UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

FLAVIA CRISTINA SEABRA PIRES

Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (*Byrsonima crassifolia*)

BELÉM-PARÁ
2021

UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

FLAVIA CRISTINA SEABRA PIRES

Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (*Byrsonima crassifolia*)

Tese de Doutorado VIII (final) apresentada ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Instituto de Tecnologia, Universidade Federal do Pará, como requisito para obtenção do título de Doutora em Ciência e Tecnologia de Alimentos.

Orientador: Prof. Dr. Raul Nunes de Carvalho Junior

BELÉM-PARÁ
2021

Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD
Sistema de Bibliotecas da Universidade Federal do Pará
Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

P667a Pires, Flávia Cristina Seabra.
Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (Byrsonima crassifolia) / Flávia Cristina Seabra Pires, Raul Nunes de Carvalho Junior . — 2021.
122 f. : il. color.

Orientador(a): Prof. Dr. Raul Nunes de Carvalho Junior
Tese (Doutorado) - Universidade Federal do Pará, Instituto de Tecnologia, Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, Belém, 2021.

1. Extração supercrítica. 2. Luteína. 3. Murici. 4. Encapsulamento. 5. Nanopartícula. I. Título.

CDD 660.28

FLAVIA CRISTINA SEABRA PIRES

Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (*Byrsonima crassifolia*)

Tese de Doutorado VIII (final) apresentada ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Instituto de Tecnologia, Universidade Federal do Pará, como requisito para obtenção do título de Doutora em Ciência e Tecnologia de Alimentos.

BANCA EXAMINADORA:

_____
Prof. Dr. Raul Nunes de Carvalho Junior
(FEA/ITEC/UFPA – Orientador)

_____
Prof. Drª. Alessandra Santos Lopes
(FEA/ITEC/UFPA – Membro interno)

_____
Profª. Drª. Lúcia de Fátima Henriques Lourenço
(FEA/ITEC/UFPA – Membro interno)

_____
Profª. Drª. Maria Regina Sarkis Peixoto Joele
(IFPA/UFPA – Suplente interno)

_____
Profª. Drª. Brenda de Nazaré do Carmo Brito
(SENAR – Membro externo)

_____
Prof. Dr. José Otavio Carrera Silva Junior
(FF/ICS/UFPA – Membro externo)

_____
Profª. Drª. Mayara Galvão Martins
(OCRIM – Suplente externo)

BELÉM-PARÁ
2021

DEDICATÓRIA

A Deus, aos meus pais, Cristina e Hildemar, aos meus irmãos Bruno e Felipe e à minha sobrinha Maria Luiza por serem os meus exemplos de amor, coragem, força e dedicação.

AGRADECIMENTOS

Agradeço primeiramente a Deus, pois ele é o responsável pela a minha saúde, fé, força e coragem, obrigada Senhor por mais essa conquista.

À minha família Cristina, Hildemar, Felipe, Bruno e Maria Luiza que me apoiam, que cuidam de mim e que me mostram que eu sempre posso ir mais longe. Amo vocês incondicionalmente!

Ao Felippe e à Dona Goreth por todo o apoio, cuidado, torcida e carinho.

À Universidade Federal do Pará (UFPA), à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA) pela oportunidade de realização do curso e pelo suporte financeiro.

Ao meu orientador Profº. Dr. Raul Nunes de Carvalho Júnior pela orientação, suporte, paciência, compreensão e por ter me dado a oportunidade de crescer profissionalmente. Aos Profs. Drs. Otavio Carrera, Alessandra Lopes, Lúcia Lourenço, Mayara Galvão, Ana Vânia Carvalho e Geormeny dos Santos por terem aceitado o convite para participar da banca da defesa e pelas sugestões, que contribuíram para a melhoria desse trabalho. Ao Profs. Drs. Herve Rogez, Julio Pieczarka, Cleusa Nagamachi, Roseane Costa e Davi Barros por me ajudarem com o suporte estrutural e laboratorial.

Aos amigos e colegas dos laboratórios LABEX, LABTECS, CVACBA, CEABIO, LACOS, Laboratório P&D Farmacêutico e Cosmético e Laboratório de Nanotecnologia Farmacêutica pela colaboração e apoio, que foram fundamentais para o desenvolvimento do presente trabalho.

Enfim... A todos vocês o meu mais sincero e profundo agradecimento, MUITO OBRIGADA!

“Leve na sua memória para o resto de sua vida as coisas boas que surgiram no meio das dificuldades. Elas serão uma prova de sua capacidade em vencer as provas e lhe darão confiança na presença divina, que nos auxilia em qualquer situação, em qualquer tempo, diante de qualquer obstáculo.”

(Chico Xavier)

RESUMO

O murici (*Byrsonima crassifolia*) é um fruto que possui um grande potencial terapêutico devido à presença de luteína, um carotenoide valorizado no mercado de suplementação alimentar voltados para a saúde ocular. Devido à sua pericibilidade, tornou-se necessária a aplicação de processos industriais que possibilitem o aumento da vida-de-prateleira, a diversificação do uso e consumo, a agregação de valor e a ampliação de mercado do murici, com o uso de tecnologias limpas associado ao incentivo à bioeconomia. Desse modo, o trabalho entitulado "Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (*Byrsonima crassifolia*)" foi desenvolvido com a finalidade de estudar e aplicar os extratos do murici, extraídos e nanoencapsulados via extração com CO_2 supercrítico, em suplemento alimentar. Para isto, o primeiro passo foi desenvolver um estudo sobre a obtenção dos extratos da polpa de murici pela tecnologia supercrítica. Foram determinadas as melhores condições de extração supercrítica da polpa de murici, com CO_2 e CO_2+etanol, através das variações de pressão (15 a 49 MPa), temperatura (323,15 a 343,15 K), densidade (654 a 946 kg/m^3), vazão de CO_2 (8.85×10^{-5} a 1.33×10^{-4} kg/s) e tempo dinâmico de extração (3600 a 10800 s), onde foram obtidas as isotermas de rendimento global, os compostos bioativos presentes nos extratos e nos leitos de extração, tais como: luteína, ácidos graxos, triglicerídeos, compostos fenólicos e flavonoides. Também foi avaliada a qualidade funcional, a capacidade/atividade antioxidante e os efeitos citotóxico e citoprotetor. O estudo da extração supercrítica, possibilitou a obtenção de óleos não-tóxicos com elevado teor de luteína, constituídos de triglicerídeos de cadeia longa ricos em ácidos graxos insaturados como os ômega 3 e 9, e com atividades antioxidante, antihipercolesterolemica, antiaterosclerogênica, antitrombogênica e citoprotetora, onde a melhor condição de extração foi obtida a 343,15 K/ 49 MPa/ 900 kg/m^3. Também possibilitou a obtenção de extratos etanólicos não-tóxicos a partir da polpa desengordurada, um subproduto nas indústrias alimentícias, contendo luteína, compostos fenólicos, flavonóides e atividade antioxidante, onde a melhor condição de extração foi obtida a 343,15 K/ 22 MPa/ 775 kg/m^3. Devido ao efeito citoprotetor, o óleo de murici foi utilizado para a produção de partículas por meio do processo de extração de emulsões por fluido supercrítico (SFEE), onde foi possível produzir nanopartículas de óleo de murici com boa concentração/retenção de luteína, tamanho reduzido de partículas e com alta estabilidade, onde a melhor formulação das emulsões de partida foi obtida com o espessante goma xantana, com o emulsificante csgel e em uma concentração de 6 mg/mL de óleo de murici, e a melhor condição do processo SFEE foi a de 8 MPa, 313,15 K, período estático de 3600 s e período dinâmico de 18000 s. A partir disso, foi possível obter emulsões SFEE em pó do óleo de murici por liofilização e por spray drying com umidades, atividades de água, tamanhos de partícula, estabilidades e teores de luteína adequados para serem utilizados em suplementos alimentares carregados em cápsulas vegetais. As cápsulas vegetais carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) por liofilização e por spray drying apresentaram-se dentro dos padrões de carregamento para comercialização, onde, foi possível obter suplementos alimentares de óleo de murici, ricos em luteína. Portanto, a presente tese evidenciou que é possível utilizar a tecnologia supercrítica na cadeia produtiva de suplementos alimentares a base de murici, para facilitar o acesso e a estabilidade dos compostos aos quais são atribuídos os potenciais terapêuticos do murici. Este trabalho verticalizou a importância de pesquisas sobre o efeito funcional do murici e sobre o uso da tecnologia supercrítica, o que contribuiu para o desenvolvimento da bieconomia da região e de novos produtos pela ciência, tecnologia e engenharia de alimentos, com um grande retorno à sociedade.

Palavras-chave: Murici, Luteína, Compostos bioativos, Extração supercrítica, Extração de emulsões por fluido supercrítico, Suplemento alimentar.

ABSTRACT

Murici (*Byrsonima crassifolia*) is a fruit that has great therapeutic potential due to the presence of lutein, a carotenoid valued in the food supplementation market focused on eye health. Due to its riskiness, it became necessary to apply industrial processes that enable the increase of shelf life, the diversification of use and consumption, the addition of value and the expansion of the murici market, with the use of clean technologies associated with the incentive to the bioeconomy. Thus, the work entitled "Application of supercritical technology in the development of a food supplement based on murici extract (*Byrsonima crassifolia*)" was developed with the purpose of studying and applying murici extracts, extracted and nanoencapsulated via extraction with supercritical CO_2, in food supplement. For this, the first step was to develop a study on obtaining extracts of murici pulp by supercritical technology. The best conditions for supercritical extraction of murici pulp were determined, with CO_2 and CO_2+ethanol, through variations in pressure (15 to 49 MPa), temperature (323.15 to 343.15 K), density (654 to 946 kg/m^3), CO_2 flow (8.85×10^{-5} to 1.33×10^{-4} kg / s) and dynamic extraction time (3600 to 10800 s), where the global yield isotherms, the bioactive compounds present in the extracts were obtained and in the extraction beds, such as: lutein, fatty acids, triglycerides, phenolic compounds and flavonoids. Functional quality, antioxidant capacity/activity, and cytotoxic and cytoprotective effects were also evaluated. The study of supercritical extraction made it possible to obtain non-toxic oils with a high content of lutein, consisting of long chain triglycerides rich in unsaturated fatty acids such as omega 3 and 9, and with antioxidant, antihypercholesterolemic, anti-atherosclerogenic, anti-thrombogenic and cytoprotective activities, where the best extraction condition was obtained at 343.15 K/ 49 MPa/ 900 kg/m^3. It also made it possible to obtain non-toxic ethanol extracts from defatted pulp, a by-product in the food industry, containing lutein, phenolic compounds, flavonoids and antioxidant activity, where the best extraction condition was obtained at 343.15 K/ 22 MPa/ 775 kg/m^3. Due to the cytoprotective effect, murici oil was used for the production of particles through the process of supercritical fluid of emulsions extraction (SFEE), where it was possible to produce murici oil nanoparticles with good concentration/retention of lutein, reduced size of particles and with high stability, where the best formulation of the starting emulsions was obtained with the thickener xanthan gum, with the emulsifier csgel and in a concentration of 6 mg / mL of murici oil, and the best condition of the SFEE process was the 8 MPa, 313.15 K, static period of 3600 s and dynamic period of 18000 s. From this, it was possible to obtain powdered SFEE emulsions of murici oil by lyophilization and spray drying with humidity, water activities, particle sizes, stability and lutein levels suitable for use in food supplements loaded in vegetable capsules. Vegetable capsules loaded with powdered SFEE emulsions of murici oil by lyophilization and spray drying were within the loading standards for commercialization, where it was possible to obtain dietary supplements of murici oil, rich in lutein. Therefore, the present thesis showed that it is possible to use supercritical technology in the murici-based food supplements production chain, to facilitate the access and stability of the compounds to which the murici's therapeutic potentials are attributed. This work verticalized the importance of research on the functional effect of murici and on the use of supercritical technology, which contributed to the development of the region's bieconomics and new products by science, technology and food engineering, with a great return to society.

