61, n. 23, p. 5600–5609, 12 jun. 2013.
- CHEMAT, F. et al. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. **Ultrasonics Sonochemistry**, v. 34, p. 540–560, 2017.
- CHRIST, A.; LAUTERBACH, M.; LATZ, E. Western Diet and the Immune System: An Inflammatory Connection. **Immunity**, v. 51, n. 5, p. 794–811, 19 nov. 2019.

DA SILVA, B. S. G. HIDRÓLISE ÁCIDA COMO ESTRATÉGIA PARA A DESPOLIMERIZAÇÃO DOS ELAGITANINOS DA CASCA E SEMENTE DA JABUTICABA (*MYRCIARIA JABOTICABA*). [s.l.] UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, 2019.

DA SILVA, J. K. et al. Functional tea from a Brazilian berry: Overview of the bioactives compounds. **LWT - Food Science and Technology**, v. 76, p. 292–298, 1 mar. 2017.

FARIA, A. et al. Absorption of anthocyanins through intestinal epithelial cells - Putative involvement of GLUT2. **Molecular Nutrition and Food Research**, v. 53, n. 11, p. 1430–1437, 2009.

FISCHER, U. A.; CARLE, R.; KAMMERER, D. R. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum L.*) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. **Food Chemistry**, v. 127, n. 2, p. 807–821, 2011.

FRACASSETTI, D. et al. Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). **Food Chemistry**, v. 139, n. 1–4, p. 578–588, 2013.

GARCÍA-VILLALBA, R. et al. Validated Method for the Characterization and Quantification of Extractable and Nonextractable Ellagitannins after Acid Hydrolysis in Pomegranate Fruits, Juices, and Extracts. **Journal of Agricultural and Food Chemistry**, v. 63, n. 29, p. 6555–6566, 2015.

GARCÍA-VILLALBA, R. et al. Comprehensive characterization by LC-DAD-MS/MS of the phenolic composition of seven *Quercus* leaf teas. **Journal of Food Composition and Analysis**, v. 63, n. June, p. 38–46, 2017.

GASC, N. et al. **4-Hydroxynonenal in foodstuffs: Heme concentration, fatty acid composition and freeze-drying are determining factors.** Redox Report. Anais...2007 Disponível em: <<https://hal.inrae.fr/hal-02655296>>. Acesso em: 17 jul. 2020

HE, J.; GIUSTI, M. M. Anthocyanins: Natural Colorants with Health-Promoting Properties. **Annual Review of Food Science and Technology**, v. 1, n. 1, p. 163–187, 2010.

INADA, K. O. P. et al. Screening of the chemical composition and occurring antioxidants in jaboticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits and their fractions. **Journal of Functional Foods**, v. 17, p. 422–433, 2015.

- INADA, K. O. P. et al. Bioaccessibility of phenolic compounds of jaboticaba (*Plinia* *jaboticaba*) peel and seed after simulated gastrointestinal digestion and gut microbiota fermentation. **Journal of Functional Foods**, v. 67, p. 103851, 1 abr. 2020.
- JANEIRO, P.; BRETT, A. M. O. Redox behavior of anthocyanins present in *Vitis vinifera L.* **Electroanalysis**, v. 19, n. 17, p. 1779–1786, 2007.
- KANESHIMA, T. et al. Antioxidative Constituents in Camu-camu Fruit Juice Residue. **Food Science and Technology Research**, v. 19, n. 2, p. 223–228, 2013.
- LAMAS, C. A. et al. A jaboticaba extract prevents prostatic damage associated with aging and high-fat diet intake. **Food and Function**, v. 11, n. 2, p. 1547–1559, 2020.
- LEITE-LEGATTI, A. V. et al. Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. **Food Research International**, v. 49, n. 1, p. 596–603, 2012.
- LENQUISTE, S. A. et al. Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. **Food Research International**, v. 77, p. 162–170, 2015.
- MARQUES-PEIXOTO, F. et al. Simulation of in vitro digestion coupled to gastric and intestinal transport models to estimate absorption of anthocyanins from peel powder of jabuticaba, jamelão and jambo fruits. **Journal of Functional Foods**, v. 24, p. 373–381, 2016.
- MATTILA, P.; KUMPULAINEN, J. Determination of Free and Total Phenolic Acids in Plant-Derived Foods by HPLC with Diode-Array Detection. **Journal of Agricultural and Food Chemistry**, v. 50, n. 13, p. 3660–3667, 2002.
- MENA, P. et al. Rapid and comprehensive evaluation of (Poly)phenolic compounds in pomegranate (*Punica granatum L.*) Juice by UHPLC-MSn. **Molecules**, v. 17, n. 12, p. 14821–14840, 2012.
- MIKKELSEN, A.; SKIBSTED, L. H. Acid-catalysed reduction of ferrylmyoglobin: product distribution and kinetics of auto-reduction and reduction by NADH. **Zeitschrift für Lebensmittel-Untersuchung und -Forschung**, v. 200, n. 3, p. 171–177, 1995.
- MINEKUS, M. et al. A standardised static in vitro digestion method suitable for food – an international consensus. **Food & Function**, v. 5, n. 5, p. 1113–1124, 2014.
- MORALES, P. et al. Non-fermented and fermented jaboticaba (*Myrciaria cauliflora* Mart.) pomaces as valuable sources of functional ingredients. **Food Chemistry**, v. 208, p. 220–227, 2016.

- NEVES, N. DE A. et al. Flavonols and ellagic acid derivatives in peels of different species of jabuticaba (*Plinia* spp.) identified by HPLC-DAD-ESI/MSn. **Food Chemistry**, v. 252, n. January, p. 61–71, 2018.
- PEREIRA, L. D. et al. Polyphenol and Ellagitannin Constituents of Jabuticaba (*Myrciaria cauliflora*) and Chemical Variability at Different Stages of Fruit Development. **Journal of Agricultural and Food Chemistry**, v. 65, n. 6, p. 1209–1219, 2017.
- PLAZA, M. et al. Characterization of antioxidant polyphenols from *Myrciaria jaboticaba* peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. **Food Chemistry**, v. 211, p. 185–197, 2016.
- PRYOR, W. A.; CASTLE, L. Chemical methods for the detection of lipid hydroperoxydes. In: PACKER, L. (Ed.). **Oxygen Radicals in Biological Systems**. Orlando, FL: Academic Press, 1984. p. 293–295.
- QUATRIN, A. et al. Characterization and quantification of tannins, flavonols, anthocyanins and matrix-bound polyphenols from jaboticaba fruit peel: A comparison between *Myrciaria trunciflora* and *M. jaboticaba*. **Journal of Food Composition and Analysis**, v. 78, n. January, p. 59–74, 2019.
- QUATRIN, A. et al. Bioaccessibility and catabolism of phenolic compounds from jaboticaba (*Myrciaria trunciflora*) fruit peel during in vitro gastrointestinal digestion and colonic fermentation. **Journal of Functional Foods**, v. 65, p. 103714, 1 fev. 2020.
- SANTOS-BUELGA, C.; GONZÁLEZ-PARAMÁS, A. M. Anthocyanins. **Reference Module in Food Science**, p. 1–12, 2018.
- SERRANO, J. et al. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. **Molecular Nutrition and Food Research**, v. 53, n. SUPPL. 2, p. 310–329, 1 set. 2009.
- SILVA, R. M. et al. Protective effect and induction of DNA repair by *Myrciaria cauliflora* seed extract and pedunculagin on cyclophosphamide-induced genotoxicity. **Mutation Research - Genetic Toxicology and Environmental Mutagenesis**, v. 810, p. 40–47, 2016.
- SUN, J. et al. Profiling polyphenols of two diploid strawberry (*Fragaria vesca*) inbred lines using UHPLC-HRMSn. **Food Chemistry**, v. 146, p. 289–298, 1 mar. 2014.
- TANAKA, T. et al. Tannins and related compounds CXVII. Isolation and characterization of three new ellagitannins, lagerstannins A, B, C, having a gluconic acid core, from *Lagerstroemia speciosa* L. Pers. **Chemical Pharmaceutical Bulletin**, v. 40, n. 11, p. 2975–2980, 1992.

TAVARES, I. M. DE C. et al. Comprehensive study of the phenolic composition of the edible parts of jambolan fruit (*Syzygium cumini* (L.) Skeels). **Food Research International**, v. 82, p. 1–13, 1 abr. 2016.

WANG, P. et al. Degradation behavior of polyphenols in model aqueous extraction system based on mechanical and sonochemical effects induced by ultrasound. **Separation and Purification Technology**, v. 247, p. 116967, 15 set. 2020.

WU, S. B. et al. Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. **Journal of Agricultural and Food Chemistry**, v. 60, n. 30, p. 7513–7525, 2012.



CAPÍTULO V

Inulin/fructooligosaccharides/pectin-based structured systems: Promising encapsulating matrices of polyphenols recovered from jabuticaba peel

Published in Food Hydrocolloids, v.111, February 2021, 106387

Inulin/fructooligosaccharides/pectin-based structured systems: Promising encapsulating matrices of polyphenols recovered from jabuticaba peel

Adriana Gadioli Tarone^a; Eric Keven Silva^b; Cinthia Baú Betim Cazarin^c; Mario Roberto Marostica Junior^{a*}

^a LANUM (Laboratory of Nutrition and Metabolism)/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

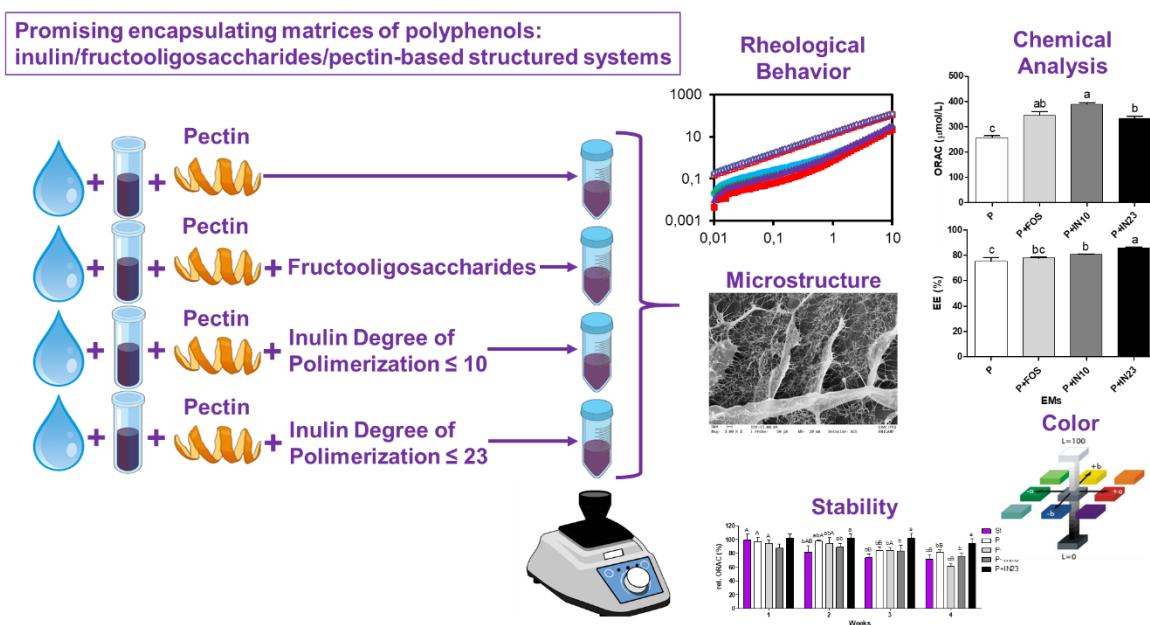
^b LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

^c LAFOP (Laboratory of Protein Source)/FEA (School of Food Engineering)/UNICAMP (University of Campinas); Rua Monteiro Lobato, 80; Campinas-SP; CEP:13083-862; Brazil

Highlights

- FOS/inulin/pectin-based structured systems were evaluated as encapsulating matrices.
- Inulin degree of polymerization influenced on rheological properties and stability.
- Jabuticaba peel is a polyphenols natural source associated with health benefits.
- FOS/inulin/pectin-based structured systems were evaluated as encapsulating matrices.

Graphical Abstract



Abstract

This paper investigated the effects of fructooligosaccharides (FOS) and inulin with different degrees of polymerization (DP) on the rheological, physical, and microstructural properties of pectin-based structured systems and their performance as encapsulating matrices of polyphenols extracted from jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) peel. The pseudoplastic pectin-based systems presented a predominantly viscous behavior. The addition of FOS diminished their apparent viscosity and the consistency index. On the other hand, the increase of inulin DP increased them. The rheological characteristics and microstructure found in the pectin-based encapsulating systems with the addition of inulin with $DP \geq 23$ (P+IN23) enable its use as a functional structuring food ingredient. The addition of inulin darkened the samples with the increase in DP, indicating better color retention in the pectin-based matrix with higher inulin DP. It also showed the highest values of chemical properties like bioactivity and encapsulation efficiency (EE) (85.7%), followed by the sample with the addition of inulin with $DP \geq 10$ (P+IN10) (80.6%), and the sample with the addition of FOS (P+FOS) (77.9%). Pectin-based sample (P) had the lowest values and EE (75.5%). Therefore, the addition of inulin improved these parameters, and this improvement was gradual with the increase of DP. During the four weeks of stability evaluation, only P+IN10 and P+IN23 samples remained stable. In the last week evaluated, P+IN23 presented the highest percentage of relative oxygen radical absorbance capacity (94%), and the values decreased with the decrease of DP (P+IN10 = 76% and P+FOS = 61%). The P encapsulating matrix presented the second-highest percentage (82%). P and P+IN23 samples were efficient in protecting the jabuticaba peel extract. They exhibited good retention of the antioxidant capacity. Likewise, the inulin addition and its molecular chain size contributed to the binding reordering of the system.

Keywords: *Myrciaria jabuticaba* (Vell.) Berg; inulin degree of polymerization; structuring food ingredient; fat replacer; colon targeted delivery system.

1. Introduction

Jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg) is a Brazilian berry whose peel is a natural source of polyphenols (Reynertson et al., 2006). These compounds are directly related to antioxidant, antimicrobial, antiproliferative, anti-inflammatory, and antimutagenic (Albuquerque et al., 2020; Lamas et al., 2020; Leite-Legatti et al., 2012) activities, besides other health benefits (Batista et al., 2014; J. K. da Silva et al., 2017; de Sá et al., 2014; Lenquiste et al., 2015; Plaza et al., 2016). Some studies suggest that these compounds may contribute to colon health by acting as prebiotics (Kawabata, Yoshioka, & Terao, 2019; Lin, Fischer, & Wicker, 2016) and modulating gut microbiota (Corrêa, Rogero, Hassimotto, & Lajolo, 2019; da Silva-Maia et al., 2019), but they require protection to go through the digestive tract and arrive in the colon intact without being absorbed into other sites or degraded. In this way, their encapsulation is a promising approach to protecting them (Munin & Edwards-Lévy, 2011).

The encapsulation of polyphenols in hydrocolloid-based systems has been widely studied with a view to their protection and targeted and controlled release in the human body (Guo, Giusti, & Kaletunç, 2018), and colon targeted delivery systems using prebiotic biopolymers as the encapsulating agent have been used successfully (Jakobek & Matić, 2019; Kosaraju, 2005; Xu et al., 2020). Besides their relatively low cost, biodegradability and renewability, the viscoelastic properties of hydrocolloids-based encapsulating matrices make them feasible for a wide variety of applications (Coviello et al., 2015; Dafe, Etemadi, Dilmaghani, & Mahdavinia, 2017; Tabernero, Baldino, Misol, Cardea, & del Valle, 2020). When used as encapsulating agents, hydrocolloids resist gastrointestinal enzymes, being fermented in the colon and liberating the encapsulated compounds into the correct site, acting also as a prebiotic (Kosaraju, 2005). Among the main hydrocolloids used to encapsulate polyphenols, some studies demonstrated that pectins (Buchweitz, Speth, Kammerer, & Carle, 2013; Chung, Rojanasasithara, Mutilangi, & McClements, 2015; Maier, Fromm, Schieber, Kammerer, & Carle, 2009) and fructans like fructooligosaccharides (FOS) and inulin (Araujo-Díaz, Leyva-Porras, Aguirre-Bañuelos, Álvarez-Salas, & Saavedra-Leos, 2017; Bakowska-Barczak & Kolodziejczyk, 2011; Bernardes et al., 2019; Lacerda et al., 2016; Nowicka & Wojdył, 2016) were efficient as encapsulating systems and

carriers, stabilizing these compounds, delaying their chemical degradation, and improving their chemical stability and other physicochemical properties.