Keywords: Murici, Lutein, Bioactive compounds, Supercritical extraction, Supercritical fluid extraction of emulsions, Dietary supplement.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1. TEXTO INTEGRADOR

O murici (*Byrsonima crassifolia*) é um fruto que possui um grande potencial terapêutico devido à presença de luteína, um carotenoide que possui bastante valor agregado no mercado de suplementação alimentar voltados para a saúde ocular, visto que a ingestão continuada de luteína possibilita a regeneração da retina dos olhos, minimizando os efeitos de degeneração macular adquirida com a idade.

Apesar deste grande potencial nutraceutico, a polpa dos frutos é normalmente consumida e comercializada para o preparo de produtos culinários como sorvetes, sucos e cremes. Além disso, possui elevada pericibilidade devido à rancificação ocasionada pela oxidação da gordura contida no fruto. Portanto, tornou-se necessária a aplicação de processos industriais que possibilitem o aumento da vida-de-prateleira, a diversificação do uso e consumo, a agregação de valor e a ampliação de mercado do murici.

Em paralelo, o apelo mundial pelo uso de tecnologias limpas associado ao incentivo à bioeconomia, que é um modelo de produção industrial baseado no uso sustentável de recursos biológicos, trouxe um novo cenário a ser explorado pela ciência, tecnologia e engenharia de alimentos, principalmente na região Amazônica. Desse modo, a tese de doutorado entitulada "Aplicação da tecnologia supercrítica no desenvolvimento de suplemento alimentar a base de extrato de murici (*Byrsonima crassifolia*)" foi desenvolvida com a finalidade de estudar e aplicar os extratos do murici, extraídos e nanoencapsulados via extração com CO_2 supercrítico, em suplemento alimentar.

Este estudo foi desenvolvido e dividido em cinco capítulos. O Capítulo I retrata uma revisão de literatura na forma de um capítulo de livro publicado sobre a "Aplicação da tecnologia supercrítica na produção de suplemento alimentar à base de extratos vegetais". Esta revisão aborda todos os principais pontos contidos na tese como: o panorama mundial do mercado de suplementos alimentares; a importância do uso de extratos vegetais em suplementos; os compostos bioativos normalmente utilizados em suplementos alimentares com enfase nos compostos que foram quantificados como carotenoides, compostos fenólicos e ácidos graxos; as técnicas de extração com enfase na extração supercrítica para a obtenção de extratos vegetais; e o uso da nanotecnologia no encapsulamento de compostos bioativos com enfase no processo de Extração de Emulsões por Fluido Supercrítico (SFEE).

Os próximos capítulos são referentes à parte experimental da tese, onde primeiro passo foi desenvolver um estudo sobre a obtenção dos extratos da polpa de murici pela tecnologia supercrítica. Devido a necessidade de serem determinadas as melhores condições de processo e os teores de compostos bioativos dos extratos de murici e dos leitos de extração, foi desenvolvido o Capítulo II, que retrata um artigo publicado sobre a "Determinação de parâmetros de processo e propriedades bioativas dos extratos da polpa de murici (*Byrsonima crassifolia*) obtidos por extração supercrítica". Neste estudo foram determinadas as melhores condições de extração supercrítica da polpa de murici, com CO_2 e CO_2+etanol, através das variações de pressão, temperatura, densidade, vazão de CO_2 e tempo dinâmico de extração, onde foram determinadas as isotermas de rendimento global, os compostos bioativos presentes nos extratos e nos leitos de extração, tais como: luteína, ácidos graxos e compostos fenólicos; e também foi avaliada a qualidade funcional e capacidade antioxidante.

Uma das respostas do estudo anterior foi a obtenção da melhor temperatura de extração, onde tornou-se necessário a aplicação de outras pressões e densidades de processo, a fim de analisar os teores de compostos bioativos dos extratos. Além disso, devido a pretensão de aplicar estes extratos em um produto para ingestão humana, foi de fundamental importância realizar o estudo da toxicidade e do efeito protetor dos extratos em modelos celulares. Devido a isto, foi desenvolvido o Capítulo III, que retrata um artigo publicado sobre os "Compostos bioativos e avaliação dos efeitos antioxidante, citotóxico e citoprotetor de extratos de polpa de murici (*Byrsonima crassifolia*) obtidos por extração supercrítica em células HepG2 tratadas com H_2O_2". Neste trabalho, baseado no primeiro artigo, foram obtidas as isotermas de rendimento global na temperatura de 343.15 K, variando a pressão e densidade, utizando CO_2 e CO_2+etanol apenas no período estático de extração. Foram obtidos os teores de luteína, compostos fenólicos, flavonoides, atividade/capacidade antioxidante e os perfis de ácidos graxos e triglicerídeos, além do potencial funcional. Também foram avaliados os efeitos citotóxico e citoprotetor em modelos celulares para determinar a seguridade alimentar dos extratos.

Portanto, a partir dos artigos anteriores, foi possível obter a melhor condição de extração supercrítica do óleo e do extrato etanólico de murici, onde para a continuidade da pesquisa foi escolhido o óleo de murici pelo seu efeito citoprotetor. Apesar do óleo ser rico em compostos bioativos, muitas reações de degradação podem ocorrer no armazenamento

como a rancificação da gordura e a redução de carotenoides. Portanto tornou-se necessário aplicar métodos de encapsulamento, onde optou-se por produzir partículas através do método de extração de emulsões por fluido supercrítico (SFEE). Com base nisso, foi desenvolvido o Capítulo IV, que retrata um artigo submetido sobre a "Produção de partículas de óleo de murici (*Byrsonima crassifolia*) por SFEE: Estudo da formulação, estabilidade, tamanho de partícula e concentração de luteína". Neste estudo foram avaliados os efeitos da formulação utilizando diferentes emulsificantes, espessantes e concentrações de óleo sobre a estabilidade, tamanhos, polidispersividade e potencial zeta das partículas. A emulsão mais estável foi submetida ao processo SFEE, onde foram avaliados os efeitos dos períodos estáticos e dinâmicos de extração sobre o conteúdo/retenção de luteína, tamanho, polidispersidade e potencial zeta das partículas do óleo de murici.

A partir disso, foi possível obter o óleo de murici encapsulado por emulsificação, onde para possibilitar o carregamento em cápsulas duras vegetais, foi necessário aplicar métodos de desidratação a fim de obter partículas em pó do óleo de murici. Portanto, foi desenvolvido o Capítulo V, que retrata uma patente submetida sobre a "Produção de partículas secas e de suplemento alimentar à base de óleo de murici". Neste pedido de patente, foi utilizada a emulsão do óleo de murici obtida na melhor condição SFEE, relatada do terceiro artigo, que foi desidratada por liofilização e por spray drying para a obtenção de partículas secas do óleo de murici. Estas partículas foram analisadas quanto ao teor de luteína, umidade, atividade de água, tamanho de partícula, polipersividade e potencial zeta, onde foram posteriormente carregadas em cápsulas duras vegetais para a produção de um suplemento alimentar à base de óleo de murici, e foram analisados o peso médio, desvio padrão relativo, variação do conteúdo teórico e tempo de desintegração.

Desse modo, a proposta da tese foi concluída e está apresentada com mais detalhes nos capítulos a seguir.

CAPÍTULO I

Application of supercritical technology in the production of dietary supplement based on plant extracts

(Aplicação da tecnologia supercrítica na produção de suplemento alimentar à base de extratos vegetais)

Flávia Cristina Seabra Pires, Ana Paula de Souza e Silva, Ivonete Quaresma da Silva, Joicy Corrêa de Oliveira, Eduardo Gama Ortiz Menezes, Wanessa Almeida da Costa, Raul Nunes de Carvalho Junior

Reprinted from Green Sustainable Process for Chemical and Environmental Engineering and Science, 2021:

PIRES, F. C. S.; SILVA, A. P. S.; SILVA, I. Q.; OLIVEIRA, J. C.; MENEZES, E. G. O.; COSTA, W. A.; CARVALHO JUNIOR, R. N. **Application of supercritical technology in the production of dietary supplement based on plant extracts**. In: INAMUDDIN; BODDULA, R.; AHAMED, M. I.; ASIRI, A. M. Green Sustainable Process for Chemical and Environmental Engineering and Science, Solvents for the Pharmaceutical Industry, chapter 8, p. 161-183, 2021. DOI: 10.1016/B978-0-12-821885-3.00006-2.

CHAPTER 8

Application of supercritical technology in the production of dietary supplement based on plant extracts

Flávia Cristina Seabra Pires[a,*], Ana Paula de Souza e Silva[a], Ivonete Quaresma da Silva[b], Joicy Corrêa de Oliveira[c], Eduardo Gama Ortiz Menezes[c], Wanessa Almeida da Costa[c], and Raul Nunes de Carvalho Jr.[a]

[a]LABEX (Extraction Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Pará), Belem, Pará, Brazil
[b]LABEX (Extraction Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Pará), UEPA (State University of Pará), Belém, Pará, Brazil
[c]LABEX (Extraction Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Pará), Belém, Pará, Brazil
Corresponding author. e-mail address: flaviapiress@gmail.com

1. Introduction

The bad habits of today's society have caused people not to eat properly. In addition, interest in physical activities and in a healthier lifestyle has increased. Due to these consumers' needs, the dietary supplements market has expanded, and a growing number of new dietary supplement products containing natural plant compounds have been produced [1, 2].

Dietary supplement is a product for oral ingestion, presented in pharmaceutical forms, and intended to supplement the diet of healthy individuals with nutrients, bioactive substances, enzymes, or probiotics, alone or in combination. These include bioactive substances, which are defined as any chemical found naturally in foods, which is not classified as a nutrient, and may confer health benefits [3].

One of the main sources of bioactive substances is plant extracts. Such compounds are known for their various therapeutic properties, widely addressed in numerous works. Due to the therapeutic potential of plants, many studies have been conducted to identify, extract, and purify bioactive substances, such as carotenoids, fatty acids, and phenolic compounds [4–7].

A very important aspect in obtaining plant extracts concerns the extraction method employed. Among these, conventional extraction techniques have been widely explored, with soxhlet being one of the most used ones. However, despite the advantages, these techniques use large amounts of toxic solvents, which leave residual amounts in the extracts, even after evaporation, and are not environmentally safe [8].

Green Sustainable Process for Chemical and Environmental Engineering and Science
https://doi.org/10.1016/B978-0-12-821885-3.00006-2

On the other hand, over the last few years, global interest in the use of clean technologies for development of edible products and pharmaceuticals has been observed. Therefore, the use of supercritical technology emerges as a potential alternative when it comes to obtaining 100% pure, solvent-free, and environmentally "green" plant extracts [9, 10].

Despite obtaining 100% pure plant extracts, its application considers characteristics such as solubility and stability. For this reason, the use of nanotechnology has emerged to revolutionize the entire industrial system, including processing, storage, and development of materials and products [11].

Nanotechnology has mainly been used for encapsulation of bioactive compounds that exhibit unstable behavior, either by degradation or bioavailability. Various nanoencapsulation methods have been studied, especially those related to innovative supercritical fluid techniques. Among these methods, supercritical fluid extraction of emulsions (SFEE) has been used to obtain nanoparticles through supercritical CO_2 nanoemulsions. This technique makes it possible to obtain solvent-free nanoencapsulated extracts in nanoemulsion form. Thus, this procedure represents a promising technique for encapsulating bioactive compounds [12].

Therefore, it is extremely important to study and apply the supercritical technology in the dietary supplements production chain to obtain 100% natural quality products obtained by environmentally safe technologies.

2. Dietary supplement

Dietary supplement can be defined as any ingestible product added to the diet for health benefits, including plant-based products (herbal or botanical extracts), minerals, and vitamins. These definitions vary from country to country, since the definition of dietary supplements adopted in the United States includes the use of metabolites. In short, they are products intended to supplement the diet only by oral administration, whether in tablets, capsules, powders, or liquids [2, 13, 14].

Often, dietary supplements are called nutraceuticals; however, it is important to note that every nutraceutical is a dietary supplement, but the opposite is not necessarily true. The difference between these products is that dietary supplements may or may not be of food origin, while nutraceuticals are derived solely from food. In addition, nutraceuticals have remarkable health claims, while dietary supplements are not allowed to have such claims on their labels [2, 13, 15].

Dietary supplement intake has been growing worldwide, mainly due to people's lifestyle. Increasing life expectancy and concerns for a healthier life conflict with the busy lifestyle of the population, which often makes it difficult to eat properly and do exercises. This daily routine has also increased the incidence of degenerative diseases associated with

these bad habits. Thus, dietary supplements have become objects of study and consumption [16, 17].

According to the Council for Responsible Nutrition [1], the innovation of supplement manufacturing industries stimulates the growing consumption of these products, which are becoming increasingly popular. The success of dietary supplement industries in providing safe and high-quality products reflects current results of consumption worldwide.

Over the past two decades, the supplement market has grown substantially. In fact, it has more than doubled its value since 1999, from US$ 49.1 billion to US$ 127.8 billion in 2017. And it is expected to continue to increase in value to over US$ 150 billion by 2020, which means it will have tripled in only 20 years. During this period, its global annual growth was consistently in the range of 5%–7%, highlighting the robustness of the sector [18].

Research indicates that supplement consumption reaches 50% in the United States, 40%–60% in Asian countries, and 30% in Europe and Latin America. In Brazil, about 75% of people complement their diet. The most prominent supplements are vitamins (48%), minerals (22%), plant extracts (19%), fatty acids (17%), proteins (16%), amino acids (14%), and oils, such as safflower, garlic, etc. (12%) [19, 20].

Dietary supplements can be related to three types of commercial appeal: health, content, and structure/function claims. Health claim is based on the prevention of possible pathologies. Content claim is based on the high concentration of ingredients or nutrients, and the structure/function claim is based on the possible physiological effects on the body. However, such products are not allowed to have label claims intended to diagnose, treat, cure, or prevent any disease [21].

The quality and effectiveness of dietary supplements, especially based on plant extracts, can be attributed to several factors such as plant type and dosage [22–24], extraction methods [25–28], encapsulation methods applied to increase stability [29–31], and pharmaceutical form of the final product [27, 32–34].

3. Plant extracts

Plant extracts have been widely studied and applied in various food and pharmaceutical products. This is due to their compositions being rich in bioactive compounds [21, 35, 36]. The presence of bioactive compounds, associated with therapeutic and nontoxic effects, provides supporting evidence for the application of such extracts to prevent and/or delay the onset of various diseases, as well as to be used in the development of safe dietary supplements [37–39].

Importantly, the relationship between the concentration of bioactive compounds and their therapeutic activity is not yet established. However, epidemiological studies have shown that continued ingestion of these compounds is associated with disease prevention [40–44].

3.1 Bioactive compounds used in dietary supplements

By definition, bioactive compounds are essential and nonessential compounds that occur in nature, are part of the food chain, and can improve human health [45]. Among these compounds, carotenoids, phenolic compounds, and polyunsaturated fatty acids have been the object of many scientific studies, aiming at their application in the development of dietary supplements [28, 39, 46].

3.1.1 Carotenoids

Carotenoids are pigments that vary from yellow to red, usually of lipophilic nature, whose main source are vegetables. These compounds are divided into two major classes, according to the structural differences of their hydrocarbons: carotenes (carbon and hydrogen) and xanthophylls (carbon, hydrogen, and oxygenated functional group) [5, 47, 48].

Xanthophylls are synthesized from carotenes through hydroxylation and epoxidation reactions. β-carotene and lycopene are examples of carotenes, while lutein and zeaxanthin are xanthophylls. There are over 600 types of carotenoids, many of which are precursors of vitamin A, including β-carotene. In the body, most of these compounds are found in tissues and plasma, and others are found in the retina (zeaxanthin and lutein) [49–51].

In the global market, there are many carotenoid supplements. The most marketed supplements are based on lutein, zeaxanthin, astaxanthin, lycopene, β-carotene, and canthaxanthin. The global dietary supplement market for these carotenoids is expected to reach US$ 1.74 billion by 2025 [52].

These compounds are used for their numerous beneficial health effects. Carotenoids have antioxidant activity against free radicals (ROS and RNS) present in cells and tissues, and some of them present provitamin A activity [53–57]. In addition, carotenoids are recognized as health-promoting compounds, presenting positive responses on the strengthening of the immune system, protection against cancer, reduction of risk of cardiovascular diseases such as arteriosclerosis, and others [47, 48, 51, 58, 59].

Regarding lutein, it is related to the prevention of age-related macular degeneration (AMD) and cataract. This is because lutein structurally forms the macula lutea of the eye. Its molecular structure can be described as a long carbon chain (40 carbon atoms) and numerous double pairs alternated with attached methyl groups on the sides. Due to the characteristics of the molecular structure of lutein, this compound has hydrophobicity and is susceptible to oxidation in the presence of light and heat [51, 60, 61], and therefore it has been applied in an encapsulated form for use in dietary supplements [62–64].

Studies have shown that acute or chronic lutein supplementation presented no toxicity, and no mutagenic or teratogenic effects [65–67]. Therefore, based on scientific data, chronic lutein supplementation at the recommended dose of 10 mg/day is safe

and nontoxic. Thus, continued intake of lutein, either fresh or supplemented, may be encouraged, especially for the elderly and for individuals at high risk of different clinical conditions [68–70].

3.1.2 Phenolic compounds

Phenolic compounds are secondary metabolites of plants, which are essential for their growth and reproduction, as they are formed under stressful conditions, such as infections, injuries, UV radiation, among others [71]. These compounds are a very diverse group, which are defined as aromatic ring-bearing substances, with one or more hydroxyl substituents, including their functional groups. Due to the diversity of their structures, phenolic compounds have a multifunctional character, being the object of studies in many research areas, such as food and pharmaceuticals [72, 73].

There are over 8000 identified phenolic compounds, which are divided into groups depending on their chemical structure. According to their carbon chain, the compounds can be simple phenols, phenolic acids, flavonoids, lignans, biflavonoids, hydrolyzable and condensed tannins, stilbenes, and lignans [74].

These compounds present various biological activities, which favor their contribution to the beneficial effects of diets rich in fruits and vegetables. They have antioxidant, antibacterial, antiviral, antiinflammatory, cardioprotective, anticarcinogenic, and chemopreventive activities. Anticarcinogenic and chemopreventive properties of phenolic compounds have been related to the inhibition of colon, esophageal, lung, liver, breast, and skin cancers [7, 75–81].

Among the phenolic compounds of interest to the pharmaceutical and food industries, flavonoids are very important for use in dietary supplements, due to their effects on the gastrointestinal tract such as: protection of the intestinal epithelium, pharmacological insults, and food toxins; modulation of the activity of enzymes involved in the absorption of lipids and carbohydrates; intestinal hormone secretion; immune system; and modulation of microbiota composition and function; and also present potential anticolorectal cancer activity [82].

The use of flavonoids in dietary supplements is usually inserted along with herbal extracts. The market has been growing, with an estimated value of US$ 1.06 billion by 2025. In general, consumption of flavonoids in dietary supplements is stimulated by possible effects such as: calorie and weight reduction, antioxidant and anticancer potential, prevention/treatment of diabetes and cardiovascular diseases [83, 84].