Pectin is a natural complex polysaccharide found in the cell wall of higher plants acting as a structural material, and its main commercial sources are citrus peel and apple pomace (Thakur, Singh, & Handa, 1997). Considered a safe food ingredient, commercial pectins used in the food industry are segregated according to their degree of methoxylation in two groups: low methyl ester (LM) pectins when their degree of esterification (DE) is less than 50% and high methyl ester (HM) pectins when their DE is greater than 50% (De Cindio, Gabriele, & Lupi, 2015; Wicker & Kim, 2015). More stable, LM pectin is typically used in the food industry as a stabilizing, thickening, and gelling agent (Rolin & De Vries, 1990; Wicker & Kim, 2015). For being highly charged, LM pectin forms strong hydrogels with divalent metal cations, usually calcium, via the egg-box structure that is stable in a wide range of pH values (from pH 3 to 6) (De Cindio et al., 2015; Rolin & De Vries, 1990; Smith, Moxon, & Morris, 2016). On the other hand, LM pectin forms soft hydrogels through hydrophobic interaction between methyl-esterified groups and/or hydrophilic interactions between undissociated carboxyl groups and hydroxyl groups via hydrogen bonds in pHs around five or below that. The pectin-based structured systems obtained in this way can be used as a thickening and fat replacer agent (De Cindio et al., 2015; Rolin & De Vries, 1990).

Inulin, a storage carbohydrate for plants, is a generic term that covers all types of linear fructans, and its primary commercial source is chicory root. Inulin consists of a disaccharide (glucose and fructose) with additional fructose units attached (the degree of polymerization can range from 2 to over 60 fructose units). When short chains are formed (degree of polymerization below about ten fructose units), they are called FOS (Roberfroid, 2005). Also considered a safe food ingredient, inulin-based structured systems are used in the food industry as sugar and fat replacement and to improve texture and spreadability (Franck, 2002). Inulin may be used in different degrees of polymerization, and some authors associate improvement in polyphenol chemical stability and physicochemical properties of the system with the size of the inulin molecular chain (Guimarães et al., 2020; Li, Shabani, et al., 2019; Ozturkoglu-Budak, Akal, Buran, & Yetişemiyen, 2019; E. K. Silva & Meireles, 2015; Tárrega, Rocafull, & Costell, 2010; Wang, Wan, Liu, Xia, & Ding, 2019).

Many studies reported good performance of pectin and fructans like FOS and inulin in polyphenol encapsulation; however, the association of both is still poorly

explored. Thus, this study aimed to evaluate the effects of the addition of FOS and inulin with different degrees of polymerization on the rheological, physical, and microstructural properties of pectin-based structured systems developed to play as structuring food ingredient and their performance as encapsulating systems of polyphenols extracted from jabuticaba peel.

2. Materials and Methods

2.1. Materials

Ethanol absolute, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine); TROLOX (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid); AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride); and gallic acid were all obtained from Sigma-Aldrich (São Paulo, Brazil). Fluorescein sodium salt was purchased from Vetec Química Fina (São Paulo, Brazil). Folin & Ciocalteu's phenol reagent was purchased from Dinâmica (Brazil). Ultrapure water (resistivity 18.2 MΩ cm⁻¹ at 25 °C) was obtained with a Millipore OPak 2 (Millipore Corporation, Bedford, MA, USA). The other reagents used were of analytical grade.

Low methylester pectin derived from the citrus peel (P) (with a degree of methyl esterification lower than 50) was kindly donated by CP Kelco (GENU®pectin type LM102-AS-Z - Limeira, Brazil) and three types of fructan obtained from chicory root were kindly donated by Beneo-Orafti (São Paulo, Brazil): fructooligosaccharide Orafti®Oligofructose with a degree of polymerization (DP) < 10 (FOS), inulin (IN) Orafti®GR with DP ≥ 10 (IN10) and inulin (IN) Orafti®HP with DP ≥ 23 (IN23).

2.2. Jabuticaba peel processing

The jabuticaba (*Myrciaria jabuticaba* (Vell.) Berg.) was kindly donated by "Indústria e Comércio Lagoa Branca Ltda", located at Fazenda Boa Vista II in the municipality of Casa Branca (São Paulo, Brazil) under the authorization of the Brazilian Management System of National Genetic Heritage and Traditional Associated Knowledge (#A72354F). The berries were washed and manually peeled, and the peel was dried in a stove with air circulation (Marconi, Piracicaba, SP, Brazil) at 40 °C for 72 h. The dried peel was ground into a fine powder with an electric mill (Marconi, MA 630/1, Piracicaba, SP, Brazil) and sifted (mash 20), and the dried jabuticaba peel powder (DJP) was stored at - 20 °C.

2.3. High-intensity ultrasound-assisted extraction of polyphenols from dried jabuticaba peel

The dried jabuticaba peel extract was obtained using a 13-mm ultrasonic probe diameter at 19 kHz (Unique, Desruptor, Indaiatuba, Brazil) for 3 min. The probe contact height with the extract was kept at 40 mm and an ice bath was used to prevent the extract from overheating. One gram of dried jabuticaba peel and 25 mL of solvent extraction (50% ethanol absolute in ultra-pure water, w/w) were used at a nominal power of 320 W. The extraction was made in triplicate and the solvent was removed in vacuo. The dried extract was reconstituted in the same volume of ultra-pure water and freeze-dried and stored at - 20 °C until use.

2.4. Production of jabuticaba peel extract-rich encapsulating systems using pectin and fructans

Four different formulations of encapsulating matrices were prepared, as shown in **Table 1**.

Table 1. Polyphenols-loaded FOS/inulin/pectin-based structured systems formulations.

Samples	Ultrapure Water (g/100 g)	P (g/100 g)	FOS (g/100 g)	IN10 (g/100 g)	IN23 (g/100 g)	Extract (g/100 g)
P	89	6	-	-	-	5
P+FOS	86	6	3	-	-	5
P+IN10	86	6	-	3	-	5
P+IN23	86	6	-	-	3	5

P = pectin; FOS = fructooligosaccharide; IN10 = inulin with a degree of polymerization (DP) ≥ 10; IN23 = inulin with DP ≥ 23 (IN23).

The hydrocolloids were placed in amber flasks and solubilized in the ultrapure water at 80 °C under mechanical agitation. After natural cooling of the systems to room temperature (24 °C), the jabuticaba peel extract was added, and the systems were homogenized manually. The lyophilized jabuticaba peel extract was reconstituted in one-third of its initial volume to present a greater amount of

polyphenols, resulting in the final concentration of 6.33 mg of lyophilized extract/mL of ultrapure water. The encapsulating systems were made in triplicate and stored for 24 h at 4° C before the analyses.

2.5. Rheology measurements

The rheological behavior and viscosity of the encapsulating systems were evaluated by a Physica MCR301 modular compact rheometer (Anton Paar, Graz, Austria), 24 h after their preparation. A cone-plate geometry (50 mm diameter, 2° angle) with a 0.5 mm gap was used for the analysis and a thin layer of silicone oil was placed around the sample to prevent evaporation. All measurements were made in triplicate at 25 °C.

2.5.1. Flow properties

Flow curves were obtained through a multistep (up-down-up) program using a shear rate varying between 0 and 300 s⁻¹ to evaluate and eliminate the thixotropy so that the relationship between viscosity (η) and shear rate ($\dot{\gamma}$) of the different samples could be evaluated. The third flow curve data were fitted to the model for shear-thinning fluids (power-law model) (Equation (1)). The parameters k and n were estimated by non-linear regression using the Quasi-Newton method with a convergence criterion of 10⁻⁴ using Statistica 13.3® software (TIBCO Software Inc., Palo Alto, CA, USA). The model fit was evaluated based on the coefficient of determination (R^2) and the mean relative percentage deviation modulus (E), as defined by Equation (2).

$$\sigma = k(\dot{\gamma})^n \quad (1)$$

where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s⁻¹), k is the consistency index (Pa.s) and n is the flow behavior index (dimensionless).

$$E = \frac{100}{N} \sum_{i=1}^N \frac{|m_i - m_p|}{m_i} \quad (2)$$

where m_i is the experimental value, m_p is the predicted value and N is the population of the experimental data.

2.5.2. Viscoelastic properties

The viscoelastic properties were evaluated by oscillatory measurements, using a frequency sweep between 0.01 and 10 Hz, within the linear viscoelastic domain

at 25 °C. The parameters determined were elastic or storage modulus (G') and viscous or dissipative modulus (G'').

2.6. Scanning electron microscopy (SEM)

Scanning electron microscopy was used to characterize the samples' microstructure, and to this end, they were frozen using liquid nitrogen and freeze-dried 24 h after their preparation. Micrographs were taken in a scanning electron microscope with Energy Dispersive X-ray Detector (Leo 440i, EDS 6070, SEM/EDS: LEO Electron Microscopy/Oxford, Cambridge, England) after coating with a thin gold film of 200 Å of thickness with the aid of a Sputter Coater (Emitech, K 450, Kent, UK). Analyses were performed with 15 kV accelerating voltage and 50 pA beam current to obtain the micrographs.

2.7. Color

Color measurements were taken in triplicate at room temperature (24 °C) after 24 h of sample preparation using a spectrophotometer (Hunter Lab - Color Quest II, Reston, USA) in reflectance mode. The CIELAB* system (D65, 10° observer) was used and parameters L^* , a^* and b^* were registered. The color measurements were expressed in terms of luminosity L^* ($L^*=0$ black and $L^*=100$ white) and chromaticity defined by a^* (+ a^* =red and - a^* =green) and b^* (+ b^* =yellow and - b^* =blue). With these parameters, the cylindrical coordinates C^* (chroma) and h (hue angle), which define the intensity and tone of the samples, were calculated according to Equations (3) and (4), respectively.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$h = \arctan \left(\frac{b^*}{a^*} \right) \quad (4)$$

2.8. Chemical and bioactivity characterization

After 24 h of preparation, the encapsulating systems had their microstructures broken to expose their bioactive compounds for the analyses. They were diluted in a phosphate buffer (pH 7.4) at 100 mg/mL.

A BioTek Synergy HT Microplate Reader (Winooski, USA) coupled to Gen5™ 2.0 data analysis software was used for fluorometric and colorimetric analyses

in 96-well microplates (transparent or dark for oxygen radical absorbance capacity (ORAC) analyses). All the analyses were made in triplicate.

2.8.1. Total reduced capacity

The Folin–Ciocalteu method adapted from Swain & Hillis (1959) was used to determine the total reduced capacity. Absorbance was measured at 725 nm, gallic acid was used in a standard curve, and the results were expressed in gallic acid equivalent (mg GAE/100 g of the encapsulating system).

2.8.2. Antioxidant capacity

The antioxidant capacity was quantified by two in vitro methods. ORAC (oxygen radical absorbance capacity) was determined according to the methodology described by Ou, Chang, Huang, & Prior (2013). Absorbance was measured with fluorescent filters at 485 nm for excitation wavelength and 520 nm for emission wavelength. Trolox was used as a standard curve and the results were expressed in Trolox equivalent ($\mu\text{mol TE}/100 \text{ g}$ of the encapsulating system). FRAP (Ferric Reducing Antioxidant Power) was determined according to the methodology described by Benzie & Strain (1996). Absorbance was measured at 595 nm, Trolox was used as a standard curve, and the results were expressed in Trolox equivalent ($\mu\text{mol TE}/100 \text{ g}$ of the encapsulating system).

2.9. Antioxidant capacity and color stability

Ten milliliters of each sample were placed in amber bottles and kept in the dark oven at 30 °C for four weeks to evaluate antioxidant and color stability. Antioxidant capacity (ORAC) and color measurements of each bottle were performed and compared to the initial time (week zero), which was considered a standard of evaluation, once a week for four weeks. Measurements were taken in triplicate after the bottles reached room temperature (24 °C).

2.9.1. Color stability

Color measurements were taken according to section 2.7., obtaining mean values of a^* , b^* , and L^* . Color difference (ΔE) was calculated according to Equation (5):

$$\Delta E = \sqrt{(L^*W_x - L^*W_0)^2 + (a^*W_x - a^*W_0)^2 + (b^*W_x - b^*W_0)^2} \quad (5)$$

where L^* , a^* , and b^* are color parameters, W_0 = week 0, standard, and W_x = 1, 2, 3 or 4 weeks after storage.

2.9.2. Antioxidant capacity stability

Measurements of ORAC were taken according to section 2.8.2 and their decrease during storage was expressed relative to week 0 (relative ORAC) according to Equation (6) (Buchweitz et al., 2013):

$$rel. ORAC = \left(\frac{rel. ORAC_{W_x}}{rel. ORAC_{W_0}} \right) \times 100 \quad (6)$$

where W_0 = week 0, standard, and W_x = 1, 2, 3 or 4 weeks after storage.

The effects of the encapsulating matrix on the ORAC measurements were evaluated and all formulations were prepared with ultrapure water rather than the jabuticaba peel extract. A formulation with ultrapure water rather than the hydrocolloids used as encapsulating matrices was also prepared as a standard.

2.10. Encapsulation efficiency

Encapsulation efficiency (EE) was performed as proposed by Robert et al. (2010), evaluating the total polyphenol content encapsulated in the samples. The EE percentage for each encapsulating system was calculated using Equation (7):

$$EE(\%) = \frac{PP}{PP_0} \times 100 \quad (7)$$

where PP is the total polyphenol content extracted from the sample and PP_0 is the total polyphenol content of the added jabuticaba peel extract (PP and PP_0 were determined by the Folin-Ciocalteau method previously described in section 2.8.2).

2.11. Statistical analysis

Analysis of variance (ANOVA) was carried out to compare the data using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) software. Tukey's

test of means at a significance level of 95% (p -value < 0.05) examined differences in the mean values obtained.

3. Results and Discussion

3.1. Rheological behavior and microstructure

The pectin/FOS/inulin-based structured systems were characterized through rheological measurements to verify their possible use as structuring agent or fat replacers in food products. **Fig. 1** shows the relationship between apparent viscosity and shear rate of the pectin/FOS/inulin-based encapsulating systems. The flow rheological properties showed in **Table 2** were fitted to the power-law model. High values of the coefficient of determination ($R^2 \geq 0.999$) and low values of relative percentage deviation modulus ($E \leq 5.03$) were obtained. Thus, the curves fitted to the rheological behavior of the samples adequately represented the experimental data.

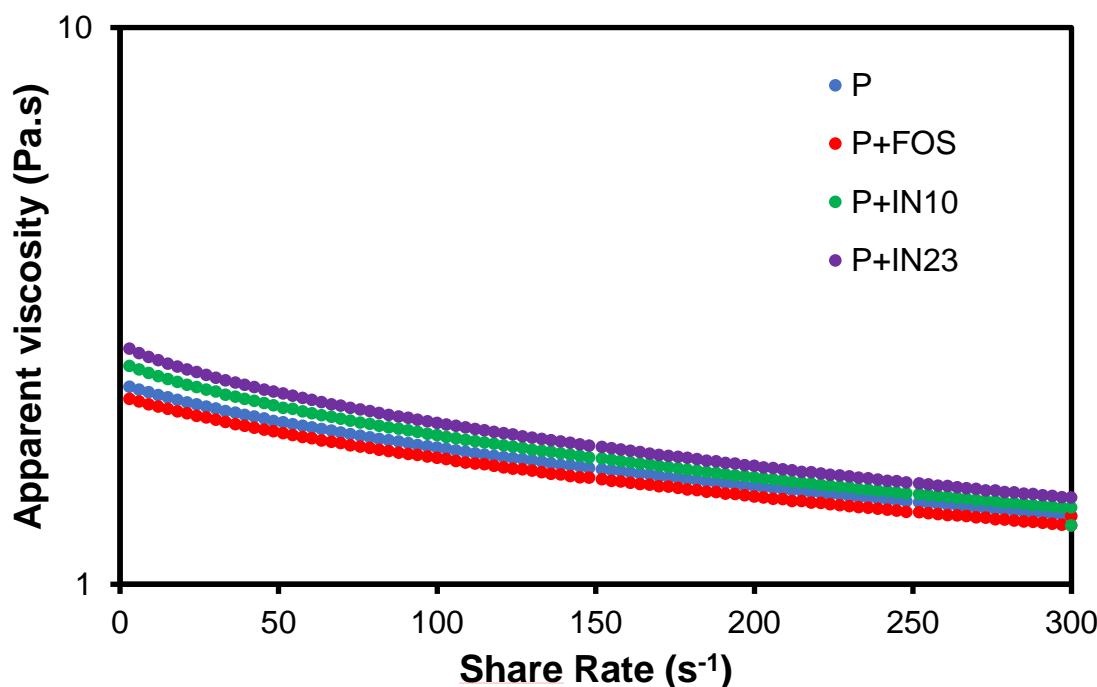


Fig. 1. Viscosity-shear rate profile at 25 °C of the polyphenols-loaded FOS/inulin/pectin-based structured systems.