Flavonoids are low molecular weight compounds that are found in the form of glycosides and aglycones [85]. Belonging to this class of compounds, quercetin is increasingly being used in functional beverages, such as energy and nutraceutical drinks, due to its benefits including energy increase, antioxidant, antiinflammatory, and antidiabetic properties. [82, 86–88].

In vivo studies found different dosages of quercetin for a positive antidiabetic effect: 50–80 mg/kg [89], 100 mg/kg [90], and 25, 50, and 75 mg/kg [91]. Therefore, these works demonstrate quercetin supplementation may be an important new strategy for the treatment/prevention of diabetic encephalopathy, diabetic nephropathy, and other diabetic complications [88].

3.1.3 Fatty acids

Vegetable fat is in the form of triglycerides, formed by a glycerol molecule, esterified to three fatty acids, called saturated, unsaturated (mono or poly), and trans. This classification of fatty acids is based on four aspects: number of double bonds, carbon chain length, double bond configuration, and fatty acid position in the glycerol molecule [92].

Polyunsaturated fatty acids are identified by the letter ω (omega). The main fatty acids belonging to the ω group, also called essentials, are ω-3 (omega-3) and ω-6 (omega-6). Omega 3 occurs when the first double bond is located on carbon 3, counting from the methyl radical (CH_3); and omega 6 occurs when the double bond is located on the sixth carbon of the chain, counting from the same radical [93, 94].

After ingestion, enzymatic synthesis occurs, resulting in other fatty acids. The main ones in the omega-3 family are alpha-linolenic (C18: 3), eicosapentaenoic-EPA (C20: 5), and docasahexaenoic-DHA (C22: 6) acids; and the most important in the omega-6 family are linoleic (C18: 2) and arachidonic (C20: 4) acids [95].

Plenty of scientific publications have confirmed the health benefits of omega-3 and omega-6. Studies have shown that their intake is related to lower prevalence of obesity [96], metabolic syndrome [97], type 2 diabetes [98], and cardiovascular events [99]. These fatty acids also present antiinflammatory [100], and anticancer (breast, prostate, and colon) activities [101–103], contributing to reduction of depression [104], and Alzheimer's disease [105].

In supplements, fatty acids are marketed in the form of natural triglycerides, free fatty acids, ethyl esters, re-esterified triglycerides, and phospholipids [46, 106]. The consumption of fatty acids in the form of supplements aims to increase the plasma levels of eicosapentaenoic and docosahexaenoic acids [106, 107].

The fatty acid supplement market is expected to grow by 12.20% by 2021. Commercially, omega-3 is present in a variety of dietary supplement formulations, including animal and vegetable oils. They are usually composed of docosahexaenoic (DHA), eicosapentaenoic (EPA), alpha linoleic (ALA), linoleic (LA), and arachidonic (AA) acids, with dosages of about 1000 mg of oil containing 180 mg EPA and 120 mg DHA, approximately [108].

Therefore, due to the many benefits of fatty acid intake, it can be stated that their supplementation is recommended.

4. Techniques for extraction of bioactive compounds from plants

Due to the therapeutic properties of some vegetables, much work has been done to identify, extract, and purify their bioactive substances [109, 110]. A very important aspect in obtaining plant extracts concerns the extraction method employed, which can be conventional or unconventional.

Conventional techniques consist of liquid-liquid and liquid-solid extractions and solid phase microextractions [111]. The conventional methods most used are: infusion, decoction, maceration, percolation, and hot extraction [112]. Among these methods, Soxhlet stands out, as it is one of the most used in scientific approaches for comparison with unconventional extractions [8, 113, 114].

Soxhlet is a solid-liquid extraction of continuous-discrete percolation which has shown, for over a century, several advantages, such as ease of mass transfer of analytes to the solvent, as the sample is repeatedly put in contact with new portions of pure solvent, which is evaporated, condensed, and recirculated. This process often causes analyte depletion. Moreover, it is a very simple method that requires little training and enables high extraction yield [115, 116].

Despite these advantages, the use of toxic solvents leaves residues in the extracts, thus altering their purity and limiting their application in food and pharmaceutical products. In addition, there is currently a call for environmental preservation, in which these types of solvent are not considered clean. For this reason, many works have approached the use of clean technologies through unconventional extraction methods to obtain plant extracts [117].

The cleanest unconventional extraction methods most currently addressed in scientific articles are: supercritical fluid, pressurized liquid, ultrasound-assisted [118], microwave-assisted, membrane-based solvent [119], high hydrostatic pressure [120], pulsed electric field assisted [121], subcritical water [122], negative pressure cavitation [123], and enzymatic extractions [124].

Among these techniques, supercritical fluid extraction has been recognized as promising because it provides high selectivity and purity, shorter extraction times compared to other extraction methods, and does not use toxic organic solvents [9, 125, 126].

4.1 Supercritical fluid extraction

Supercritical extraction is a sustainable green technology that has presented a wide range of applications, some already implemented at the industrial level and others still emerging [127–129]. Supercritical fluid techniques are considered clean, primarily because of the use of generally recognized as safe (GRAS) solvents. In addition, many supercritical fluid extraction equipments present solvent recirculation, making it reusable and reducing environmental impacts [130].

According to Brunner [131], in the supercritical extraction process, the equilibrium between liquid and gas phases is established, causing them to have identical properties

such as density, viscosity, refractive index, thermal conductivity, and others. This equilibrium point is found above the critical point and is characterized by presenting, instead of two phases (liquid and gaseous), a single phase (supercritical). Thus, by definition, supercritical fluid is any substance that is under conditions of pressure and temperature above the critical point.

Supercritical fluids have some properties that make them particularly attractive. They are highly compressible and have high solubility and selectivity, which are modified with density. This is due to their physical properties as they present an arrangement between liquid and gas properties, which enables increased mass transfer and shorter extraction times [132, 133].

The most used supercritical fluid is carbon dioxide (CO_2), as it is nonflammable, nontoxic, inert, nonpolluting, and selective, as well as has low cost and is easy to recover. [9]. Besides, CO_2 is also widely used due to its moderate critical properties (temperature = 31.1°C; pressure = 73 bar), thus avoiding the degradation of volatile, thermolabile, and oxidizable substances [134–136]. A noteworthy fact is that CO_2 is generated as a byproduct of the production of ammonia, ethanol, hydrogen, and natural gases, and the application of CO_2 is a way to reduce its emission into the atmosphere, thus helping to solve environmental problems [137]. Thus, the advantage of CO_2 in supercritical extraction fits into green industrial production processes, especially in food and pharmaceutical industries.

According to Brunner [131], supercritical extraction takes place after confinement of the plant matrix and supercritical fluid under certain conditions of pressure and temperature. It is first necessary to assemble the fixed bed according to the porosity and density of the plant solid matrix. Then, the process goes through a static extraction period and a dynamic extraction period. During the static extraction period, the supercritical fluid is injected into the extractor and flows through the solid matrix in a closed system. Then, the membranes of the plant matrix break down, causing mass transport of compounds toward the surface. The dynamic extraction period occurs in an open system and the fluid drags the extract into a container at room temperature. The separation of the extract from the solvent occurs through precipitation caused by system depressurization, which leads to fluid solvating power to reduce, causing separation of the extract and release of the gas.

Because supercritical extraction is based on fluid solvation properties, it becomes strongly influenced by intrinsic and extrinsic process characteristics such as temperature, pressure, extraction time, solvent type, solvent flow rate, sample size, and cosolvent use [138].

Temperature and pressure are important factors that affect the final extract composition and yield, as well as the solubility of each component in the supercritical fluid [139, 140]. Generally, at constant pressure, increasing temperature increases solubility and diffusivity, and reduces fluid viscosity and surface tension of the solute, allowing

the supercritical fluid to move freely through small pores and structures, and inducing the transport phenomena in the process. Under isothermal conditions, the increase in pressure increases the density and, consequently, the solvating power [126].

According to the results obtained by Pires et al. [4], extraction times reduce with increasing solvent flow rates. In addition, the use of supercritical CO_2 made it possible to obtain extracts with lipophilic or nonpolar bioactive compounds, such as carotenoids and fatty acids. Since the use of cosolvents modifies fluid solubility, with the use of CO_2 (90%) and ethanol (10%) as cosolvents, it was possible to obtain extracts of hydrophilic or polar compounds, such as phenolic compounds.

Regarding sample size, according to Brunner [131], the solid matrix must be composed of particles small enough to increase the contact area with the fluid, thus facilitating the mass transfer of the particles center toward the surface, but not so small as to cause leaks and clogging in the process line.

Importantly, the application of extracts with active ingredients considers characteristics such as solubility and stability. After extraction, bioactive compounds are more vulnerable to degradation. For this reason, the use of nanotechnology emerges to revolutionize the entire food and pharmaceutical systems, including production, processing, storage, and development of materials and products [11].

5. Nanotechnology

Nanotechnology is one of the leading emerging technologies that support sustainable competitiveness in various industrial sectors. In the agro-industrial area, nanotechnology is applied to increase agricultural productivity and economic growth of industries, aiming at improving the nutritional value and quality of products [141, 142].

By definition, nanotechnology is any natural, incidental, or manufactured material that contains particles in an unbound/aggregate/agglomerate state, in which 50% or more of the particles present one or more nanoscale dimensions [143]. The nanoparticle diameters range from 1 to 100 nm. However, for use in biomedical areas, these diameters can be in the range from 1 to 1000 nm [144].

The range of possible fields of application is wide and includes food, medical, cosmetics, biotechnology, and energy production industries. In the food industry, nanotechnology is used to extend shelf life, customize flavors, and promote health and well-being [145–147].

The development of new dietary supplements is one of the main areas of food and pharmaceutical industries that have been constantly under innovation by the development of nanotechnology [148]. Among the many benefits of applying nanotechnology, controlled drug delivery system can be highlighted, which allows bioactive compounds to be delivered to their specific action sites in the body [149, 150].

This occurs because the properties of nanomaterials are very different from those of conventional materials, as they have high surface to volume ratio, altering or improving properties such as tensile strength, reactivity, electrical characteristics, and others [151]. In addition, they enable greater absorption, ability to create porous structures, greater solubility, and better controlled release of bioactive compounds into the body [142, 152].

Thus, nanotechnology offers many opportunities for industrial applications and can be applied in food/pharmaceutical products, nutritional supplements, and food packaging [147, 153].