Table 2. Rheological characteristics (apparent viscosity, flow and consistency indices at 25 °C) of the polyphenols-loaded FOS/inulin/pectin-based structured systems.

	Apparent viscosity at 100 s ⁻¹ (Pa.s)	Consistency index, k (Pa.s)	Flow behavior index, n	R ²	E (%)
P	1.33 ± 0.04 ^{bc}	5.1 ± 0.2 ^{bc}	0.7667 ± 0.0004 ^a	0.999	5.03
P+FOS	1.28 ± 0.07 ^b	4.8 ± 0.7 ^c	0.77 ± 0.01 ^a	0.999	4.94
P+IN10	1.373 ± 0.004 ^{ac}	5.8 ± 0.1 ^{ab}	0.750 ± 0.003 ^b	0.999	5.00
P+IN23	1.43 ± 0.02 ^a	6.3 ± 0.4 ^a	0.74 ± 0.01 ^b	0.999	4.86

Results are expressed as mean ± standard deviation. Different superscript letters in the same column indicate a significant statistical difference ($P < 0.05$) between the samples.

All samples exhibited a non-Newtonian and pseudoplastic behavior (flow behavior index $n < 1$), where apparent viscosity decreased with increasing shear rate. This shear-thinning behavior is typical in suspensions with more than 1 g/100 g of pectin (Huang et al., 2017; Rolin & De Vries, 1990). The addition of inulin decreased the n value, and the higher the inulin DP, the higher the pseudoplasticity (smaller is the n value). The apparent viscosity and the fluid consistency index (k) presented the same behavior, decreasing when FOS was added and increasing the higher the DP of the inulin added. The addition of FOS was efficient in decreasing apparent viscosity and consistency of the pectin-based structured system. The addition of IN10 did not change these properties, and the IN23 addition conferred higher apparent viscosity and consistency.

These rheological changes occurred due to inulin's tendency to break the pectin microstructure to promote a new and better-ordered binding, reducing the freedom of polymeric pectin chains (Bouaziz, Rassaoui, & Besbes, 2014). The inulin-based structured system's microstructure was formed by a network of small crystalline particles compounded of high molecular weight chains (taking into account the high polydispersity of inulin) (Bot, Erle, Vreeker, & Agterof, 2004). With the increase in DP of the added inulin, more particles with high molecular weight chains are available in the medium and more crystalline particles are formed, changing and improving the chain structure of the system and its rheological properties (Tárrega et al., 2010). Bouaziz et al. (2014) reported this synergic effect between pectin and inulin. Similar behavior was shown by Li, Ma, & Liu (2019) with cross-linked inulin with different DPs,

and by Wang et al. (2019) with hydrogels of rice starch pure and with added inulin (same DPs used in this study).

The oscillatory tests provided rheological behavior of the samples, as shown in **Fig. 2**.

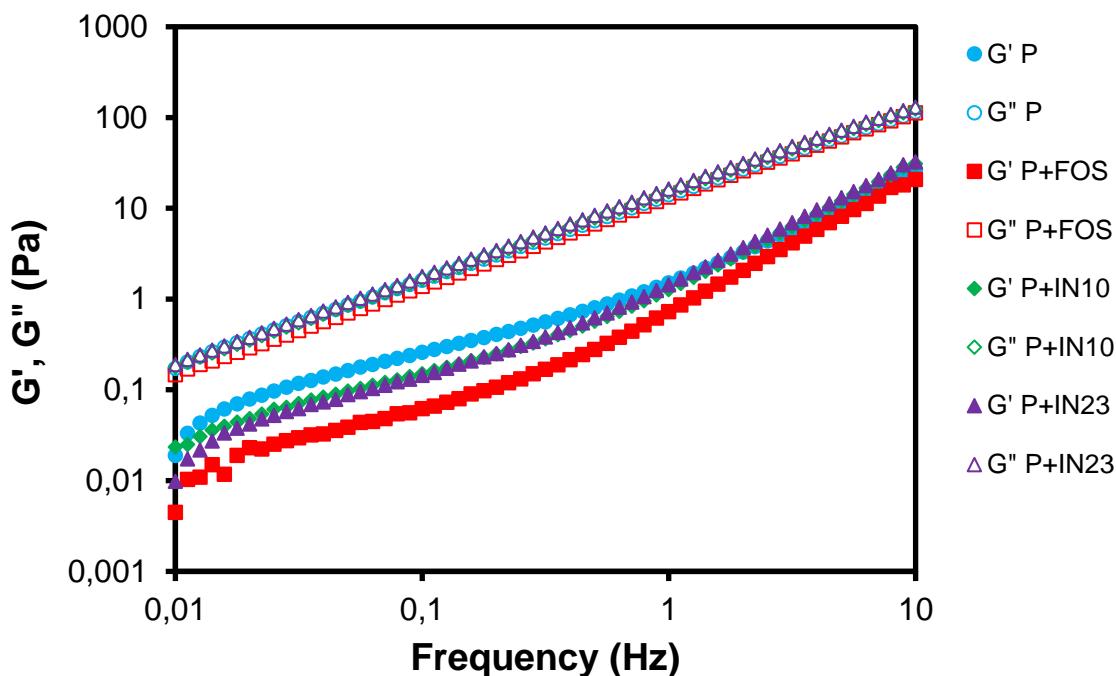


Fig. 2. Frequency sweep of storage modulus (G') and loss modulus (G'') for the polyphenols-loaded FOS/inulin/pectin-based structured systems.

The four formulations exhibited a similar trend in viscoelastic properties. Both G' and G'' moduli curves increased with the increase of frequency, showing a frequency-dependent behavior. G'' was greater than G' over the measured frequency sweep for all samples, indicating that viscous behavior predominated over elastic behavior. Therefore, all encapsulating systems exhibited a predominantly viscous response ($G' < G''$). The formation of a strong gel from LM pectin is dependent on the addition of calcium ions (Ca^{2+}) to the system (Rolin & De Vries, 1990). The absence of additional Ca^{2+} and a pH relatively low (4.5) permitted the formation of the pectin-based structured systems by hydrophobic interactions or hydrogen bonds (De Cindio et al., 2015; Rolin & De Vries, 1990). However, these systems were not characterized as hydrogels due to their predominant viscous behavior ($G' < G''$). The results indicated that the addition of inulin to the system did not affect the samples' viscoelastic behavior,

since the addition of inulin did not change the system's pH. This rheological behavior allowed the application of these pectin-based structured systems as structuring food ingredient (fat replacer agent or improving texture) in food products as can be seen in **Fig. 3.**

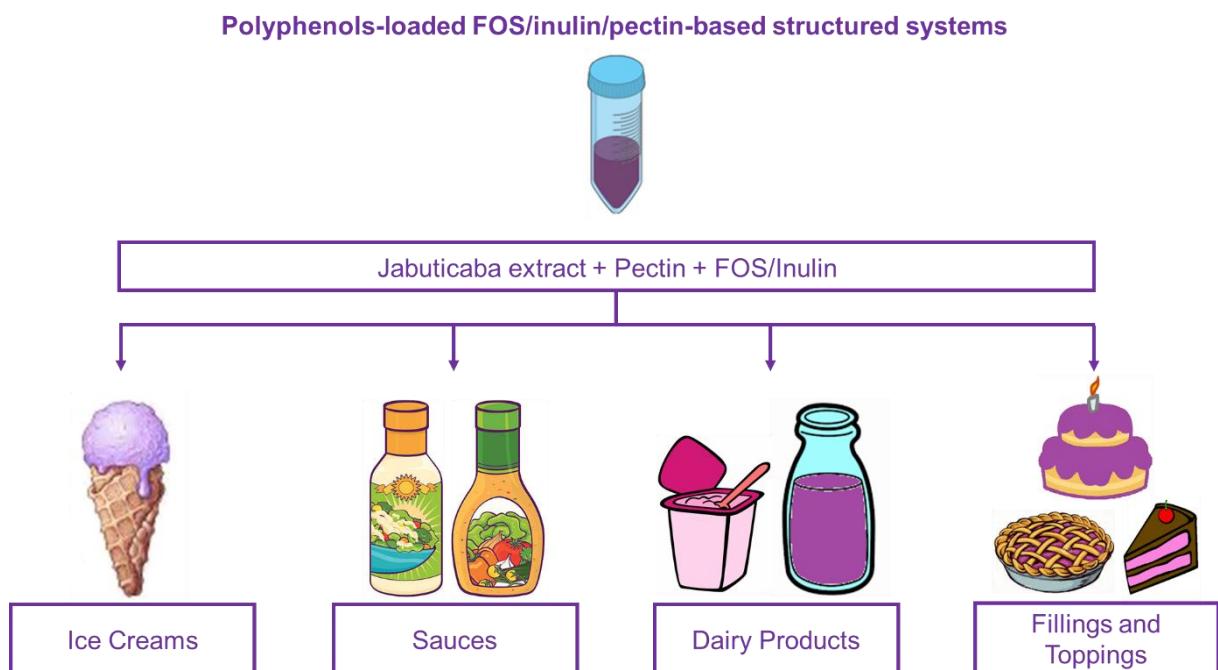


Fig. 3. Technological applications of polyphenols-loaded FOS/inulin/pectin-based structured systems as structuring ingredients (fat replacer agent or improving texture) in food products.

Nevertheless, the reduction of inulin DP promoted a slight decrease in G'' values. Ye et al. (2018) observed that the addition of inulin with lower DP resulted in a decreased G'' value compared to inulin with medium and high DP. They obtained a softer and more liquid-like structure of rice starch gels, similar to results of this study. According to the results obtained by Xu et al. (2020) and Zimeri & Kokini (2003), inulin was able to change the viscoelastic behavior of double-polysaccharide systems in concentrations higher than 2%. Witczak, Witczak, & Ziobro (2014) observed that higher concentrations of inulin increased system viscosity and that G' and G'' are increased with low DP inulin and with low concentrations of high DP inulin. This behavior leads us to believe that both inulin concentration and its DP directly affect systems' viscoelastic properties.

SEM in micrographs was used to characterize the morphologies of the encapsulating systems (**Fig. 4**). Microstructures of P and P+IN23 samples are similar. However, the network constructed by P+IN23 was more structured, probably due to its higher apparent viscosity and consistency than P. The P+FOS and P+IN10 have a denser microstructure, and P+FOS had an almost imperceptible network formation, corroborating the rheological properties found (lower apparent viscosity and consistency for P+FOS, followed by P+IN10). The above-mentioned synergic effect between pectin and inulin and the potential effect of inulin DP on the interaction between the system particles and the network structure formed were verified.

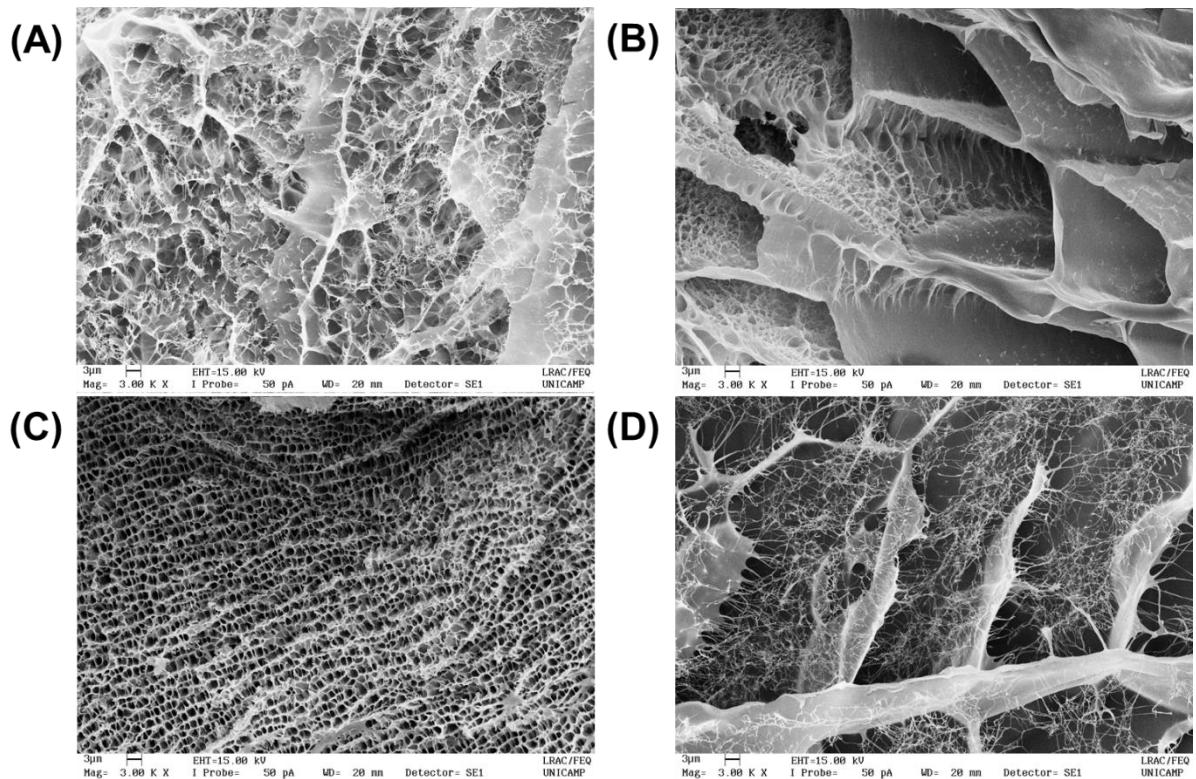


Fig. 4. SEM micrographs of the structured systems P (A), P+FOS (B), P+IN10 (C) and P+IN23 (D).

These rheological characteristics and microstructure make it possible to use them as structuring agents (replacing fat in dairy products or improving sauces' texture, for example (**Fig. 3**)). The P+IN23 encapsulating system is the most suitable for these purposes, as shown before.

3.2. Color parameters

The color parameters were evaluated to verify their change with the addition of inulin and the effect of the different DP of inulin. **Table 3** presents the results for the color parameters of the pectin/FOS/inulin-based encapsulating systems.

Table 3. Color parameters of the polyphenols-loaded FOS/inulin/pectin-based structured systems.

	L*	a*	b*	C*	h
P	33.50±0.14 ^a	5.09±0.05 ^a	-1.77±0.02 ^a	5.39±0.05 ^a	359.667±0.003 ^a
P+FOS	33.14±0.05 ^b	5.00±0.03 ^b	-1.91±0.02 ^c	5.35±0.03 ^{ab}	359.635±0.005 ^c
P+IN10	32.44±0.24 ^c	4.91±0.02 ^c	-1.92±0.01 ^c	5.27±0.02 ^c	359.627±0.002 ^d
P+IN23	32.96±0.08 ^b	5.01±0.03 ^b	-1.82±0.01 ^b	5.32±0.02 ^{bc}	359.652±0.004 ^b

Results are expressed as mean ± standard deviation. Different superscript letters in the same column indicate a significant statistical difference ($P < 0.05$) between the samples.

The addition of inulin promoted a decrease in color values: P sample presented the highest values for all color parameters studied and they decreased with the inulin addition. The samples showed a predominantly red hue with h close to 360° (or 0°), a* values ranging from 5.09 (P) to 4.91 (P+IN10) and b* values ranging from -1.77 (P) to -1.92 (P+IN10). Cyanidin, the major compound of jabuticaba peel (Lee, Durst, & Wrolstad, 2005), is probably responsible for dark red colors (low L* values) (Lacerda et al., 2016). The low L* values obtained with the addition of inulin suggest that its addition improved color retention and, consequently, the chemical compound. Faded color intensity is observed with Croma's low values (C*), which is characteristic of hydrocolloid suspensions (Pathare, Opara, & Al-said, 2013). It was more accentuated in samples with added inulin, where the hydrocolloid concentration was higher than in the P sample. Inulin DP influenced color parameters, with the P+IN23 sample showing the best results given the above.

3.3. Bioactivity characterization and encapsulation efficiency

Since a possible change in the phenolic compounds' chemical structure could modify their bioactivity properties and interaction with the hydrocolloids used, the pectin-based structured systems were characterized according to their chemical

properties, bioactivity, and encapsulation efficiency. Thus, we assessed the effect of the interaction between the extract and the addition of fructans with different DP on the structure of the phenolic compounds and, consequently, in their retention by these encapsulating matrices. The samples were evaluated according to total reduced capacity, antioxidant capacity (FRAP and ORAC) and encapsulation efficiency (**Table 4**).