5.1 Nanoencapsulation

The ingestion of bioactive compounds from plants is a mechanism that makes it possible to improve the therapeutic properties of plants, with minimization of possible side effects. However, such compounds can undergo enzymatic, thermal degradation, oxidation due to exposure to light, heat, pH, moisture, and oxygen [150]. Therefore, it is necessary to use techniques to protect sensitive bioactive molecules, in which nanoencapsulation emerges as one of the most widely used branches of nanotechnology to increase bioavailability, solubility, and stability of bioactive compounds. In addition, this technique provides products with high load capacity, controlled sustained release, ease of handling, oxidation protection, and volatile ingredient retention [11, 154, 155].

Generally, the various bioactive encapsulation techniques are developed in three steps: (1) entrapment of the material to be encapsulated; (2) maintenance of trapped material; (3) entry of unwanted materials prevention. In summary, the function of incorporating active ingredients into nanoparticles is to protect the compound that may be exhibiting unstable behavior in the presence of other food components [152, 156].

Several works in the scientific literature address various types of particles obtained by different nanoencapsulation techniques: nanostructures, such as nanospheres (NSs), nanocapsules (NCs), solid lipid nanoparticles (SLNs), cyclodextrins (CDs), liposomes (LSs), and micelles (MCs) [157]. These nanoparticles have been obtained by numerous techniques such as self-assembly, high-pressure homogenization, nanoemulsification, nanocomplexity, nanoprecipitation, coacervation, lipid nanoencapsulation, supercritical fluids, etc. [158–161].

Among these, the techniques for obtaining nanoparticles by supercritical CO_2 are being increasingly exploited due to the increasing interest in clean technologies and the inherent characteristics of the supercritical process, such as rapid mass transfer, nontoxicity, low processing temperature, and production of uniform and small particles with controllable morphology [162].

5.1.1 Techniques of nanoencapsulation using supercritical CO_2

Various techniques for obtaining nanoparticles have used supercritical CO_2 (SC-CO_2). According to Temelli [163], they can be classified according to the CO_2 role in the

process, as follows: SC-CO_2 as solvent; SC-CO_2 as cosolvent; SC-CO_2 as antisolvent; and SC-CO_2 as solute.

In processes in which SC-CO_2 is used as a solvent, the solutes contained in the plant matrix are solubilized in the SC-CO_2, and then they are separated by precipitation after leaving the system by depressurization [9].

The use of SC-CO_2 as a cosolvent means that it is not used as the main solvent but as an assistant to facilitate the solute solubilization [164].

In techniques that use SC-CO_2 as an antisolvent, solute is extracted with an organic solvent, in which SC-CO_2 solubilizes the organic solvent, thus forming an expanded liquid (SC-CO_2 + organic solvent). The formation of this expanded liquid makes the organic solvent immiscible in the solute, and SC-CO_2 drags the solvent out of the system and solute is precipitated [165]. The main processes that use SC-CO_2 as an antisolvent are: ASES (aerosol solvent extraction system), and SFEE.

In processes that use SC-CO_2 as a solute, SC-CO_2 solubilization occurs in a liquid solution (usually lipids), which is subsequently depressurized. This results in a significant reduction in melting point, viscosity, and interfacial tension of the solution (SC-CO_2 + lipids), which facilitates its pulverization [166].

The methods that use SC-CO_2 for the formation of nanoparticles can also be classified according to the techniques of SC-CO_2 assisted drying, which can be: CO_2 for drying, CO_2 for atomization, use of gas-expanded liquids, and aerogels. Among the processes most addressed in the technical-scientific literature, the CPF (concentrated powder form) stands out over the processes for SC-CO_2 for drying.

Importantly, among all these techniques, SFEE has been the subject of many studies involving plant bioactive compounds for the formation of nanoparticles [12, 167, 168].

Supercritical fluid extraction of emulsions

SFEE consists of producing nanoparticles through nanoemulsions. By definition, nanoemulsions are heterogeneous arrangements in which one liquid (the inner phase) is dissolved into another (the outer phase), producing uniformly small sized nanoparticles (100–1000 nm) [169–171].

Nanoparticles can be named nanocapsules or nanospheres. Nanocapsules are systems in which the active principle is confined in the particle nucleus, surrounded by a polymeric membrane, while in nanospheres, the active principle is on the polymeric surface. Both are encapsulated nanoparticles [172, 173].

Nanoemulsions are usually transparent, in which turbidity is influenced by particle size. These systems have particular characteristics, such as low viscosity, high kinetic stability, and large surface area, which make nanoemulsions attractive for food, cosmetic, and drug applications [174, 175]. However, their physicochemical properties are influenced by the qualitative and quantitative composition of bioactives, and by the preparation conditions. Studies aiming at system stability and bioavailability are necessary [176].

In formulations of emulsions applied in SFEE, a biopolymer, an organic solvent, an emulsifier, water, and the bioactive compound are used [177]. The use of emulsifiers in SFEE is of fundamental importance due to the thermodynamic instability of nanoemulsions [149, 178]. Importantly, despite being thermodynamically unstable, some studies describe that nanoemulsions may reduce toxicity, increase therapeutic activity and bioavailability, and, in some cases, allow controlled or targeted release of incorporated bioactives [179].

The SFEE technique involves five steps: (1) Preparation of the starting emulsion; (2) Addition of the starting emulsion to the reactor (closed system); (3) Addition of CO_2 and adjustment of temperature and pressure; (4) Outlet valve opening and flow adjustment (open system); (5) Slow system depressurization and recovery of the nanoemulsions (suspension of nanoparticles in water) [180].

In preparation of the starting emulsion (usually oil in water), the organic phase (organic solvent, emulsifier, and bioactive compound) is dissolved in the aqueous phase (water and biopolymer) by stirring and with/without the use of ultrasound. [12, 167]. This emulsion is added to the reactor, the valves are closed, and CO_2 is added. The system is subjected to a certain temperature and pressure according to the conditions of thermodynamic equilibrium between SC-CO_2 and the organic solvent used. After stabilization, the system is opened, and the flow of CO_2 + organic solvent is adjusted. After the end of the process, the system is cooled and depressurized, and solvent-free nanoemulsion is recovered [181].

SFEE applications in the food sector have focused on production of nanoparticles with bioactive compounds, such as natural carotenoids, including astaxanthin [182], lutein [144], β-carotene, and lycopene [183], as well as vitamins such as vitamin E [12].

Nanoemulsions can be used in liquid, solid, or powder forms. The use of encapsulated dry extracts for dietary supplement application has been the subject of several studies [38, 39, 184]. For this, it is necessary to use dehydration techniques after emulsification, such as lyophilization or spray-drying, which are widely used to obtain powder nanoemulsions [185].

Among the advantages of this technology, the versatility stands out because encapsulating hydrophilic and lipophilic compounds is possible [186, 187]. In addition, SFEE is able to eliminate organic solvents due to the high solubility of organic molecules in supercritical fluid, rapid mass transfer, and moderate temperature and pressure [136, 188]. Therefore, this method represents a promising technique for encapsulating bioactive compounds for use in dietary supplements.

6. Conclusion

The use of supercritical technology for obtaining and encapsulating plant extracts demonstrates the concern with the environment, and with the quality of bioactives obtained,

thus increasing their applicability and the sustainability of food and pharmaceutical industries.

The study and application of supercritical CO_2 in the dietary supplements production chain reveals a new proposal that highlights the importance of using supercritical technology in the development of a high-quality product, which presents the stability that bioactive compounds, which are attributed to the therapeutic potentials of plant extracts, need. This verticalizes and contributes to the development of new food and pharmaceutical products, with a great return to society.

References

[1] Council for Responsible Nutrition, Dietary supplement use more popular than ever: CRN study, CRN Sci. Behind Suppl. (2019). https://www.nutraingredients-usa.com/Article/2019/09/30/Dietary-supplement-use-more-popular-than-ever-CRN-study. Accessed 21 November 2019.

[2] B. Gurley, Dietary Supplement, Encycl. Br. Inc., 2019. https://academic-eb-britannica.ez3.periodicos.capes.gov.br/levels/collegiate/article/dietary-supplement/601294

[3] Parlamento Europeu e do Conselho da União Européia, Directiva 2002/46/CE do Parlamento Europeu e do Conselho de 10 de Junho de 2002 relativa à aproximação das legislações dos Estados-Membros respeitantes aos suplementos alimentares, J. Comunidades Eur. 183 (2002) 51–57.

[4] F.C.S. Pires, A.P.D.S.E. Silva, M.D.L.A.R. Salazar, W.A.D. Costa, H.S.C.D. Costa, A. S. Lopes, H. Rogez, R.N.D. Carvalho Junior, Determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction. J. Supercrit. Fluids 146 (2019) 128–135, https://doi.org/10.1016/j.supflu.2019.01.014.

[5] D.B. Rodrigues, L.R.B. Mariutti, A.Z. Mercadante, Two-step cleanup procedure for the identification of carotenoid esters by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. J. Chromatogr. A 1457 (2016) 116–124, https://doi.org/10.1016/j.chroma.2016.06.039.

[6] R.H.H. Pinto, C. Sena, O.V. Santos, W.A. Da Costa, A.M.C. Rodrigues, R.N.C. Junior, Extraction of bacaba (*Oenocarpus bacaba*) oil with supercritical CO_2: global yield isotherms, fatty acid composition, functional quality, oxidative stability, spectroscopic profile and antioxidant activity. Extracción de aceite de bacaba (*Oenocarpus bacaba*, Grasas y Aceites) 69 (2018) 1–8, https://doi.org/10.3989/gya.0883171.

[7] C.E. Aruwa, S. Amoo, T. Kudanga, Phenolic compound profile and biological activities of southern African *Opuntia ficus-indica* fruit pulp and peels. LWT 111 (2019) 337–344, https://doi.org/10.1016/j.lwt.2019.05.028.

[8] E.O. Faboro, L. Wei, S. Liang, A.G. McDonald, C.A. Obafemi, Characterization of dichloromethane and methanol extracts from the leaves of a medicinal plant: *Globimetula oreophila*. Ind. Crop. Prod. 83 (2016) 391–399, https://doi.org/10.1016/j.indcrop.2016.01.008.

[9] C.G. Pereira, M.A.A. Meireles, Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol. 3 (2010) 340–372, https://doi.org/10.1007/s11947-009-0263-2.

[10] M. Herrero, M. Castro-Puyana, J.A. Mendiola, E. Ibañez, Compressed fluids for the extraction of bioactive compounds. Trends Anal. Chem. 43 (2013) 67–83, https://doi.org/10.1016/j.trac.2012.12.008.

[11] P.N. Ezhilarasi, D. Indrani, B.S. Jena, C. Anandharamakrishnan, Freeze drying technique for microencapsulation of Garcinia fruit extract and its effect on bread quality. J. Food Eng. 117 (2013) 513–520, https://doi.org/10.1016/j.jfoodeng.2013.01.009.

[12] C. Prieto, L. Calvo, Supercritical fluid extraction of emulsions to nanoencapsulate vitamin E in polycaprolactone. J. Supercrit. Fluids 119 (2017) 274–282, https://doi.org/10.1016/j.supflu.2016.10.004.

[13] Food and Drug Administration, Dietary Supplement Products & Ingredients, FDA Food Drug Adm, https://www.fda.gov/food/dietary-supplements/dietary-supplement-products-ingredients, 2019. Accessed 21 November 2019.
[14] FDA, Guidance for Industry: Questions and Answers Regarding Adverse Event Reporting and Recordkeeping for Dietary Supplements as Required by the Dietary Supplement and Nonprescription Drug Consumer Protection Act, Food Drug Adm. (2014) pp. 1–9
[15] M. Heinrich, Nutraceutical, Encycl. Br. Inc, 2019. https://academic-eb-britannica.ez3.periodicos.capes.gov.br/levels/collegiate/article/nutraceutical/601293.
[16] R.L. Bailey, J.J. Gahche, P.E. Miller, P.R. Thomas, J.T. Dwyer, Why US adults use dietary supplements. JAMA Intern. Med. 173 (2013) 355–361, https://doi.org/10.1001/jamainternmed.2013.2299.
[17] N. Trent, Global Dietary Supplement Market—2019-2026, https://www.wiseguyreports.com/sample-request/3792103-global-dietary-supplement-market-2019-2026, 2019.
[18] IADSA (International Alliance of Dietary/Food Supplement Associations), The Evolution of the Health Supplements Sector From the 1990S To the Current Day, © IADSA 2018, London, (2018).
[19] S. Rautiainen, J.E. Manson, A.H. Lichtenstein, H.D. Sesso, Dietary supplements and disease prevention–a global overview. Nat. Rev. Endocrinol. 12 (2016) 407–420, https://doi.org/10.1038/nrendo.2016.54.
[20] Abiad (Associação Brasileira da Indústria de Alimentos Para Fins Especiais e Congêneres), Consumption of Food Supplements in Brazil, http://abiad.org.br/pb/tatiana-pires-explica-em-entrevista-o-consumo-de-suplementos-alimentares-no-brasil/, 2016.
[21] J.T. Dwyer, R.A. Bailen, L.G. Saldanha, J.J. Gahche, R.B. Costello, J.M. Betz, C.D. Davis, R. L. Bailey, N. Potischman, A.G. Ershow, B.C. Sorkin, A.J. Kuszak, L. Rios-Avila, F. Chang, J. Goshorn, K.W. Andrews, P.R. Pehrsson, P.A. Gusev, J.M. Harnly, C.J. Hardy, N. J. Emenaker, K.A. Herrick, The dietary supplement label database: recent developments and applications. J. Nutr. 148 (2018) 1428S–1435S, https://doi.org/10.1093/jn/nxy082.
[22] A.C. Patterson, R.C. Hogg, D.M. Kishi, K.D. Stark, Biomarker and dietary validation of a Canadian food frequency questionnaire to measure Eicosapentaenoic and docosahexaenoic acid intakes from whole food, functional food, and nutraceutical sources. J. Acad. Nutr. Diet. 112 (2012) 1005–1014, https://doi.org/10.1016/j.jand.2012.03.030.
[23] K.B. Arun, J. Chandran, V.V. Venugopal, T.S. Madhavankutty, P. Nisha, Spent cumin seeds generated from ayurvedic industry as a source of bioactive compounds for nutraceutical/functional food applications. J. Food Process. Preserv. 42 (2018) 1–9, https://doi.org/10.1111/jfpp.13392.
[24] P. Nasirullah, R. Kumar, Shariff, development of nutraceutical carriers for functional food applications. Nutr. Food Sci. 41 (2011) 34–43, https://doi.org/10.1108/00346651111102874.
[25] B.Y. Chen, C.H. Kuo, Y.C. Liu, L.Y. Ye, J.H. Chen, C.J. Shieh, Ultrasonic-assisted extraction of the botanical dietary supplement resveratrol and other constituents of *Polygonum cuspidatum*. J. Nat. Prod. 75 (2012) 1810–1813, https://doi.org/10.1021/np300392n.
[26] G. Joana Gil-Chávez, J.A. Villa, J. Fernando Ayala-Zavala, J. Basilio Heredia, D. Sepulveda, E. M. Yahia, G.A. González-Aguilar, Technologies for Extraction and Production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Compr. Rev. Food Sci. Food Saf. 12 (2013) 5–23, https://doi.org/10.1111/1541-4337.12005.
[27] K. Saravanan, S.M. Aradhya, Potential nutraceutical food beverage with antioxidant properties from banana plant bio-waste (pseudostem and rhizome). Food Funct. 2 (2011) 603–610, https://doi.org/10.1039/c1fo10071h.
[28] M. Khalil, J. Raila, M. Ali, K.M.S. Islam, R. Schenk, J.-P. Krause, F.J. Schweigert, H. Rawel, Stability and bioavailability of lutein ester supplements from tagetes flower prepared under food processing conditions. J. Funct. Foods 4 (2012) 602–610, https://doi.org/10.1016/J.JFF.2012.03.006.
[29] J. Haider, H. Majeed, P.A. Williams, W. Safdar, F. Zhong, Formation of chitosan nanoparticles to encapsulate krill oil (*Euphausia superba*) for application as a dietary supplement. Food Hydrocoll. 63 (2017) 27–34, https://doi.org/10.1016/j.foodhyd.2016.08.020.
[30] S. Kanokpanont, R. Yamdech, P. Aramwit, Stability enhancement of mulberry-extracted anthocyanin using alginate/chitosan microencapsulation for food supplement application. Artif. Cells Nanomed. Biotechnol. 46 (2018) 773–782, https://doi.org/10.1080/21691401.2017.1339050.

[31] A.E. Edris, D. Kalemba, J. Adamiec, M. Piaotkowski, Microencapsulation of Nigella sativa oleoresin by spray drying for food and nutraceutical applications. Food Chem. 204 (2016) 326–333, https://doi.org/10.1016/j.foodchem.2016.02.143.
[32] M. Saifullah, Y.A. Yusof, N.L. Chin, M.G. Aziz, M.A.P. Mohammed, N.A. Aziz, Dissolution profiling and its comparison of natural fruit powder effervescent tablets. J. Food Eng. 178 (2016) 60–70, https://doi.org/10.1016/J.JFOODENG.2016.01.007.
[33] B.D. Adiba, B. Salem, S. Nabil, M. Abdelhakim, Preliminary characterization of food tablets from date (*Phoenix dactylifera* L.) and spirulina (*Spirulina sp.*) powders. Powder Technol. 208 (2011) 725–730, https://doi.org/10.1016/j.powtec.2011.01.016.
[34] T. Vyas, R. Prasad, S. Anandjiwala, M. Nivsarkar, Formulation and pharmacokinetic evaluation of hard gelatin capsule encapsulating lyophilized *Vasa swaras* for improved stability and oral bioavailability of vasicine. Fitoterapia 82 (2011) 446–453, https://doi.org/10.1016/j.fitote.2010.12.005.
[35] C.R.G. Lira, F. Zucco, A.N. Negrão, M.A.S. Silva, F.S. Murakami, Nutraceuticals : an overview on safety, quality control and legislation, Rev. Bras. Farmácia. 90 (2009) 45–49.
[36] I.A. Neri-Numa, R.A.S. Sancho, A.P.A. Pereira, G.M. Pastore, Small Brazilian wild fruits: nutrients, bioactive compounds, health-promotion properties and commercial interest. Food Res. Int. 103 (2018) 345–360, https://doi.org/10.1016/j.foodres.2017.10.053.
[37] M. Kaneria, S. Chanda, Evaluation of antioxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves by sequential soxhlet extraction method. Asian Pac. J. Trop. Biomed. 2 (2012) S1526–S1533, https://doi.org/10.1016/S2221-1691(12)60448-1.
[38] R. Ksouri, W.M. Ksouri, I. Jallali, A. Debez, C. Magné, I. Hiroko, C. Abdelly, Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications. Crit. Rev. Biotechnol. 32 (2012) 289–326, https://doi.org/10.3109/07388551.2011.630647.
[39] L. Salvia-Trujillo, D.J. McClements, Improvement of β-carotene bioaccessibility from dietary supplements using excipient Nanoemulsions. J. Agric. Food Chem. 64 (2016) 4639–4647, https://doi.org/10.1021/acs.jafc.6b00804.
[40] C. Yang, M.A.K. Chowdhury, Y. Hou, J. Gong, Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens 4 (2015) 137–156, https://doi.org/10.3390/pathogens4010137.
[41] R.M. Pérez-Gutiérrez, A.M. Ramirez, Hypoglycemic effects of Sesquiterpene lactones from *Byrsonima crassifolia.* Food Sci. Biotechnol. 25 (2016) 1135–1145, https://doi.org/10.1007/s10068-016-0182-8.
[42] R.M. Perez-Gutierrez, A. Muñiz-Ramirez, Y.G. Gomez, E.B. Ramírez, Antihyperglycemic, antihyperlipidemic and antiglycation effects of *Byrsonima crassifolia* fruit and seed in normal and streptozotocin-induced diabetic rats. Plant Foods Hum. Nutr. 65 (2010) 350–357, https://doi.org/10.1007/s11130-010-0181-5.
[43] S. Kang, E. Kim, I. Kang, M. Lee, Y. Lee, Anti-diabetic effects and anti-inflammatory effects of *Laminaria japonica* and *Hizikia fusiforme* in skeletal muscle : in vitro and in vivo model. Nutrients 10 (2018) 1–12, https://doi.org/10.3390/nu10040491.
[44] F. Guilhon-Simplicio, C.C.D.S. Pinheiro, G.G. Conrado, G.D.S. Barbosa, P.A. Dos Santos, M.D. M. Pereira, E.S. Lima, Anti-inflammatory, anti-hyperalgesic, antiplatelet and antiulcer activities of *Byrsonima japurensis* A. Juss. (Malpighiaceae). J. Ethnopharmacol. 140 (2012) 282–286, https://doi.org/10.1016/j.jep.2012.01.018.
[45] J.M. Carbonell-Capella, M. Buniowska, F.J. Barba, M.J. Esteve, A. Frígola, Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: a review. Compr. Rev. Food Sci. Food Saf. 13 (2014) 155–171, https://doi.org/10.1111/1541-4337.12049.
[46] E. Cunningham, Are krill oil supplements a better source of n-3 fatty acids than fish oil supplements?. J. Acad. Nutr. Diet. 112 (2012) 344, https://doi.org/10.1016/j.jand.2011.12.016.
[47] E. Fernández-García, I. Carvajal-Lérida, M. Jarén-Galán, J. Garrido-Fernández, A. Pérez-Gálvez, D. Hornero-Méndez, Carotenoids bioavailability from foods: from plant pigments to efficient biological activities. Food Res. Int. 46 (2012) 438–450, https://doi.org/10.1016/J.FOODRES.2011.06.007.
[48] N.I. Krinsky, E.J. Johnson, Carotenoid actions and their relation to health and disease. Mol. Asp. Med. 26 (2005) 459–516, https://doi.org/10.1016/J.MAM.2005.10.001.