Table 4. Total polyphenolic content, antioxidant capacity (FRAP and ORAC) and encapsulation efficiency (EE) of the polyphenols-loaded FOS/inulin/pectin-based structured systems.

	Polyphenols (mg GAE/100g)	FRAP (µmol TE/100g)	ORAC (µmol TE/100g)	EE (%)
P	39.4±1.5 ^c	294.3±7.3 ^c	256.7±12.3 ^c	75.5±2.8 ^c
P+FOS	40.7±0.3 ^{bc}	315.6±8.7 ^b	346.2±24.8 ^{ab}	77.9±0.6 ^b
P+IN10	42.1±0.2 ^b	326.2±12.4 ^b	389.8±10.8 ^a	80.6±0.3 ^b
P+IN23	44.7±0.3 ^a	343.1±5.7 ^a	332.6±17.3 ^b	85.7±0.7 ^a

Results are expressed as mean ± standard deviation. Different superscript letters in the same column indicate a significant statistical difference ($P < 0.05$) between the samples.

P+IN23 sample presented the highest value of total reduced capacity, followed by P+IN10 and P+FOS, and the same profile was found for FRAP and EE. For ORAC, the P+IN10 sample presented the highest value, followed by P+FOS and P+IN23. The P sample had the lowest values for all parameters measured.

The changes in the encapsulating systems' microstructure caused by the addition of inulin may have promoted larger retention of compounds, explaining the better results for P IN23 compared with P samples. The association of inulin and pectin creates a highly branched structure, resulting in higher availability of sites for hydrogen bondings (Lacerda et al., 2016), hydrophobic interactions (Buchweitz et al., 2013; Chung et al., 2015; Holzwarth, Korhummel, Siekmann, Carle, & Kammerer, 2013) and electrostatic interactions (Fredes, Osorio, Parada, & Robert, 2018), which are the main mechanisms for interaction between the phenolic compounds (present in the added extract) and the microstructure of the encapsulating systems (Jakobek & Matić, 2019). The higher the inulin DP, the greater the formed structure's branching, and the higher the number of the sites available (Wang et al., 2019).

These results showed that inulin addition improved the parameters studied and inulin DP had an influence on them, where the larger the size of the inulin chain (DP), the stronger its effect. The use of a buffer solution (pH 7.4) to break the microstructures and expose the compounds for analyses could have contributed to degrade or modify some pH-sensitive compounds, like anthocyanins (the major compound of jabuticaba peel, as cited previously) (He & Giusti, 2010; Prior & Wu, 2006; Santos-Buelga & González-Paramás, 2018). In this way, some results may have been underestimated and some compounds not identified.

3.4. Antioxidant capacity and color stability

The stability investigation aimed to evaluate the pectin-based structured systems' possible destabilization over four weeks, exposing and degrading the encapsulated compounds. **Fig. 5** shows the results for the samples' stability based on their antioxidant capacity (ORAC) and color parameters.

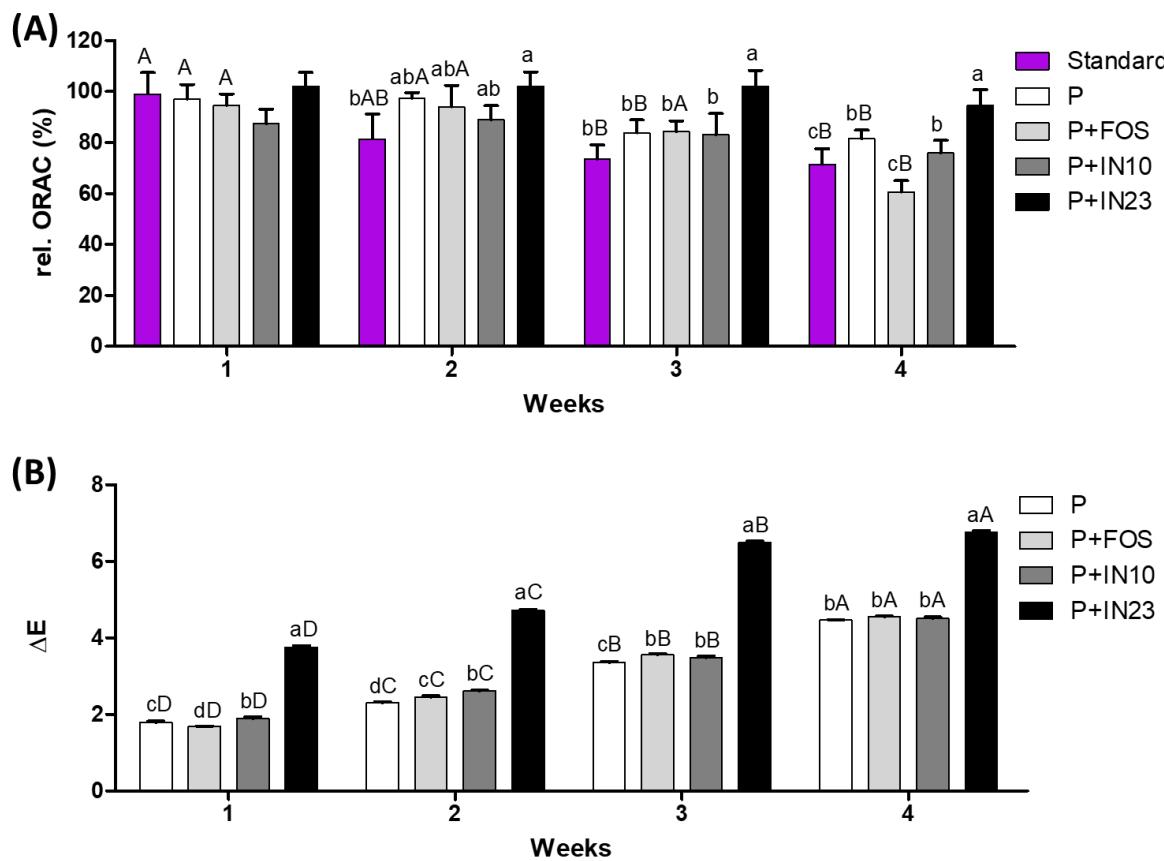


Fig. 5. Antioxidant capacity stability (A) and color stability (B) after storage of the polyphenols-loaded FOS/inulin/pectin-based structured systems in a dark oven at 30 °C for four weeks.

rel.ORAC (%) = relative ORAC values expressed relative to the initial values, ΔE = color difference, Standard = a formulation prepared with ultrapure water instead of the biopolymeric particles used as wall material. Results are expressed as mean \pm standard deviation. Different letters in the columns indicate a statistical difference ($P < 0.05$) between the samples. Capital letters indicate statistical difference ($P < 0.05$) between the weeks for the same samples and small letters indicate statistical difference ($P < 0.05$) between the different samples in the same week.

3.4.1. Antioxidant capacity stability

During the four weeks of evaluation, Standard, P, and P+FOS samples had their relative ORAC percentages reduced. P+IN10 and P+IN23 did not show a statistical difference between the weeks remaining stable during the storage time. In the first week, there was no statistical difference between the samples ($p\text{-value} > 0.05$). In the second week, Standard had its relative ORAC percentages reduced and showed a statistical difference concerning the other samples ($p\text{-value} < 0.05$). In the third week, Standard, P, P+FOS, and P+IN10 samples had their relative ORAC percentages reduced and showed a statistical difference ($p\text{-value} < 0.05$) concerning the P+IN23 sample. In the last week evaluated, week 4, the highest percentage of relative ORAC was for P+IN23 (94%), remaining statistically different from the others and indicating

the best retention of antioxidant capacity and the highest efficiency to protect the jabuticaba peel extract during the stability evaluation (p -value < 0.05). Although no statistical differences were found between P (82%) and P+IN10 (76%), P presented the second-highest percentage of relative ORAC, exhibiting good retention of antioxidant capacity and being efficient to protect the jabuticaba peel extract during the stability evaluation. The values between Standard (71%) and P+FOS (61%) did not show a statistical difference (p -value > 0.05), demonstrating that this encapsulating system was not efficient in protecting the jabuticaba peel extract during the stability evaluation. The decreasing values of P+IN10 (76%) and P+FOS (61%) compared to P+IN23 (94%) show that the P+IN23 sample presented the best retention of antioxidant capacity, corroborating the previously observed effect of the size of inulin chain on the binding reordering of the system.

According to many authors, the link between some phenolic compounds present in anthocyanin-rich materials (like the jabuticaba peel) and pectin is created by hydrophobic interactions and by the hydrogen bonds formed between pectin amide groups and the hydroxyl groups present on these compounds (Buchweitz et al., 2013; Chung et al., 2015; Holzwarth et al., 2013). This kind of link is stronger as cation availability increases (electrostatic interactions) (Fernandes, Brás, Mateus, & De Freitas, 2014; Lin et al., 2016). When inulin was added to the system, it broke the pectin structure to promote a newly ordered binding that changes the hydrophobic interactions (Bouaziz et al., 2014). These interactions are poorly reordered with FOS addition and become more ordered with the added inulin chain's increased size, creating more interaction sites for these compounds in P+IN23 and, consequently, conferring more stability to their antioxidant capacity in this encapsulating system network. The improvement in antioxidant stability makes this sample potentially useful as a functional structuring agent. Besides being used to improve food texture, it also has stable compounds with health-promoting properties. Also, no statistical differences were observed in ORAC for all formulations prepared with ultrapure water instead of the extract (p -value > 0.05).

3.4.2. Color stability

The measurement of color stability ΔE describes the human eye's ability to distinguish the color of two samples and is assumed that a person can only distinguish

it when $\Delta E \geq 5$ (Pérez-Magariño & González-Sanjosé, 2003). During the four weeks of evaluation, all samples had the ΔE enlarged, showing a statistical difference between the weeks. In the first three weeks evaluated, all the encapsulating systems were statistically different from each other, with P+IN23 showing the highest values of ΔE (p-value < 0.05). In the 4th week, P+IN23 (6.75) showed the highest ΔE , which can be identified by the human eye more easily. P (4.47), P+FOS (4.55), and P+IN10 (4.51) samples did not show any statistical difference between them, and their color variation may not have been noticed because their values were below 5 (p-value > 0.05). Although the values of color variation increased for all pectin-based structured systems over the four weeks of evaluation, only P+IN23 presented a noticeable difference to the human eye.

4. Conclusions

Combing of pectin and inulin with different DP proved to be a promising encapsulating system of jabuticaba peel polyphenols and a functional structuring food ingredient. Inulin DP greatly influenced the rheological characteristics and morphology of the pectin-based structured systems. The samples with the highest inulin DP can be used as an encapsulating system of jabuticaba peel polyphenols, protecting their chemical characteristics and bioactivity during storage and exhibiting synergy between pectin and inulin. This synergic effect was weakened when FOS was added to the formulation, demonstrating that polyphenols protection and the system's structuring properties were enhanced with the increase of inulin DP.

CRediT authorship contribution statement

Adriana Gadioli Tarone: Writing - original draft, Conceptualization, Investigation. Eric Keven Silva: Writing - original draft, Conceptualization, Methodology, Validation. Cinthia Baú Betim Cazarin: Writing - review & editing, Validation, Project administration. Mario Roberto Marostica Junior: Writing - review & editing, Resources, Project administration, Supervision.

Declaration of competing interest

The authors confirm that there are no known conflicts of interest associated with this publication.

Acknowledgements

Mario R. Marostica Junior is grateful to the National Council for Scientific and Technological Development - CNPQ for his productivity grant (301496/2019-6). Adriana G. Tarone thanks CNPQ (140942/2016-5) for the Ph.D. scholarship. Eric Keven Silva thanks CAPES (88887.473261/2020-00) for his postdoctoral assistantship at the University of Campinas. This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FAPESP (2015/50333-1; 2015/13320-9). MRMJ acknowledges Red Iberamericana de Alimentos Autoctonos Subutilizados (ALSUB-CYTED, 118RT0543).

References

- Albuquerque, B. R., Pereira, C., Calhelha, R. C., José Alves, M., Abreu, R. M. V., Barros, L., ... Ferreira, I. C. F. R. (2020). Jabuticaba residues (*Myrciaria jaboticaba* (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. *Food Chemistry*, 309, 125735. <https://doi.org/10.1016/j.foodchem.2019.125735>
- Araujo-Díaz, S. B., Leyva-Porras, C., Aguirre-Bañuelos, P., Álvarez-Salas, C., & Saavedra-Leos, Z. (2017). Evaluation of the physical properties and conservation of the antioxidants content, employing inulin and maltodextrin in the spray drying of blueberry juice. *Carbohydrate Polymers*, 167, 317–325. <https://doi.org/10.1016/j.carbpol.2017.03.065>
- Bakowska-Barczak, A. M., & Kolodziejczyk, P. P. (2011). Black currant polyphenols: Their storage stability and microencapsulation. *Industrial Crops and Products*, 34(2), 1301–1309. <https://doi.org/10.1016/J.INDCROP.2010.10.002>
- Batista, Â. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., Luiz-Ferreira, A., Bogusz, S., ... Maróstica Junior, M. R. (2014). Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods*, 6(1), 450–461. <https://doi.org/10.1016/j.jff.2013.11.011>
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Bernardes, A. L., Moreira, J. A., Tostes, M. das G. V., Costa, N. M. B., Silva, P. I., &

- Costa, A. G. V. (2019). In vitro bioaccessibility of microencapsulated phenolic compounds of jussara (*Euterpe edulis* Martius) fruit and application in gelatine model-system. *LWT - Food Science and Technology*, 102, 173–180. <https://doi.org/10.1016/j.lwt.2018.12.009>
- Bot, A., Erle, U., Vreeker, R., & Agterof, W. G. M. (2004). Influence of crystallisation conditions on the large deformation rheology of inulin gels. *Food Hydrocolloids*, 18(4), 547–556. <https://doi.org/10.1016/j.foodhyd.2003.09.003>
- Bouaziz, M. A., Rassaoui, R., & Besbes, S. (2014). Chemical composition, functional properties, and effect of inulin from tunisian *Agave americana* L. Leaves on textural qualities of pectin gel. *Journal of Chemistry*, 2014. <https://doi.org/10.1155/2014/758697>
- Buchweitz, M., Speth, M., Kammerer, D. R., & Carle, R. (2013). Impact of pectin type on the storage stability of black currant (*Ribes nigrum* L.) anthocyanins in pectic model solutions. *Food Chemistry*, 139(1–4), 1168–1178. <https://doi.org/10.1016/j.foodchem.2013.02.005>
- Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J. (2015). Enhanced stability of anthocyanin-based color in model beverage systems through whey protein isolate complexation. *Food Research International*, 76, 761–768. <https://doi.org/10.1016/j.foodres.2015.07.003>
- Corrêa, T. A. F., Rogero, M. M., Hassimotto, N. M. A., & Lajolo, F. M. (2019). The Two-Way Polyphenols-Microbiota Interactions and Their Effects on Obesity and Related Metabolic Diseases. *Frontiers in Nutrition*, 6(December), 1–15. <https://doi.org/10.3389/fnut.2019.00188>
- Coviello, T., Trotta, A. M., Marianelli, C., Carafa, M., Di Marzio, L., Rinaldi, F., ... Matricardi, P. (2015). Gel-embedded niosomes: Preparation, characterization and release studies of a new system for topical drug delivery. *Colloids and Surfaces B: Biointerfaces*, 125, 291–299. <https://doi.org/10.1016/j.colsurfb.2014.10.060>
- da Silva-Maia, J. K., Batista, Â. G., Cazarin, C. B. B., Soares, E. S., Junior, S. B., Leal, R. F., ... Maróstica Junior, M. R. (2019). Aqueous extract of Brazilian berry (*Myrciaria jaboticaba*) peel improves inflammatory parameters and modulates *Lactobacillus* and *Bifidobacterium* in rats with induced-colitis. *Nutrients*, 11(2776), 1–13. <https://doi.org/10.3390/nu11112776>
- da Silva, J. K., Batista, Â. G., Cazarin, C. B. B., Dionísio, A. P., de Brito, E. S., Marques, A. T. B., & Maróstica Junior, M. R. (2017). Functional tea from a Brazilian berry:

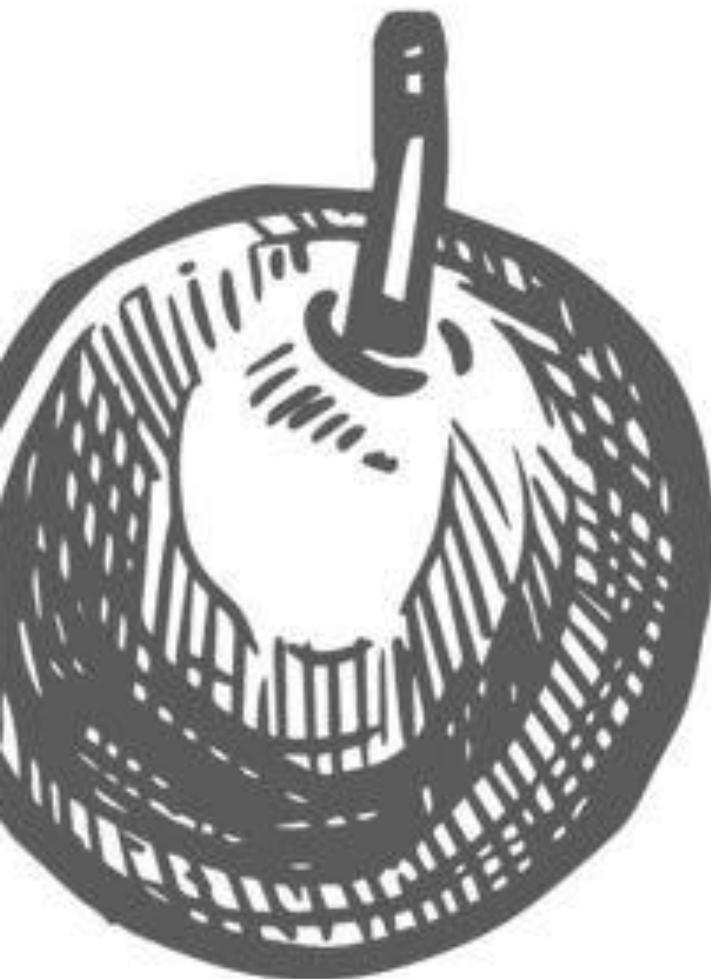
- Overview of the bioactives compounds. *LWT - Food Science and Technology*, 76, 292–298. <https://doi.org/10.1016/j.lwt.2016.06.016>
- Dafe, A., Etemadi, H., Dilmaghani, A., & Mahdavinia, G. R. (2017). Investigation of pectin/starch hydrogel as a carrier for oral delivery of probiotic bacteria. *International Journal of Biological Macromolecules*, 97, 536–543. <https://doi.org/10.1016/j.ijbiomac.2017.01.060>
- De Cindio, B., Gabriele, D., & Lupi, F. R. (2015). Pectin: Properties Determination and Uses. In *Encyclopedia of Food and Health* (pp. 294–300). Elsevier. <https://doi.org/10.1016/B978-0-12-384947-2.00531-6>
- de Sá, L. Z. C. M., Castro, P. F. S., Lino, F. M. A., Bernardes, M. J. C., Viegas, J. C. J., Dinis, T. C. P., ... Gil, E. S. (2014). Antioxidant potential and vasodilatory activity of fermented beverages of jabuticaba berry (*Myrciaria jahoticaba*). *Journal of Functional Foods*, 8, 169–179. <https://doi.org/10.1016/j.jff.2014.03.009>
- Fernandes, A., Brás, N. F., Mateus, N., & De Freitas, V. (2014). Understanding the Molecular Mechanism of Anthocyanin Binding to Pectin. *Langmuir*, 30(28), 8516–8527. <https://doi.org/10.1021/la501879w>
- Franck, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition*, 87(S2), S287–S291. <https://doi.org/10.1079/bjn/2002550>
- Fredes, C., Osorio, M. J., Parada, J., & Robert, P. (2018). Stability and bioaccessibility of anthocyanins from maqui (*Aristotelia chilensis* [Mol.] Stuntz) juice microparticles. *LWT - Food Science and Technology*, 91(June 2017), 549–556. <https://doi.org/10.1016/j.lwt.2018.01.090>
- Guimarães, J. T., Silva, E. K., Arruda, H. S., Freitas, M. Q., Pastore, G. M., Meireles, M. A. A., & Cruz, A. G. (2020). How does the degree of inulin polymerization affect the bioaccessibility of bioactive compounds from soursop whey beverage during in vitro gastrointestinal digestion? *Food Hydrocolloids*, 101, 105511. <https://doi.org/10.1016/j.foodhyd.2019.105511>
- Guo, J., Giusti, M. M., & Kaletunç, G. (2018). Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. *Food Research International*, 107, 414–422. <https://doi.org/10.1016/j.foodres.2018.02.035>
- He, J., & Giusti, M. M. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annual Review of Food Science and Technology*, 1(1), 163–187. <https://doi.org/10.1146/annurev.food.080708.100754>

- Holzwarth, M., Korhummel, S., Siekmann, T., Carle, R., & Kammerer, D. R. (2013). Influence of different pectins, process and storage conditions on anthocyanin and colour retention in strawberry jams and spreads. *LWT - Food Science and Technology*, 52(2), 131–138. <https://doi.org/10.1016/J.LWT.2012.05.020>
- Huang, T., Tu, Z. cai, Wang, H., Shangguan, X., Zhang, L., Zhang, N. hai, & Bansal, N. (2017). Pectin and enzyme complex modified fish scales gelatin: Rheological behavior, gel properties and nanostructure. *Carbohydrate Polymers*, 156, 294–302. <https://doi.org/10.1016/j.carbpol.2016.09.040>
- Jakobek, L., & Matić, P. (2019). Non-covalent dietary fiber - Polyphenol interactions and their influence on polyphenol bioaccessibility. *Trends in Food Science and Technology*, 83, 235–247. <https://doi.org/10.1016/j.tifs.2018.11.024>
- Kawabata, K., Yoshioka, Y., & Terao, J. (2019). Role of intestinal microbiota in the bioavailability and physiological functions of dietary polyphenols. *Molecules*, 24(2). <https://doi.org/10.3390/molecules24020370>
- Kosaraju, S. L. (2005). Colon targeted delivery systems: Review of polysaccharides for encapsulation and delivery. *Critical Reviews in Food Science and Nutrition*, 45(4), 251–258. <https://doi.org/10.1080/10408690490478091>
- Lacerda, E. C. Q., Calado, V. M. de A., Monteiro, M., Finotelli, P. V., Torres, A. G., & Perrone, D. (2016). Starch, inulin and maltodextrin as encapsulating agents affect the quality and stability of jussara pulp microparticles. *Carbohydrate Polymers*, 151, 500–510. <https://doi.org/10.1016/j.carbpol.2016.05.093>
- Lamas, C. A., Kido, L. A., Montico, F., Collares-Buzato, C. B., Maróstica, M. R., & Cagnon, V. H. A. (2020). A jaboticaba extract prevents prostatic damage associated with aging and high-fat diet intake. *Food and Function*, 11(2), 1547–1559. <https://doi.org/10.1039/c9fo02621e>
- Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, 88(5), 1269–1278. <https://doi.org/10.5555/jaoi.2005.88.5.1269>
- Leite-Legatti, A. V., Batista, A. G., Dragano, N. R. V., Marques, A. C., Malta, L. G., Riccio, M. F., ... Maróstica, M. R. (2012). Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. *Food Research International*, 49(1), 596–603. <https://doi.org/10.1016/j.foodres.2012.07.044>
- Lenquiste, S. A., Marineli, R. da S., Moraes, É. A., Dionísio, A. P., Brito, E. S. de, &

- Maróstica Junior, M. R. (2015). Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. *Food Research International*, 77, 162–170. <https://doi.org/10.1016/j.foodres.2015.07.023>
- Li, Y., Ma, X., & Liu, X. (2019). Physicochemical and rheological properties of cross-linked inulin with different degree of polymerization. *Food Hydrocolloids*, 95, 318–325. <https://doi.org/10.1016/j.foodhyd.2018.11.026>
- Li, Y., Shabani, K. I., Qin, X., Yang, R., Jin, X., Ma, X., & Liu, X. (2019). Effects of cross-linked inulin with different polymerisation degrees on physicochemical and sensory properties of set-style yoghurt. *International Dairy Journal*, 94, 46–52. <https://doi.org/10.1016/j.idairyj.2019.02.009>
- Lin, Z., Fischer, J., & Wicker, L. (2016). Intermolecular binding of blueberry pectin-rich fractions and anthocyanin. *Food Chemistry*, 194, 986–993. <https://doi.org/10.1016/j.foodchem.2015.08.113>
- Maier, T., Fromm, M., Schieber, A., Kammerer, D. R., & Carle, R. (2009). Process and storage stability of anthocyanins and non-anthocyanin phenolics in pectin and gelatin gels enriched with grape pomace extracts. *European Food Research and Technology*, 229(6), 949–960. <https://doi.org/10.1007/s00217-009-1134-9>
- Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics3040793>
- Nowicka, P., & Wojdyło, A. (2016). Stability of phenolic compounds, antioxidant activity and colour through natural sweeteners addition during storage of sour cherry puree. *Food Chemistry*, 196, 925–934. <https://doi.org/10.1016/j.foodchem.2015.10.019>
- Ou, B., Chang, T., Huang, D., & Prior, R. L. (2013). Determination of total antioxidant capacity by oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescence probe: First action 2012.23. *Journal of AOAC International*, 96(6), 1372–1376. <https://doi.org/10.5740/jaoacint.13-175>
- Ozturkoglu-Budak, S., Akal, H. C., Buran, İ., & Yetişemiyen, A. (2019). Effect of inulin polymerization degree on various properties of symbiotic fermented milk including Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12. *Journal of Dairy Science*, 102(8), 6901–6913. <https://doi.org/10.3168/jds.2019-16479>
- Pathare, P. B., Opara, U. L., & Al-said, F. A. (2013). Colour Measurement and Analysis

- in Fresh and Processed Foods : A Review. *Food and Bioprocess Technology*, 6, 36–60. <https://doi.org/10.1007/s11947-012-0867-9>
- Pérez-Magariño, S., & González-Sanjosé, M. L. (2003). Application of absorbance values used in wineries for estimating CIELAB parameters in red wines. *Food Chemistry*, 81(2), 301–306. [https://doi.org/10.1016/S0308-8146\(02\)00509-5](https://doi.org/10.1016/S0308-8146(02)00509-5)
- Plaza, M., Batista, Â. G., Cazarin, C. B. B., Sandahl, M., Turner, C., Ostman, E., & Maróstica Junior, M. R. (2016). Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. *Food Chemistry*, 211, 185–197. <https://doi.org/10.1016/j.foodchem.2016.04.142>
- Prior, R. L., & Wu, X. (2006). Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research*, 40(10), 1014–1028. <https://doi.org/10.1080/10715760600758522>
- Reynertson, K. A., Wallace, A. M., Adachi, S., Gil, R. R., Yang, H., Basile, M. J., ... Kennelly, E. J. (2006). Bioactive depsides and anthocyanins from jaboticaba (*Myrciaria cauliflora*). *Journal of Natural Products*, 69(8), 1228–1230. <https://doi.org/10.1021/np0600999>
- Roberfroid, M. B. (2005). Introducing inulin-type fructans. *British Journal of Nutrition*, 93(S1), S13–S25. <https://doi.org/10.1079/bjn20041350>
- Robert, P., Gorena, T., Romero, N., Sepulveda, E., Chavez, J., & Saenz, C. (2010). Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. *International Journal of Food Science and Technology*, 45(7), 1386–1394. <https://doi.org/10.1111/j.1365-2621.2010.02270.x>
- Rolin, C., & De Vries, J. (1990). Pectin. In P. Harris (Ed.), *Food gels* (pp. 401–434). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-009-0755-3_10
- Santos-Buelga, C., & González-Paramás, A. M. (2018). Anthocyanins. *Reference Module in Food Science*, 1–12. <https://doi.org/10.1016/B978-0-08-100596-5.21609-0>
- Silva, E. K., & Meireles, M. A. A. (2015). Influence of the degree of inulin polymerization on the ultrasound-assisted encapsulation of annatto seed oil. *Carbohydrate Polymers*, 133, 578–586. <https://doi.org/10.1016/j.carbpol.2015.07.025>
- Smith, A. M., Moxon, S., & Morris, G. A. (2016). Biopolymers as wound healing materials. In M. S. Ågren (Ed.), *Wound Healing Biomaterials* (Vol. 2, pp. 261–287). Elsevier Inc. <https://doi.org/10.1016/B978-1-78242-456-7.00013-1>

- Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), 63–68. <https://doi.org/10.1002/jsfa.2740100110>
- Tabernero, A., Baldino, L., Misol, A., Cardea, S., & del Valle, E. M. M. (2020). Role of rheological properties on physical chitosan aerogels obtained by supercritical drying. *Carbohydrate Polymers*, 233, 115850. <https://doi.org/10.1016/j.carbpol.2020.115850>
- Tárrega, A., Rocafull, A., & Costell, E. (2010). Effect of blends of short and long-chain inulin on the rheological and sensory properties of prebiotic low-fat custards. *LWT - Food Science and Technology*, 43(3), 556–562. <https://doi.org/10.1016/j.lwt.2009.10.002>
- Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and Uses of Pectin - A Review. *Critical Reviews in Food Science and Nutrition*, 37(1), 47–73. <https://doi.org/10.1080/10408399709527767>
- Wang, R., Wan, J., Liu, C., Xia, X., & Ding, Y. (2019). Pasting, thermal, and rheological properties of rice starch partially replaced by inulin with different degrees of polymerization. *Food Hydrocolloids*, 92, 228–232. <https://doi.org/10.1016/j.foodhyd.2019.02.008>
- Wicker, L., & Kim, Y. (2015). Pectin and Health. In *Encyclopedia of Food and Health* (pp. 289–293). Elsevier. <https://doi.org/10.1016/B978-0-12-384947-2.00532-8>
- Witczak, T., Witczak, M., & Ziobro, R. (2014). Effect of inulin and pectin on rheological and thermal properties of potato starch paste and gel. *Journal of Food Engineering*, 124, 72–79. <https://doi.org/10.1016/j.jfoodeng.2013.10.005>
- Xu, W., Xiong, Y., Li, Z., Luo, D., Wang, Z., Sun, Y., & Shah, B. R. (2020). Stability, microstructural and rheological properties of complex prebiotic emulsion stabilized by sodium caseinate with inulin and konjac glucomannan. *Food Hydrocolloids*, 105, 105772. <https://doi.org/10.1016/j.foodhyd.2020.105772>
- Ye, J., Yang, R., Liu, C., Luo, S., Chen, J., Hu, X., & Wu, J. (2018). Improvement in freeze-thaw stability of rice starch gel by inulin and its mechanism. *Food Chemistry*, 268, 324–333. <https://doi.org/10.1016/j.foodchem.2018.06.086>
- Zimeri, J. E., & Kokini, J. L. (2003). Rheological properties of inulin-waxy maize starch systems. *Carbohydrate Polymers*, 52(1), 67–85. [https://doi.org/10.1016/S0144-8617\(02\)00268-0](https://doi.org/10.1016/S0144-8617(02)00268-0)



CAPÍTULO VI - Discussão Geral

Uma revisão da literatura sobre antocianinas (principal classe de compostos fenólicos encontrada na casca da jabuticaba), seu método de absorção pelo organismo humano e quais as tecnologias utilizadas e desafios enfrentados para sua encapsulação foram apresentados no Capítulo II da tese, evidenciando a importância de se trabalhar com tecnologias ou metodologias que proporcionem maior proteção às antocianinas, assim como a outros compostos bioativos. A partir disso, o trabalho foi estruturado para se obter um extrato feito com a farinha da casca de jabuticabas rico em antocianinas e outros compostos fenólicos e para a encapsulação desse extrato da forma mais eficiente possível. A **Figura 2** representa o fluxograma de todo o trabalho realizado.

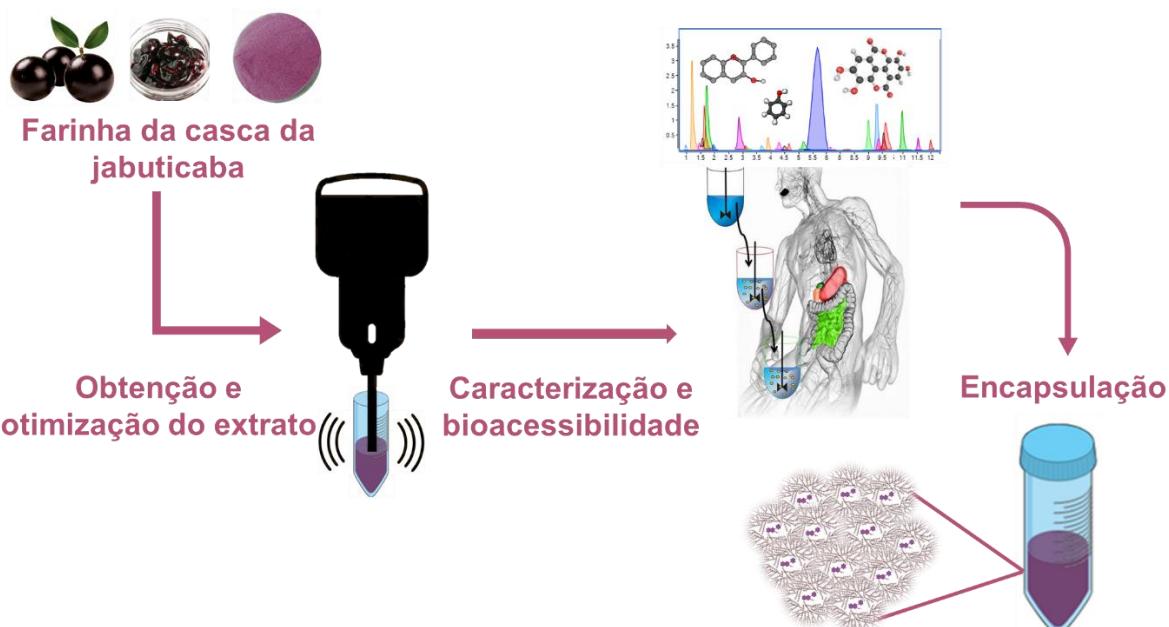


Figura 2. Fluxograma do trabalho realizado para compor a tese.

A tecnologia de extração assistida por ultrassom de alta intensidade foi escolhida para a produção do extrato da farinha da casca da jabuticaba rico em antocianinas e outros compostos fenólicos. Para se obter a melhor condição de extração, foi realizado um delineamento experimental onde foram examinados os efeitos da intensidade de ultrassom ($1,1$, $3,7$, $7,3$, e $13,0\text{ W/cm}^2$) e da composição do solvente em relação à proporção de água/etanol (0 , 25 , 50 , 75 e 100 g de água/ 100 g de solvente) na extração (resultados no Capítulo III).

Os extremos de concentração de água (0 e 100 g de água/ 100 g) apresentaram intensidade de cor desbotada e os menores valores para as respostas

estudadas, mostrando uma baixa eficiência de extração quando utilizado um sistema monosolvente. A mistura dos solventes etanol e água abrange a polaridade de diversas classes de compostos fenólicos (LAO; GIUSTI, 2018) presentes na casca da jabuticaba, explicando a interação positiva observada entre os dois solventes através da alta eficiência de extração na concentração de água de 50 g/100 g, onde foram encontrados os melhores resultados para os parâmetros estudados e a cor vermelha foi estabelecida, que é normalmente encontrada em extratos ricos em antocianinas (SIGURDSON et al., 2018). Foi observado no presente estudo um aumento de temperatura decorrente do aumento da intensidade de ultrassom, seguido por uma diminuição de 4 de 6 respostas estudadas (antocianinas monoméricas, flavonoides totais, taninos condensados e FRAP). Antocianinas monoméricas (HE; GIUSTI, 2010), flavonoides totais e taninos condensados são sensíveis a altas temperaturas (MUNIN; EDWARDS-LÉVY, 2011) seguidos por sua capacidade antioxidante anexada (FRAP). Já algumas classes de polifenóis são muito resistentes (AL-DHABI; PONMURUGAN; MARAN JEGANATHAN, 2017), sendo observadas nos valores mais altos de polifenóis para as intensidades de ultrassom mais altas seguidas por sua capacidade antioxidante anexada (ORAC). Os efeitos da intensidade de ultrassom alteram várias propriedades do material através da cavitação acústica produzida (RASTOGI, 2011), que melhora significativamente a extração de compostos orgânicos do material vegetal e tem o seu efeito destacado com o aumento da intensidade do ultrassom (MASON; PANIWNYK; LORIMER, 1996). Por outro lado, a intensificação desse fenômeno resulta na geração de pressão, cisalhamento e gradiente de temperatura, que podem promover a degradação dos compostos extraídos pelos mecanismos de oxidação (WANG et al., 2020). Consequentemente, a taxa de extração de alguns compostos de interesse pode ser menor para intensidades de ultrassom mais altas devido à degradação destes. O aumento da intensidade de ultrassom também causou a diminuição de todos os parâmetros de cor analisados, resultando na perda da cor vermelha e no aparecimento da cor acastanhada, indicando a presença de compostos provenientes da quebra/degradação das antocianinas (SIGURDSON et al., 2018). Essas conclusões nos levam a estabelecer a intensidade de ultrassom de 3,7 W/cm² como a melhor a ser utilizada.

A fim de verificar a eficiência do método de extração, análises microscópicas confocais foram realizadas na farinha da casca de jabuticaba antes e após as extrações e foram baseadas nas propriedades naturais de autofluorescência

dos compostos fenólicos (TALAMOND; VERDEIL; CONÉJÉRO, 2015). Duas regiões distintas de emissão foram encontradas nas amostras deste estudo: região do espectro da cor verde com emissão de fluorescência em 524 nm indicando a presença de polifenóis e/ou taninos e/ou flavonóides (BUSCHMANN; LANGSDORF; LICHTENTHALER, 2000) e região do espectro da cor vermelha com emissão em 690 nm indicando a presença de antocianinas e/ou antocianidinas (AGATI; TRAVERSI; CEROVIC, 2008). A maioria dos experimentos mostrou uma emissão mais proeminente que o padrão nas duas regiões, indicando que um grande número de compostos foi eficientemente exposto pelo método de extração utilizado. A região do espectro da cor verde apresentou menor fluorescência na condição de 3,7 W/cm² e a concentração de água de 50 g/100 g indicando uma retirada mais eficiente dos compostos expostos e, consequentemente, melhor extração nessa condição. Na região do espectro da cor vermelha, as concentrações de água de 0 e de 100 g de água/100 g de solvente apresentaram menor fluorescência apesar dos menores resultados encontrados para o conteúdo de antocianinas monoméricas, indicando que a alteração sofrida no seu estado conformacional pela exposição aos solventes (LAO; GIUSTI, 2018) causou a diminuição em sua emissão de fluorescência (GARCÍA-PLAZAOLA et al., 2015). A mudança de polaridade causada pela mistura de solventes contribuiu para maior emissão na concentração de água de 50 g/100 g, o que indica melhor exposição (e extração) de antocianinas. Nesta concentração de água temos uma fluorescência menor a 3,7 W/cm², significando uma retirada mais eficiente das antocianinas exposta e, consequentemente, uma melhor extração. Esses resultados corroboram os encontrados anteriormente, mostrando que este é um método eficiente para se avaliar a eficiência do método de extração.

A extração assistida por ultrassom de alta intensidade realizada neste estudo se mostrou mais eficiente que outros métodos de extração de compostos fenólicos da casca da jabuticaba encontrados na literatura (ALBUQUERQUE et al., 2020; BATISTA et al., 2014; DA SILVA et al., 2017; SANTOS; VEGGI; MEIRELES, 2010, 2012), de acordo com os parâmetros estudados. A fim de melhor verificar essa eficiência, foi feita a identificação e quantificação dos compostos fenólicos presentes no extrato otimizado no Capítulo III e em um extrato obtido através de tecnologia convencional por análise de UPLC/DAD/ESI-MSⁿ para comparar a eficiência de extração de compostos fenólicos dos dois métodos de extração (resultados no Capítulo IV). Na comparação dos perfis fenólicos dos dois extratos, os mesmos 58

compostos fenólicos foram identificados em ambos, dos quais 11 foram relatados pela primeira vez na espécie de jabuticaba. Ambos os métodos de extração exibiram a mesma eficiência para extrair o ácido elágico. A extração assistida por ultrassom de alta intensidade apresentou maior eficiência para extrair derivados de ácido gálico, enquanto a extração convencional apresentou maior eficiência para extrair derivados de antocianinas e de flavonóis. Apesar de haver diferenças significativas entre as classes de compostos, a soma dos compostos fenólicos não apresentou diferenças estatísticas entre os métodos de extração utilizados. Apesar de apresentarem a mesma eficiência na extração de compostos fenólicos, cada método de extração se mostrou mais eficiente na extração de determinada(s) classe(s) de compostos, mostrando que a escolha do método de extração também deve levar em conta a classe de compostos que se deseja extrair.

Uma hidrólise ácida do extrato otimizado no Capítulo III liofilizado e da farinha da casca de jabuticaba seguida de identificação e quantificação dos compostos fenólicos por análise de UPLC/DAD/ESI-MSⁿ foi conduzida para revelar as estruturas dos ácidos fenólicos presentes covalentemente ligados às hexoses (resultados no Capítulo IV). Como esses compostos fenólicos podem não ser solúveis em meio aquoso, foi adicionada uma etapa adicional de lavagem do pellet com uma mistura de MeOH/DMSO conforme proposto por (GARCÍA-VILLALBA et al., 2015). O ácido gálico e o ácido elágico (e seus derivados) foram os principais produtos liberados após a hidrólise. Os maiores teores de ácido gálico foram liberados na parte solúvel do extrato, enquanto o ácido elágico foi liberado na parte insolúvel, após extração com MeOH/DMSO. Vale ressaltar que o ácido gálico não estava presente na casca da jabuticaba e apareceu somente após a hidrólise ácida. No geral, os produtos hidrolíticos de elagitaninos totalizaram 70,3 mg/g de farinha da casca de jabuticaba antes da extração e 42,4 mg/g de farinha da casca de jabuticaba após a extração. Os resultados da hidrólise sugerem que a tecnologia de extração assistida por ultrassom de alta intensidade não foi capaz de extrair todos os compostos presentes na farinha da casca de jabuticaba. As quantidades de compostos encontradas são marcadamente superiores às obtidas pela análise UPLC de compostos individuais para o extrato, que foi de 10,3 mg/g de farinha da casca de jabuticaba. A elevada quantidade de produtos hidrolíticos de elagitaninos em comparação com a quantidade total de compostos fenólicos encontrados antes da hidrólise sugere que a hidrólise ácida, que se baseia principalmente na titulação de dois padrões (ácido elágico e ácido

gálico), é uma técnica mais precisa para quantificar os compostos fenólicos da casca de jabuticaba do que a técnica de UPLC na ausência de padrões individuais. A lavagem subsequente do pellet com MeOH/DMSO forneceu mais de um terço dos produtos de hidrólise. É importante notar que as moléculas hidrofóbicas, como ácido elágico e dilactona de ácido valoneico, foram recuperadas principalmente com esta etapa adicional, enquanto a dilactona de ácido sanguisórbico se mostrou solúvel apenas neste sistema de solventes.

A fim de ampliar a sua caracterização, também foi avaliada a capacidade antioxidante do extrato otimizado no Capítulo III na oxidação lipídica e a bioacessibilidade de suas antocianinas sob digestão gastrointestinal *in vitro* em modelo com emulsão óleo-em-água simulando dieta tipo *Western* (resultados no Capítulo IV). Segundo pesquisa feita na literatura, este é o primeiro estudo de capacidade antioxidante *in vitro* feito com extrato de casca de jabuticaba no modelo proposto (considerando as condições de digestão alimentar e gástrica) realizado até agora.

O extrato foi capaz de inibir quase que totalmente a oxidação lipídica nas duas concentrações utilizadas ($E1 = 1\text{ mg de extrato liofilizado/mL de fluido gástrico}$ e $E5 = 5\text{ mg de extrato liofilizado/mL de fluido gástrico}$) durante toda a fase gástrica, apresentando baixos níveis dos marcadores de oxidação lipídica analisados, dienos conjugados (CD) e 4-hydroxy-2-nonenal (4-HNE). Uma inibição estatisticamente significativa na formação dos dois marcadores foi observada para ambos os extratos quando comparados ao ensaio controle, suportando uma proteção total dos lipídeos poli-insaturados por E1 e E5. O acúmulo relativamente linear de CDs, que são constituídos principalmente por hidroperóxidos derivados de lipídios provenientes da cadeia de ácido graxo do ácido linoleico, está correlacionado com um aumento de 4-HNE, um marcador terminal específico da oxidação do ácido linoleico. A baixa proporção de 4-HNE/CDs livres observada no final desta fase pode ser explicada pela formação de vários outros produtos de oxidação secundária, como aldeídos de cadeia curta, epóxidos e álcoois, bem como pela alta reatividade do 4-HNE. O 4-HNE eletrofílico reage rapidamente com os aminoácidos nucleofílicos, embora essa reação possa ser favorecida em pH mais alto, como encontrado na fase intestinal (GASC et al., 2007). Um nível mais alto de polifenóis, como observado em E5 em comparação com E1, parece estar correlacionado a uma maior proteção da oxidação de lipídios, embora não haja diferença significativa entre as concentrações de extrato para os

acúmulos de CDs e 4-HNE. A queda do nível de CDs entre a amostra final na fase gástrica e a primeira amostra na fase intestinal pode ser potencialmente atribuída a uma rápida degradação dos CDs em condições intestinais ou, mais provavelmente, a uma extração incompleta de triglicerídeos por hexano em micelas formadas após a adição de sais biliares. Na presença nas concentrações de extrato E1 e E5, a oxidação lipídica em condições intestinais resulta em níveis mais elevados de CDs em comparação com o controle. Esse aumento de CDs, que não é suportado pela formação de 4-HNE, poderia ser atribuído à extração de substâncias que absorvem a 234 nm e são liberadas dos extratos durante a fase intestinal. A concentração de 4-HNE diminuiu drasticamente com a mudança nas condições de digestão tanto para o controle como para as concentrações de extrato E1 e E5. Um pH mais alto e a presença adicional de proteínas na adição de pancreatina podem favorecer a ligação de 4-HNE a peptídeos e proteínas (BOLÉA et al., 2019), explicando essa diminuição. Novamente não foram encontradas diferenças estatísticas entre E1 e E5 para ambos os marcadores na fase intestinal, embora tenha havido maior proteção da oxidação lipídica com o uso de E5.

Ao contrário das análises de oxidação lipídica, a concentração do extrato utilizada teve grande influência nos resultados de bioacessibilidade das antocianinas. A cianidina-3-O-glicosídeo (COG) provou ser amplamente estável quando adicionada na concentração de extrato E5, mostrando uma recuperação de 73% no final da fase gástrica. Com a COG presente em um nível 5 vezes menor, como apresentado na concentração de extrato E1, ela diminui exponencialmente com apenas 21% de recuperação no final da fase gástrica. Na maior concentração do extrato (E5), também foi recuperada a delphinidina-3-O-glicosídeo (DOG), embora em uma taxa inferior (22%) do que a COG. Essa baixa recuperação se deve principalmente à maior oxidabilidade da fração 1,2,3-trihidroxifenila da delphinidina, quando comparada à do núcleo 1,2-dihidroxifenila da cianidina (JANEIRO; BRETT, 2007). As antocianinas presentes no final da fase gástrica mostraram-se completamente degradadas poucos minutos após a mudança nas condições de digestão, independentemente da concentração de extrato empregada. Esta degradação que ocorre na fase intestinal surge da hidratação do cátion flavylium em pH mais alto levando à formação de hemicetal, uma reação que é seguida por rápida clivagem da estrutura principal das antocianinas. Além disso, a desprotonação leva a formação de pseudobases de carbinol instáveis do cátion flavylium (SANTOS-BUELGA; GONZÁLEZ-PARAMÁS, 2018).

Após a sua caracterização, uma forma de encapsulação foi estudada a fim de proteger e dar uma aplicação ao extrato da farinha da casca de jabuticaba. Então, no Capítulo V foram investigados os efeitos de frutooligossacarídeos (FOS) e inulinas com diferentes graus de polimerização (GP) nas propriedades reológicas, físicas e microestruturais de sistemas estruturados à base de pectina e sua atuação como matrizes encapsulantes do extrato da farinha da casca de jabuticaba otimizado no Capítulo III e analisado no Capítulo IV.

As quatro formulações estudadas exibiram um comportamento não newtoniano e pseudoplástico, típico de suspensões com mais de 1 g de pectina/100 g de suspensão (HUANG et al., 2017). A adição de FOS foi eficiente para diminuir a viscosidade aparente e a consistência do sistema à base de pectina, a adição de IN10 (inulina com GP ≥ 10) não alterou essas propriedades e a adição de IN23 (inulina com GP ≥ 23) aumentou-as. Essas alterações ocorreram devido à tendência da inulina em quebrar a microestrutura da pectina para promover uma nova ligação mais ordenada, reduzindo a liberdade das cadeias poliméricas de pectina (BOUAZIZ; RASSAOUI; BESBES, 2014). A microestrutura de sistemas estruturados à base de inulina é formada por uma rede de pequenas partículas cristalinas compostas por cadeias de alto peso molecular (levando em consideração a alta polidispersividade da inulina) (BOT et al., 2004). Com o aumento do GP da inulina adicionada ao sistema, mais partículas com cadeias de alto peso molecular ficam disponíveis no meio e mais partículas cristalinas são formadas, alterando e melhorando a estrutura da cadeia do sistema em que foi adicionada e suas propriedades reológicas (TÁRREGA; ROCAFULL; COSTELL, 2010), como observado neste estudo.