[49] J. Widomska, Why has nature chosen lutein and zeaxanthin to protect the retina?. J. Clin. Exp. Ophthalmol. 05 (2014) 1–22, https://doi.org/10.4172/2155-9570.1000326.
[50] D. Rodriguez-Amaya, M. Kimura, HarvestPlus Handbook for Carotenoid Analysis, first ed., Breeding Crops for Better Nutrition, Washington, 2004.
[51] A. Azqueta, A.R. Collins, Carotenoids and DNA damage. Mutat. Res. Fundam. Mol. Mech. Mutagen. 733 (2012) 4–13, https://doi.org/10.1016/j.mrfmmm.2012.03.005.
[52] Research and Markets, The World's Largest Market Research Store, Carotenoids Market Analysis By Source (Natural, Synthetic), By Product (Beta-Carotene, Lutein, Lycopene, Astaxanthin, Zeaxanthin, Canthaxanthin), By Application (Food, Supplements, Feed, Pharmaceuticals, Cosmetics), And Segment Forecasts, 2014-2025, https://www.researchandmarkets.com/reports/4031963/carotenoids-market-analysis-by-source-natural#relb1-4437244, 2016.
[53] L.R.B. Mariutti, E. Rodrigues, A.Z. Mercadante, Carotenoids from *Byrsonima crassifolia*: identification, quantification and in vitro scavenging capacity against peroxyl radicals. J. Food Compos. Anal. 31 (2013) 155–160, https://doi.org/10.1016/j.jfca.2013.05.005.
[54] E. Rodrigues, L.R.B. Mariutti, A.Z. Mercadante, Carotenoids and phenolic compounds from *Solanum sessilif lorum*, an unexploited amazonian fruit, and their scavenging capacities against reactive oxygen and nitrogen species. J. Agric. Food Chem. 61 (2013) 3022–3029, https://doi.org/10.1021/jf3054214.
[55] E. Rodrigues, L.R.B. Mariutti, A.F. Faria, A.Z. Mercadante, Microcapsules containing antioxidant molecules as scavengers of reactive oxygen and nitrogen species. Food Chem. 134 (2012) 704–711, https://doi.org/10.1016/j.foodchem.2012.02.163.
[56] E. Rodrigues, L.R.B. Mariutti, R.C. Chisté, A.Z. Mercadante, Development of a novel micro-assay for evaluation of peroxyl radical scavenger capacity: application to carotenoids and structure–activity relationship. Food Chem. 135 (2012) 2103–2111, https://doi.org/10.1016/J.FOODCHEM.2012.06.074.
[57] L.R.B. Mariutti, E. Rodrigues, R.C. Chisté, E. Fernandes, A.Z. Mercadante, The amazonian fruit *Byrsonima crassifolia* effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage. Food Res. Int. 64 (2014) 618–625, https://doi.org/10.1016/j.foodres.2014.07.032.
[58] G.J. Handelman, The evolving role of carotenoids in human biochemistry, Nutrition 17 (2001) 818–822.
[59] I.D. Nwachukwu, C.C. Udenigwe, R.E. Aluko, Lutein and zeaxanthin: production technology, bioavailability, mechanisms of action, visual function, and health claim status. Trends Food Sci. Technol. 49 (2016) 74–84, https://doi.org/10.1016/j.tifs.2015.12.005.
[60] C. Lygia, B. Ambrósio, Carotenoids as an alternative against hypovitaminosis A, Rev. Nutr. 19 (2006) 233–243.
[61] A. Kijlstra, Y. Tian, E.R. Kelly, T.T.J.M. Berendschot, Lutein: more than just a filter for blue light. Prog. Retin. Eye Res. 31 (2012) 303–315, https://doi.org/10.1016/j.preteyeres.2012.03.002.
[62] H. Chen, J. Weiss, F. Shahidi, Nanotechnology in nutraceuticals and functional foods, Food Technol. 60 (2006) 30–36.
[63] C. Quan, C. Johan, T. Charlotta, Carotenoids particle formation by supercritical fluid technologies. Chin. J. Chem. Eng. 17 (2009) 344–349, https://doi.org/10.1016/S1004-9541(08)60214-1.
[64] C.D.S. Anselmo, T.D.C. Mendes, T.d.S. Honorio, F.A. do Carmo, L.M. Cabral, V.P. De Sousa, Development and validation of a dissolution test for lutein tablets and evaluation of intestinal permeability. Food Chem. 210 (2016) 63–69, https://doi.org/10.1016/j.foodchem.2016.04.081.
[65] E.F.S.A. (EFSA), Scientific opinion on the re-evaluation of lutein (E 161b) as a food additive. EFSA J. 8 (2010)https://doi.org/10.2903/j.efsa.2010.1678.
[66] R. Ravikrishnan, S. Rusia, G. Ilamurugan, U. Salunkhe, J. Deshpande, J. Shankaranarayanan, M. L. Shankaranarayana, M.G. Soni, Safety assessment of lutein and zeaxanthin (Lutemax^{TM} 2020): subchronic toxicity and mutagenicity studies. Food Chem. Toxicol. 49 (2011) 2841–2848, https://doi.org/10.1016/J.FCT.2011.08.011.
[67] K.B. Harikumar, C.V. Nimita, K.C. Preethi, R. Kuttan, M.L. Shankaranarayana, J. Deshpande, Toxicity profile of lutein and lutein ester isolated from marigold flowers (*Tagetes erecta*). Int. J. Toxicol. 27 (2008) 1–9, https://doi.org/10.1080/10915810701876265.
[68] S. Buscemi, D. Corleo, F. Di Pace, M.L. Petroni, A. Satriano, G. Marchesini, The effect of lutein on eye and extra-eye health. Nutrients 10 (2018) 1–24, https://doi.org/10.3390/nu10091321.

[69] G. Dagnelie, I.S. Zorge, T.M. McDonald, Lutein improves visual function in some patients with retinal degeneration: a pilot study via the internet, Optometry 71 (2000) 147–164.
[70] J.T. Landrum, R.A. Bone, H. Joa, M.D. Kilburn, L.L. Moore, K.E. Sprague, A one year study of the macular pigment: the effect of 140 days of a lutein supplement. Exp. Eye Res. 65 (1997) 57–62, https://doi.org/10.1006/exer.1997.0309.
[71] R. Randhir, Y.T. Lin, K. Shetty, Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors, Asia Pac. J. Clin. Nutr. 13 (2004) 295–307.
[72] V. Cheynier, G. Comte, K.M. Davies, V. Lattanzio, S. Martens, Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiol. Biochem. 72 (2013) 1–20, https://doi.org/10.1016/j.plaphy.2013.05.009.
[73] A. Savran, G. Zengin, A. Aktumsek, A. Mocan, J. Glamoćlija, A. Ćirić, M. Soković, Phenolic compounds and biological effects of edible: *Rumex scutatus* and *Pseudo sempervivum*: potential sources of natural agents with health benefits. Food Funct. 7 (2016) 3252–3262, https://doi.org/10.1039/c6fo00695g.
[74] W. Vermerris, R. Nicholson, Families of phenolic compounds and means of classification. in: R. Nicholson (Ed.), Phenolic Compd. Biochem., Springer, Dordrecht, 2008, pp. 1–34, https://doi.org/10.1007/978-1-4020-5164-7_1.
[75] O.R. Alara, N.H. Abdurahman, C.I. Ukaegbu, N.H. Azhari, *Vernonia cinerea* leaves as the source of phenolic compounds, antioxidants, and anti-diabetic activity using microwave-assisted extraction technique. Ind. Crops Prod. 122 (2018) 533–544, https://doi.org/10.1016/j.indcrop.2018.06.034.
[76] C. Moliner, L. Barros, M.I. Dias, I. Reigada, I.C.F.R. Ferreira, V. López, E. Langa, C. G. Rincón, Viola cornuta and Viola x wittrockiana: phenolic compounds, antioxidant and neuroprotective activities on *Caenorhabditis elegans*. J. Food Drug Anal. 27 (2019) 849–859, https://doi.org/10.1016/j.jfda.2019.05.005.
[77] J.Q. Liu, C.L. Lian, T.Y. Hu, C.F. Wang, Y. Xu, L. Xiao, Z.Q. Liu, S.Q. Qiu, B.H. Cheng, Two new farnesyl phenolic compounds with anti-inflammatory activities from *Ganoderma duripora*. Food Chem. 263 (2018) 155–162, https://doi.org/10.1016/j.foodchem.2018.04.097.
[78] S.N. Lou, C.T. Ho, Phenolic compounds and biological activities of small-size citrus: Kumquat and calamondin. J. Food Drug Anal. 25 (2017) 162–175, https://doi.org/10.1016/j.jfda.2016.10.024.
[79] X.K. Zheng, Y.G. Cao, Y.Y. Ke, Y.L. Zhang, F. Li, J.H. Gong, X. Zhao, H.X. Kuang, W. S. Feng, Phenolic constituents from the root bark of *Morus alba* L. and their cardioprotective activity in vitro. Phytochemistry 135 (2017) 128–134, https://doi.org/10.1016/j.phytochem.2016.12.006.
[80] M. Čulenová, A. Sychrová, S.T.S. Hassan, K. Berchová-Bímová, P. Svobodová, A. Helclová, H. Michnová, J. Hošek, H. Vasilev, P. Suchý, G. Kuzminová, E. Švajdlenka, J. Gajdziok, A. Čížek, V. Suchý, K. Šmejkal, Multiple in vitro biological effects of phenolic compounds from *Morus alba* root bark. J. Ethnopharmacol. 248 (2020) 1–12, https://doi.org/10.1016/j.jep.2019.112296.
[81] A. Golonko, T. Pienkowski, R. Swislocka, R. Lazny, M. Roszko, W. Lewandowski, Another look at phenolic compounds in cancer therapy the effect of polyphenols on ubiquitin-proteasome system. Eur. J. Med. Chem. 167 (2019) 291–311, https://doi.org/10.1016/j.ejmech.2019.01.044.
[82] P.I. Oteiza, C.G. Fraga, D.A. Mills, D.H. Taft, Flavonoids and the gastrointestinal tract: local and systemic effects. Mol. Aspects Med. 61 (2018) 41–49, https://doi.org/10.1016/j.mam.2018.01.001.
[83] Research and Markets, The World's Largest Market Research Store, Flavonoids in Dietary Supplements Market: By form; By Flavonoid Class; By Application & By Geography—Forecast 2017-2022, https://www.researchandmarkets.com/reports/4532576/flavonoids-in-dietary-supplements-market-by#pos-1, 2018.
[84] Research and Markets, The World's Largest Market Research Store, Flavonoids Market Size & Trend Analysis By Product, By Application, By Region and Segment Forecasts, 2014-2025, https://www.researchandmarkets.com/reports/4031966/flavonoids-market-size-and-trend-analysis-by#pos-0, 2016.
[85] J.A. Ross, C.M. Kasum, Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22 (2002) 19–34, https://doi.org/10.1146/annurev.nutr.22.111401.144957.
[86] Research and Markets, The World's Largest Market Research Store, Global Quercetin—Procurement Market Intelligence Report, https://www.researchandmarkets.com/reports/4613139/global-quercetin-procurement-market#pos-0, 2018.