As quatro formulações estudadas também exibiram uma tendência semelhante nas propriedades viscoelásticas. Todos os sistemas de encapsulamento exibiram uma resposta predominantemente viscosa, onde G'' foi maior que G' . A formação de um gel forte a partir da pectina LM é dependente da adição de íons cálcio (Ca^{2+}) ao sistema (ROLIN; DE VRIES, 1990). A ausência de Ca^{2+} adicional e um pH relativamente baixo (4,5) permitiram a formação de sistemas estruturados à base de pectina por interações hidrofóbicas ou ligações de hidrogênio (DE CINDIO; GABRIELE; LUPI, 2015; ROLIN; DE VRIES, 1990). No entanto, esses sistemas não foram caracterizados como hidrogéis devido ao seu comportamento viscoso predominante ($G' < G''$). Os resultados indicaram que a adição de inulina ao sistema não afetou o comportamento viscoelástico das amostras, uma vez que a adição de

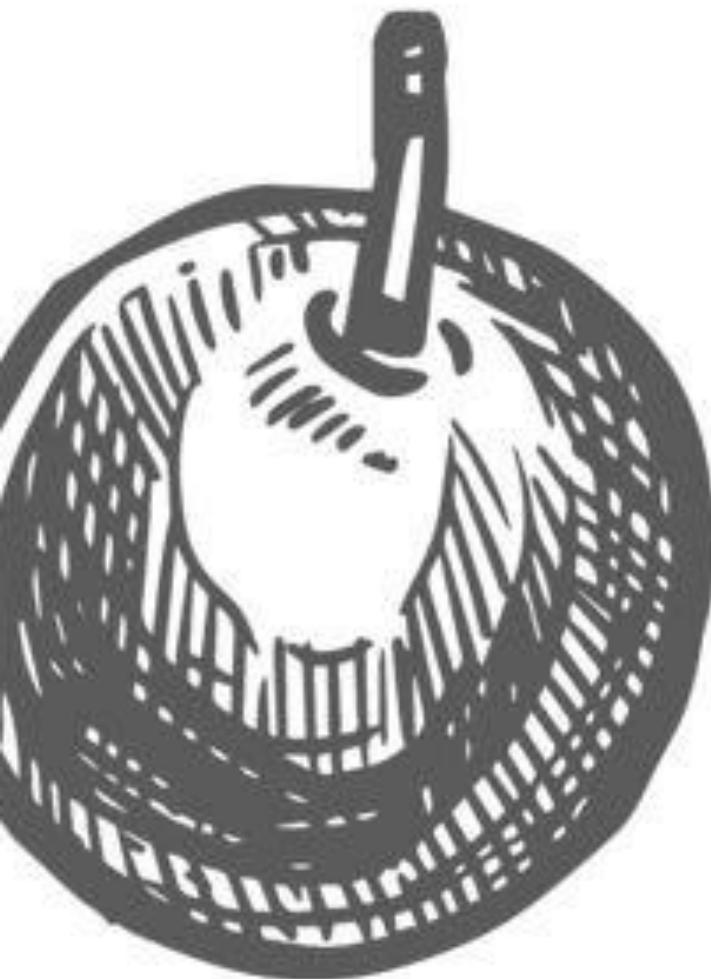
inulina não alterou o pH do sistema. No entanto, a redução do GP da inulina promoveu leve diminuição nos valores de G". Alguns estudos observaram que maiores concentrações de inulina foram capazes de aumentar a viscosidade de sistemas encapsulantes (XU et al., 2020; ZIMERI; KOKINI, 2003), e que G' e/ou G" podem aumentar com a adição de maiores concentrações de inulina de baixo GP e menores concentrações de inulina de alto GP (WITCZAK; WITCZAK; ZIOBRO, 2014; YE et al., 2018). Esse comportamento nos leva a acreditar que tanto as concentrações de inulina adicionadas como seu GP podem afetar diretamente as propriedades viscoelásticas dos sistemas.

A microscopia eletrônica de varredura foi utilizada para caracterizar a morfologia dos sistemas encapsulantes, mostrando microestruturas diferentes. As microestruturas do sistema à base de pectina (P) e do sistema P+IN23 foram semelhantes, mas a rede construída pelo sistema P+IN23 foi mais estruturada, provavelmente devido à sua maior viscosidade aparente e consistência. O sistema P+FOS e o sistema P+IN10 apresentaram microestruturas mais densas, sendo que P+FOS teve uma formação de rede quase imperceptível, corroborando as propriedades reológicas encontradas (menor viscosidade aparente e consistência para P+FOS, seguido de P+IN10). Foi verificado um efeito sinérgico entre a pectina e a inulina e um potencial efeito do GP da inulina na interação entre as partículas do sistema e a estrutura da rede formada. Essas características reológicas e microestrutura conferem aos sistemas características que permitem com que sejam utilizados como agentes estruturantes (substituindo a gordura em produtos lácteos ou melhorando a textura dos molhos, por exemplo), sendo P + IN23 o mais adequado para esses fins.

As amostras apresentaram uma tonalidade predominantemente vermelha, conferida pela presença da cianidina-3-O-glicosídeo, principal composto encontrado na casca da jabuticaba. A adição de inulina escureceu os sistemas. Os baixos valores de luminosidade obtidos com a adição da inulina sugerem que sua adição melhorou a retenção da cor e, consequentemente, do composto químico (cianidina) (LACERDA et al., 2016). Quanto maior o GP da inulina adicionada, mais escuros ficaram os sistemas, indicando melhor retenção de cor em P+IN23. A adição de inulina também promoveu um aumento na capacidade redutora total, na atividade antioxidante e na eficiência de encapsulação dos hidrogéis, e esse aumento foi gradual com o aumento do seu GP. As alterações na microestrutura dos sistemas encapsulantes causadas

pela adição de inulina podem ter promovido maior retenção de compostos, explicando os melhores resultados para o sistema P+IN23 em comparação com o sistema P. A associação de inulina e pectina cria uma estrutura altamente ramificada, resultando em maior disponibilidade de locais para ligações de hidrogênio (LACERDA et al., 2016), interações hidrofóbicas (BUCHWEITZ et al., 2013; CHUNG et al., 2015; HOLZWARTH et al., 2013) e interações eletrostáticas (FREDES et al., 2018), que são os principais mecanismos de interação entre os compostos fenólicos (presentes no extrato adicionado) e a microestrutura dos sistemas (JAKOBÉK; MATIĆ, 2019). Quanto maior o GP da inulina, maior a ramificação da estrutura formada e maior o número de sítios disponíveis (WANG et al., 2019). Esses resultados mostraram que a adição de inulina melhorou os parâmetros estudados e o GP da inulina teve influência sobre eles, sendo que quanto maior o tamanho da cadeia de inulina (GP), mais forte seu efeito.

Durante as 4 semanas de avaliação da estabilidade, o sistema P+IN23 apresentou a melhor retenção da capacidade antioxidante, indicando maior eficiência para proteger o extrato de farinha da casca de jabuticaba. Esse resultado mostra que a adição de inulina influenciou na retenção da capacidade antioxidante do sistema, e que o maior GP da inulina moveu o melhor resultado, corroborando o efeito previamente observado do tamanho da cadeia de inulina no reordenamento de ligação do sistema. Como já citado anteriormente, esse reordenamento promovido pela inulina com maior GP cria mais sítios de interação entre o sistema e os compostos fenólicos presentes no extrato e, consequentemente, confere maior estabilidade à sua capacidade antioxidante nesta rede de sistema encapsulante. A melhoria na estabilidade antioxidante torna esta amostra potencialmente útil como um agente estruturante funcional. Além de ser usado para melhorar a textura dos alimentos, também possui compostos estáveis com propriedades promotoras da saúde. Os sistemas não apresentaram boa estabilidade de cor, tendo os valores da variação de cores aumentado para todos eles durante as 4 semanas de avaliação.



CAPÍTULO VII - Conclusão Geral

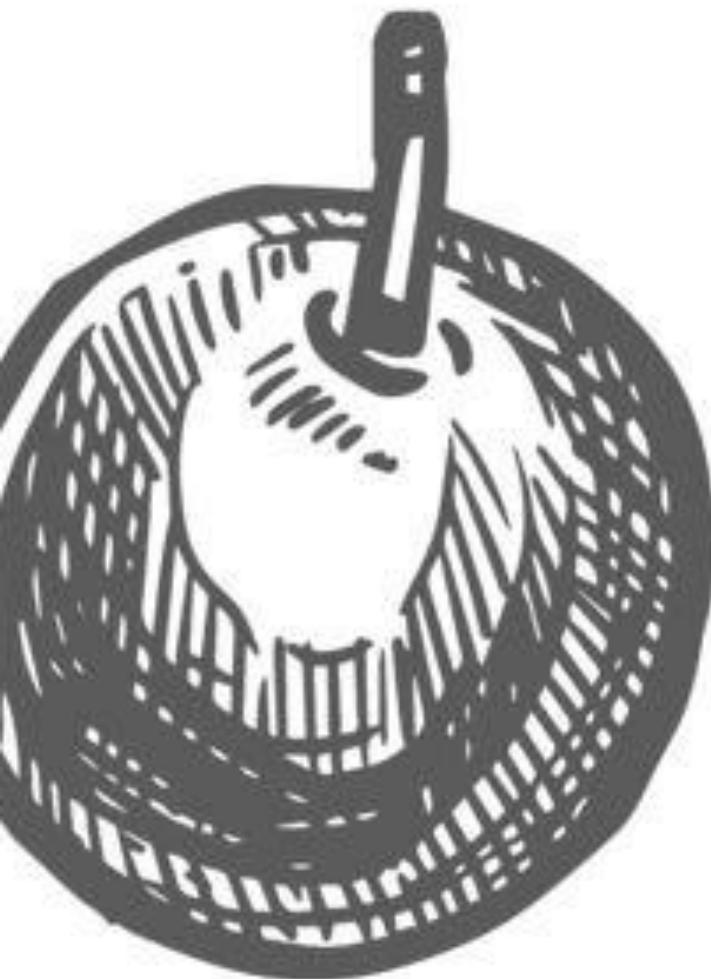
A tecnologia de ultrassom de alta intensidade se mostrou uma alternativa promissora para a obtenção de um extrato rico em antocianinas e outros compostos fenólicos proveniente do resíduo da cadeia de processamento da jabuticaba, uma vez que pode reduzir o consumo de energia, o tempo de extração e aumentar a taxa de recuperação desses compostos mantendo sua cor característica. As diferentes condições de intensidade de ultrassom e concentração de água exerceram forte influência sobre os compostos bioativos, capacidade antioxidant e cor dos extratos obtidos. A mistura de solventes (água e etanol) se mostrou mais interessante para extrair esses compostos, manter sua capacidade antioxidant e cor vermelha característica de extratos ricos em antocianinas, com os melhores resultados encontrados na concentração de água de 50 g/100 g de solvente. Quanto maior a intensidade de ultrassom, menores os valores para compostos bioativos e capacidade antioxidant, e mais acastanhada a cor dos extratos, indicando degradação dos compostos e perda de cor provavelmente devido ao aumento do efeito da cavitação e da temperatura, sendo encontrados os melhores resultados na intensidade de ultrassom de 3,7 W/cm². Nestas condições, a microscopia confocal de varredura a laser mostrou uma exaustão dos compostos avaliados na farinha da casca de jabuticaba após o processo de extração por ultrassom, comprovando sua melhor recuperação.

Foram identificados 58 compostos fenólicos em ambos os extratos analisados (extrato otimizado no Capítulo III obtido por tecnologia de ultrassom de alta intensidade e extrato obtido por tecnologia convencional) e, apesar de cada tipo de extração ser mais eficiente para certas classes de compostos, ambas extraíram a mesma quantidade de compostos fenólicos. A hidrólise ácida se mostrou uma alternativa eficaz para quantificar os compostos fenólicos da casca de jabuticaba, que são hidrolisados principalmente em ácido gálico e o ácido elágico, necessitando de um número menor de padrões analíticos. Ambas as concentrações de DJPE-HIUS utilizadas se mostraram eficazes para inibir a oxidação lipídica. Elas também apresentaram alta bioacessibilidade das antocianinas, sendo que a maior concentração apresentou valores mais altos e a recuperação de delphinidina-3-O-glicosídeo, não observada na menor concentração.

A combinação de pectina e inulina com diferentes graus de polimerização (GP) na forma de sistemas estruturados provou ser um promissor sistema de encapsulamento dos polifenóis extraídos da farinha da casca de jabuticaba, bem

como um ingrediente alimentar estruturador funcional. O GP da inulina apresentou uma grande influência em todos os parâmetros estudados. O sistema estruturado à base de pectina com a adição de inulina com maior GP apresentou as melhores características reológicas e morfológicas a serem utilizadas como ingrediente alimentar estruturador funcional e os melhores resultados para os parâmetros químicos estudados, eficiência de encapsulação, cor e estabilidade. Os resultados desse sistema foram melhores que os resultados do sistema estruturado à base de pectina, mostrando uma sinergia entre a pectina e a inulina. Este efeito sinérgico foi enfraquecido quando o FOS foi adicionado à formulação do sistema, demonstrando que a proteção dos polifenóis e as propriedades estruturantes do sistema foram aprimoradas com o aumento do GP da inulina.

Concluindo, otimizamos a melhor condição de extração de compostos fenólicos da farinha da casca de jabuticaba através da tecnologia de ultrassom de alta intensidade; identificamos 58 compostos no extrato otimizado e quantificamos 32 deles; identificamos alto poder antioxidante *in vitro* independente da concentração utilizada, demonstramos que as antocianinas presentes no extrato são bioacessíveis e encapsulamos o extrato com sucesso em uma matriz com potencial para ser utilizada como agente estruturante (espessante e substituto de gorduras) em alimentos como molhos, produtos lácteos, sorvetes e outros.



REFERÊNCIAS |

- AGATI, G.; TRAVERSI, M. L.; CEROVIC, Z. G. Chlorophyll Fluorescence Imaging for the Noninvasive Assessment of Anthocyanins in ... **Photochemistry and Photobiology**, v. 84, p. 1431–1434, 2008.
- AL-DHABI, N. A.; PONMURUGAN, K.; MARAN JEGANATHAN, P. Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds. **Ultrasonics Sonochemistry**, v. 34, p. 206–213, 2017.
- ALBUQUERQUE, B. R. et al. Jaboticaba residues (*Myrciaria jaboticaba* (Vell.) Berg) are rich sources of valuable compounds with bioactive properties. **Food Chemistry**, v. 309, p. 125735, out. 2020.
- ANAL, A. K.; SINGH, H. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. **Trends in Food Science and Technology**, v. 18, n. 5, p. 240–251, 2007.
- ARAUJO-DÍAZ, S. B. et al. Evaluation of the physical properties and conservation of the antioxidants content, employing inulin and maltodextrin in the spray drying of blueberry juice. **Carbohydrate Polymers**, v. 167, p. 317–325, 2017.
- BAGHDIKIAN, B. et al. Extraction by solvent using microwave and ultrasound-assisted techniques followed by HPLC analysis of Harpagoside from *Harpagophytum procumbens* and comparison with conventional solvent extraction methods. **Comptes Rendus Chimie**, v. 19, n. 6, p. 692–698, 2016.
- BATISTA, Â. G. et al. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. **Journal of Functional Foods**, v. 6, n. 1, p. 450–461, 2014.
- BERNARDES, A. L. et al. In vitro bioaccessibility of microencapsulated phenolic compounds of jussara (*Euterpe edulis* Martius) fruit and application in gelatine model-system. **LWT - Food Science and Technology**, v. 102, p. 173–180, 1 mar. 2019.
- BETZ, M.; KULOZIK, U. Microencapsulation of bioactive bilberry anthocyanins by means of whey protein gels. **Procedia Food Science**, v. 1, p. 2047–2056, 2011.
- BOLÉA, G. et al. Lipid protection by polyphenol-rich apple matrices is modulated by pH and pepsin in in vitro gastric digestion. **Food & Function**, v. 10, p. 3942–3954, 2019.
- BOT, A. et al. Influence of crystallisation conditions on the large deformation rheology of inulin gels. **Food Hydrocolloids**, v. 18, n. 4, p. 547–556, 1 jul. 2004.
- BOUAZIZ, M. A.; RASSAOUI, R.; BESBES, S. Chemical composition, functional properties, and effect of inulin from tunisian *Agave americana* L. Leaves on textural

- qualities of pectin gel. **Journal of Chemistry**, v. 2014, 2014.
- BUCHWEITZ, M. et al. Impact of pectin type on the storage stability of black currant (*Ribes nigrum L.*) anthocyanins in pectic model solutions. **Food Chemistry**, v. 139, n. 1–4, p. 1168–1178, ago. 2013.
- BUSCHMANN, C.; LANGSDORF, G.; LICHTENTHALER, H. K. Imaging of the Blue, Green, and Red Fluorescence Emission of Plants: An Overview. **Photosynthetica**, v. 38, n. 4, p. 483–491, 2000.
- CHEMAT, F. et al. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. **Ultrasonics Sonochemistry**, v. 34, p. 540–560, 2017.
- CHUNG, C. et al. Enhanced stability of anthocyanin-based color in model beverage systems through whey protein isolate complexation. **Food Research International**, v. 76, p. 761–768, 1 out. 2015.
- CROZIER, A. et al. Secondary metabolites as dietary components in plant-based foods and beverages. In: CROZIER A; CLIFFORD MN; ASHIHARA H. (Eds.). . **Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet**. Oxford: Blackwell Publishing, 2006. p. 208–302.
- DA SILVA, J. K. et al. Functional tea from a Brazilian berry: Overview of the bioactives compounds. **LWT - Food Science and Technology**, v. 76, p. 292–298, 1 mar. 2017.
- DE ALMEIDA, P. L. et al. Effect of jabuticaba peel extract on lipid oxidation, microbial stability and sensory properties of Bologna-type sausages during refrigerated storage. **Meat Science**, v. 110, p. 9–14, 2015.
- DE CINDIO, B.; GABRIELE, D.; LUPI, F. R. Pectin: Properties Determination and Uses. In: **Encyclopedia of Food and Health**. [s.l.] Elsevier, 2015. p. 294–300.
- DE MOURA, S. C. S. R. et al. Encapsulating anthocyanins from *Hibiscus sabdariffa L.* calyces by ionic gelation: Pigment stability during storage of microparticles. **Food Chemistry**, v. 241, n. August 2017, p. 317–327, 2018.
- DE SÁ, L. Z. C. M. et al. Antioxidant potential and vasodilatory activity of fermented beverages of jabuticaba berry (*Myrciaria jahoticaba*). **Journal of Functional Foods**, v. 8, p. 169–179, 2014.
- DEL RIO, D. et al. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. **Antioxidants & redox signaling**, v. 18, n. 14, p. 1818–92, 10 maio 2013.
- DONADIO, L. **Jabuticaba (Myrciaria jahoticaba (Vell.) Berg)**. Jaboticabal: FUNEP,

2000.

FREDES, C. et al. Stability and bioaccessibility of anthocyanins from maqui (*Aristotelia chilensis* [Mol.] Stuntz) juice microparticles. **LWT - Food Science and Technology**, v. 91, n. June 2017, p. 549–556, 2018.

GARCÍA-PLAZAOLA, J. I. et al. **Autofluorescence: Biological functions and technical applications**Plant ScienceElsevier Ireland Ltd, , 1 jul. 2015.

GARCÍA-VILLALBA, R. et al. Validated Method for the Characterization and Quantification of Extractable and Nonextractable Ellagitannins after Acid Hydrolysis in Pomegranate Fruits, Juices, and Extracts. **Journal of Agricultural and Food Chemistry**, v. 63, n. 29, p. 6555–6566, 2015.

GASC, N. et al. **4-Hydroxynonenal in foodstuffs: Heme concentration, fatty acid composition and freeze-drying are determining factors.** Redox Report. Anais...2007Disponível em: <<https://hal.inrae.fr/hal-02655296>>. Acesso em: 17 jul. 2020

GUO, J.; GIUSTI, M. M.; KALETUNÇ, G. Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. **Food Research International**, v. 107, p. 414–422, 1 maio 2018.

HE, J.; GIUSTI, M. M. Anthocyanins: Natural Colorants with Health-Promoting Properties. **Annual Review of Food Science and Technology**, v. 1, n. 1, p. 163–187, 2010.

HOLZWARTH, M. et al. Influence of different pectins, process and storage conditions on anthocyanin and colour retention in strawberry jams and spreads. **LWT - Food Science and Technology**, v. 52, n. 2, p. 131–138, 1 jul. 2013.

HUANG, T. et al. Pectin and enzyme complex modified fish scales gelatin: Rheological behavior, gel properties and nanostructure. **Carbohydrate Polymers**, v. 156, p. 294–302, 2017.

JAKOBEK, L.; MATIĆ, P. Non-covalent dietary fiber - Polyphenol interactions and their influence on polyphenol bioaccessibility. **Trends in Food Science and Technology**, v. 83, p. 235–247, 1 jan. 2019.

JANEIRO, P.; BRETT, A. M. O. Redox behavior of anthocyanins present in *Vitis vinifera* L. **Electroanalysis**, v. 19, n. 17, p. 1779–1786, 2007.

LACERDA, E. C. Q. et al. Starch, inulin and maltodextrin as encapsulating agents affect the quality and stability of jussara pulp microparticles. **Carbohydrate Polymers**,

v. 151, p. 500–510, 20 out. 2016.

LAO, F.; GIUSTI, M. M. Extraction of purple corn (*Zea mays L.*) cob pigments and phenolic compounds using food-friendly solvents. **Journal of Cereal Science**, v. 80, p. 87–93, 1 mar. 2018.

LEITE-LEGATTI, A. V. et al. Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. **Food Research International**, v. 49, n. 1, p. 596–603, 2012.

LENQUISTE, S. A. et al. Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. **Food Research International**, v. 77, p. 162–170, 2015.

MAIER, T. et al. Process and storage stability of anthocyanins and non-anthocyanin phenolics in pectin and gelatin gels enriched with grape pomace extracts. **European Food Research and Technology**, v. 229, n. 6, p. 949–960, 30 out. 2009.

MASON, T. J.; PANIWNYK, L.; LORIMER, J. P. The uses of ultrasound in food technology. **Ultrasonics Sonochemistry**, v. 3, n. 3, 1996.

MORALES, P. et al. Non-fermented and fermented jabuticaba (*Myrciaria cauliflora Mart.*) pomaces as valuable sources of functional ingredients. **Food Chemistry**, v. 208, p. 220–227, 2016.

MUNIN, A.; EDWARDS-LÉVY, F. **Encapsulation of natural polyphenolic compounds; a review** *Pharmaceutics*, 2011.

NERI-NUMA, I. A. et al. Small Brazilian wild fruits: Nutrients, bioactive compounds, health-promotion properties and commercial interest. **Food Research International**, v. 103, p. 345–360, 1 jan. 2018.

NEVES, N. DE A. et al. Flavonols and ellagic acid derivatives in peels of different species of jabuticaba (*Plinia* spp.) identified by HPLC-DAD-ESI/MSn. **Food Chemistry**, v. 252, n. January, p. 61–71, 2018.

PINELA, J. et al. Optimization of heat- and ultrasound-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces for natural food colorants. **Food Chemistry**, v. 275, n. September 2018, p. 309–321, 2019.

PLAZA, M. et al. Characterization of antioxidant polyphenols from *Myrciaria jaboticaba* peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. **Food Chemistry**, v. 211, p. 185–197, 2016.

PLAZA, M.; KARIUKI, J.; TURNER, C. Quantification of individual phenolic compounds' contribution to antioxidant capacity in apple: A novel analytical tool based

on liquid chromatography with diode array, electrochemical, and charged aerosol detection. **Journal of Agricultural and Food Chemistry**, v. 62, n. 2, p. 409–418, 2014.

QUATRIN, A. et al. Characterization and quantification of tannins, flavonols, anthocyanins and matrix-bound polyphenols from jaboticaba fruit peel: A comparison between *Myrciaria trunciflora* and *M. jaboticaba*. **Journal of Food Composition and Analysis**, v. 78, n. January, p. 59–74, 2019.

RASTOGI, N. K. Opportunities and challenges in application of ultrasound in food processing. **Critical Reviews in Food Science and Nutrition**, v. 51, n. 8, p. 705–722, 2011.

ROLIN, C.; DE VRIES, J. Pectin. In: HARRIS, P. (Ed.). **Food gels**. Dordrecht: Springer Netherlands, 1990. p. 401–434.

SANTOS-BUELGA, C.; GONZÁLEZ-PARAMÁS, A. M. Anthocyanins. **Reference Module in Food Science**, p. 1–12, 2018.

SANTOS, D. T.; VEGGI, P. C.; MEIRELES, M. A. A. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation. **Journal of Food Engineering**, v. 101, n. 1, p. 23–31, 2010.

SANTOS, D. T.; VEGGI, P. C.; MEIRELES, M. A. A. Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. **Journal of Food Engineering**, v. 108, n. 3, p. 444–452, 2012.

SIGURDSON, G. T. et al. Impact of location, type, and number of glycosidic substitutions on the color expression of o-dihydroxylated anthocyanidins. **Food Chemistry**, v. 268, p. 416–423, 1 dez. 2018.

TALAMOND, P.; VERDEIL, J. L.; CONÉJERO, G. Secondary metabolite localization by autofluorescence in living plant cells. **Molecules**, v. 20, n. 3, p. 5024–5037, 2015.

TARONE, A. G.; CAZARIN, C. B. B.; MAROSTICA JUNIOR, M. R. Anthocyanins: New techniques and challenges in microencapsulation. **Food Research International**, v. 133, p. 109092, fev. 2020.

TÁRREGA, A.; ROCAFULL, A.; COSTELL, E. Effect of blends of short and long-chain inulin on the rheological and sensory properties of prebiotic low-fat custards. **LWT - Food Science and Technology**, v. 43, n. 3, p. 556–562, 1 abr. 2010.

TSAI, F. H.; KITAMURA, Y.; KOKAWA, M. Effect of gum arabic-modified alginate on physicochemical properties, release kinetics, and storage stability of liquid-core hydrogel beads. **Carbohydrate Polymers**, v. 174, p. 1069–1077, 2017.

- WANG, P. et al. Degradation behavior of polyphenols in model aqueous extraction system based on mechanical and sonochemical effects induced by ultrasound. **Separation and Purification Technology**, v. 247, p. 116967, 15 set. 2020.
- WANG, R. et al. Pasting, thermal, and rheological properties of rice starch partially replaced by inulin with different degrees of polymerization. **Food Hydrocolloids**, v. 92, p. 228–232, 1 jul. 2019.
- WANG, W. et al. Optimization of solvent and ultrasound-assisted extraction for different anthocyanin rich fruit and their effects on anthocyanin compositions. **LWT - Food Science and Technology**, v. 72, p. 229–238, 2016.
- WITCZAK, T.; WITCZAK, M.; ZIOBRO, R. Effect of inulin and pectin on rheological and thermal properties of potato starch paste and gel. **Journal of Food Engineering**, v. 124, p. 72–79, 1 mar. 2014.
- WU, L. et al. Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from *Moringa oleifera* L. leaves: Optimization, comparison and antioxidant activity. **Separation and Purification Technology**, v. 247, p. 117014, 15 set. 2020.
- XU, W. et al. Stability, microstructural and rheological properties of complex prebiotic emulsion stabilized by sodium caseinate with inulin and konjac glucomannan. **Food Hydrocolloids**, v. 105, p. 105772, 1 ago. 2020.
- YE, J. et al. Improvement in freeze-thaw stability of rice starch gel by inulin and its mechanism. **Food Chemistry**, v. 268, p. 324–333, 1 dez. 2018.
- ZIMERI, J. E.; KOKINI, J. L. Rheological properties of inulin-waxy maize starch systems. **Carbohydrate Polymers**, v. 52, n. 1, p. 67–85, 1 abr. 2003.



ANEXOS

Anexo 1 - Cadastro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado.



Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Certidão
Cadastro nº A72354F

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

Número do cadastro: **A72354F**
 Usuário: **UNICAMP**
 CPF/CNPJ: **46.068.425/0001-33**
 Objeto do Acesso: **Patrimônio Genético**
 Finalidade do Acesso: **Pesquisa e Desenvolvimento Tecnológico**

Espécie**Myrciaria jaboticaba**

Título da Atividade: **CASCA DE JABOTICABA: EXTRAÇÃO, ENCAPSULAÇÃO, COMPOSIÇÃO FENÓLICA, BIOACESSIBILIDADE E ATIVIDADE ANTIOXIDANTE NA DIGESTÃO GASTROINTESTINAL IN VITRO**

Equipe

Adriana Gadioli Tarone	UNICAMP
Mário Roberto Maróstica Júnior	UNICAMP
Cinthia Baú betim Cazarin	Unicamp
Claire Dufour	INRAE

Parceiras no Exterior**Institut National de la Recherche Agronomique - INRA****Envios de Amostra**

Espécie:	Myrciaria jaboticaba
Tipo do Patrimônio Genético:	-
Forma do Patrimônio Genético:	Outra
Instituição Destinatária:	INRA - Institut National de la Recherche Agronomique
Sede da Instituição Destinatária:	UMR408 SQPOV, Centre de recherche Paca, 228 route de l'Aérodrome, Domaine Saint Paul - Myrciaria jaboticaba
Espécie:	-
Tipo do Patrimônio Genético:	Outra
Forma do Patrimônio Genético:	INRA - Institut National de la Recherche Agronomique
Instituição Destinatária:	UMR408 SQPOV, Centre de recherche Paca, 228 route de l'Aérodrome, Domaine Saint Paul - Myrciaria jaboticaba
Sede da Instituição Destinatária:	INRA - Institut National de la Recherche Agronomique
Espécie:	-
Tipo do Patrimônio Genético:	Outra
Forma do Patrimônio Genético:	INRA - Institut National de la Recherche Agronomique
Instituição Destinatária:	UMR408 SQPOV, Centre de recherche Paca, 228 route de l'Aérodrome, Domaine Saint Paul - Myrciaria jaboticaba
Sede da Instituição Destinatária:	INRA - Institut National de la Recherche Agronomique
Espécie:	-
Tipo do Patrimônio Genético:	Outra
Forma do Patrimônio Genético:	INRA - Institut National de la Recherche Agronomique
Instituição Destinatária:	UMR408 SQPOV, Centre de recherche Paca, 228 route de l'Aérodrome, Domaine Saint Paul - Myrciaria jaboticaba

Data do Cadastro: **09/02/2018 16:43:00**
 Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético
 Situação cadastral conforme consulta ao SisGen em **12:53 de 10/09/2020**.



**SISTEMA NACIONAL DE GESTÃO
 DO PATRIMÔNIO GENÉTICO
 E DO CONHECIMENTO TRADICIONAL
 ASSOCIADO - SISGEN**

Anexo 2 - Permissão para reprodução dos artigos.

05/05/2020

Rightslink® by Copyright Clearance Center

Copyright
Clearance
Center**RightsLink®**

Anthocyanins: New techniques and challenges in microencapsulation

Author: Adriana Gadioli Tarone, Cinthia Baú Betim Cazarin, Mario Roberto Marostica Junior

Publication: Food Research International

Publisher: Elsevier

Date: July 2020

© 2020 Elsevier Ltd. All rights reserved.

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: <https://www.elsevier.com/about/our-business/policies/copyright#Author-rights>

BACK

CLOSE WINDOW

© 2020 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Terms and Conditions
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

08/01/2021

Rightslink® by Copyright Clearance Center

Copyright
Clearance
Center**RightsLink®**

High-intensity ultrasound-assisted recovery of anthocyanins from jabuticaba by-products using green solvents: Effects of ultrasound intensity and solvent composition on the extraction of phenolic compounds

Author:

Adriana Gadioli Tarone, Eric Keven Silva, Helena Dias de Freitas Queiroz Barros, Cinthia Baú Betim Cazarin, Mario Roberto Marostica Junior

Publication: Food Research International

Publisher: Elsevier

Date: February 2021

© 2020 Elsevier Ltd. All rights reserved.

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: <https://www.elsevier.com/about/our-business/policies/copyright#Author-rights>

BACK

CLOSE WINDOW



04/11/2020

Rightslink® by Copyright Clearance Center



RightsLink®

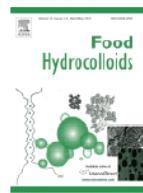
Home

Help

Email Support

Sign in

Create Account



Inulin/fructooligosaccharides/pectin-based structured systems: Promising encapsulating matrices of polyphenols recovered from jabuticaba peel

Author:

Adriana Gadioli Tarone, Eric Keven Silva, Cinthia Baú Betim Cazarin, Mario Roberto Marostica Junior

Publication: Food Hydrocolloids

Publisher: Elsevier

Date: February 2021

© 2020 Elsevier Ltd. All rights reserved.

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: <https://www.elsevier.com/about/our-business/policies/copyright#Author-rights>

BACK

CLOSE WINDOW