[87] B. Fernández-Rojas, G. Gutiérrez-Venegas, Flavonoids exert multiple periodontic benefits including anti-inflammatory, periodontal ligament-supporting, and alveolar bone-preserving effects. Life Sci. 209 (2018) 435–454, https://doi.org/10.1016/j.lfs.2018.08.029.
[88] G.J. Shi, Y. Li, Q.H. Cao, H.X. Wu, X.Y. Tang, X.H. Gao, J.Q. Yu, Z. Chen, Y. Yang, In vitro and in vivo evidence that quercetin protects against diabetes and its complications: a systematic review of the literature. Biomed. Pharmacother. 109 (2019) 1085–1099, https://doi.org/10.1016/j.biopha.2018.10.130.
[89] T. Mahesh, V.P. Menon, Quercetin allievates oxidative stress in streptozotocin-induced diabetic rats. Phyther. Res. 18 (2004) 123–127, https://doi.org/10.1002/ptr.1374.
[90] J.H. Kim, M.J. Kang, H.N. Choi, S.M. Jeong, Y.M. Lee, J.I. Kim, Quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. Nutr. Res. Pract. 5 (2011) 107–111, https://doi.org/10.4162/nrp.2011.5.2.107.
[91] P. Srinivasan, S. Vijayakumar, S. Kothandaraman, M. Palani, Anti-diabetic activity of quercetin extracted from *Phyllanthus emblica* L. fruit: in silico and in vivo approaches. J. Pharm. Anal. 8 (2018) 109–118, https://doi.org/10.1016/j.jpha.2017.10.005.
[92] A.M. Lottenberg, Importance of the dietary fat on the prevention and control of metabolic disturbances and cardiovascular disease, Arq. Bras. Endocrinol. Metab. 53 (2009) 595–607.
[93] A. Paula de Souza e Silva, W. Almeida da Costa, M. de Los Angeles Rodriguez Salazar, P. do Nascimento Bezerra, F. Cristina Seabra Pires, M. Caroline Rodrigues Ferreira, E. Gama Ortiz Menezes, G. Rafael Olivo Urbina, J. Rodrigues Barbosa, d.C.R. Nunes, Commercial and therapeutic potential of plant-based fatty acids. in: Biochemistry and Health Benefits of Fatty Acids, IntechOpen, 2018, https://doi.org/10.5772/intechopen.81122.
[94] I.S. Newton, Long chain fatty acids in health and nutrition. J. Food Lipids. 3 (1996) 233–249, https://doi.org/10.1111/j.1745-4522.1996.tb00071.x.
[95] C.V.M.B. Pimentel, V.M. Francki, A.P.B. Gollücke, Alimentos Funcionais: Introdução as Principais Substâncias Bioativas em Alimentos. first ed., (2005), https://doi.org/10.1016/j.bbapap.2017.07.007Varela, São Paulo.
[96] R.C. Gaspar, C.B. Veiga, M.P. Bessi, M.N. Dátilo, M.R. Sant'Ana, P.B. Rodrigues, L.P. de Moura, A.S.R. da Silva, G.A. Santos, R.R. Catharino, E.R. Ropelle, J.R. Pauli, D.E. Cintra, Unsaturated fatty acids from flaxseed oil and exercise modulate GPR120 but not GPR40 in the liver of obese mice: a new anti-inflammatory approach. J. Nutr. Biochem. 66 (2019) 52–62, https://doi.org/10.1016/j.jnutbio.2018.12.003.
[97] H. Jang, K. Park, Omega-3 and omega-6 polyunsaturated fatty acids and metabolic syndrome: a systematic review and meta-analysis. Clin. Nutr. (2019) 1–9, https://doi.org/10.1016/j.clnu.2019.03.032.
[98] N. Li, Y. Qiu, Y. Wu, M. Zhang, Z. Lai, Q. Wang, Y. Du, L. Guo, S. Liu, Z. Li, Association of serum total fatty acids with type 2 diabetes. Clin. Chim. Acta. 23 (2019) 1–10, https://doi.org/10.1016/j.cca.2019.09.018.
[99] H. Nayeri, G.A. Naderi, S. Asgari, M. Sadeghi, M. Boshtam, S. Mohamadzadeh, N. Babaknejad, LDL fatty acids composition as a risk biomarker of cardiovascular disease. Artery Res. 20 (2017) 1–7, https://doi.org/10.1016/j.artres.2017.08.001.
[100] S.A. Khatib, E.L. Rossi, L.W. Bowers, S.D. Hursting, Reducing the burden of obesity-associated cancers with anti-inflammatory long-chain omega-3 polyunsaturated fatty acids. Prostaglandins Other Lipid Mediat. 125 (2016) 100–107, https://doi.org/10.1016/j.prostaglandins.2016.07.011.
[101] V. Gasperi, C. Vangapandu, I. Savini, G. Ventimiglia, G. Adorno, M.V. Catani, Polyunsaturated fatty acids modulate the delivery of platelet microvesicle-derived microRNAs into human breast cancer cell lines. J. Nutr. Biochem. 74 (2019) 1–11, https://doi.org/10.1016/j.jnutbio.2019.108242.
[102] C.E. Roynette, P.C. Calder, Y.M. Dupertuis, C. Pichard, n-3 polyunsaturated fatty acids and colon cancer prevention. Clin. Nutr. 23 (2004) 139–151, https://doi.org/10.1016/j.clnu.2003.07.005.
[103] C.D. Williams, B.M. Whitley, C. Hoyo, D.J. Grant, J.D. Iraggi, K.A. Newman, L. Gerber, L. A. Taylor, M.G. Mckeever, S.J. Freedland, A high ratio of dietary n-6/n-3 polyunsaturated fatty acids is associated with increased risk of prostate cancer. Nutr. Res. 31 (2011) 1–8, https://doi.org/10.1016/j.nutres.2011.01.002.

[104] L. Arbabi, M.T.H. Baharuldin, M.A.M. Moklas, S. Fakurazi, S.I. Muhammad, Antidepressant-like effects of omega-3 fatty acids in postpartum model of depression in rats. Behav. Brain Res. 271 (2014) 65–71, https://doi.org/10.1016/j.bbr.2014.05.036.
[105] L. Yan, Y. Xie, S.K. Satyanarayanan, H. Zeng, Q. Liu, M. Huang, Y. Ma, J.-B. Wan, X. Yao, K.-P. Su, H. Su, Omega-3 polyunsaturated fatty acids promote brain-to-blood clearance of β-Amyloid in a mouse model with Alzheimer's disease, Brain. Behav. Immun. (2019) 1–11.
[106] J. Dyerberg, P. Madsen, J.M. Møller, I. Aardestrup, E.B. Schmidt, Bioavailability of marine n-3 fatty acid formulations. Prostaglandins Leukot. Essent. Fat. Acids. 83 (2010) 137–141, https://doi.org/10.1016/j.plefa.2010.06.007.
[107] M.H. Davidson, D. Kling, K.C. Maki, Novel developments in omega-3 fatty acid-based strategies. Curr. Opin. Lipidol. 22 (2011) 437–444, https://doi.org/10.1097/MOL.0b013e32834bd642.
[108] Research and Markets, The World's Largest Market Research Store, Global Fatty Acid Supplements Market 2017-2021, https://www.researchandmarkets.com/reports/4420436/global-fatty-acid-supplements-market-2017-2021, 2017.
[109] F.A. Espinosa-Pardo, V.M. Nakajima, G.A. Macedo, J.A. Macedo, J. Martínez, Extraction of phenolic compounds from dry and fermented orange pomace using supercritical CO_2 and cosolvents. Food Bioprod. Process. 101 (2017) 1–10, https://doi.org/10.1016/j.fbp.2016.10.002.
[110] C.M. Marinho, C.O.T. Lemos, S. Arvelos, M.A.S. Barrozo, C.E. Hori, E.O. Watanabe, Extraction of corn germ oil with supercritical CO_2 and cosolvents. J. Food Sci. Technol. 56 (2019) 4448–4456, https://doi.org/10.1007/s13197-019-03923-2.
[111] N.A. Yahya, N. Attan, R.A. Wahab, An overview of cosmeceutically relevant plant extracts and strategies for extraction of plant-based bioactive compounds. Food Bioprod. Process. 112 (2018) 69–85, https://doi.org/10.1016/j.fbp.2018.09.002.
[112] M. Devgan, A. Nanda, S.H. Ansari, Comparative evaluation of the anti-diabetic activity of *Pterocarpus marsupium* Roxb. heartwood in alloxan induced diabetic rats using extracts obtained by optimized conventional and non conventional extraction methods, Pak. J. Pharm. Sci. 26 (2013) 973–976.
[113] M.A.R. Salazar, J.V. Costa, G.R.O. Urbina, V.M.B. Cunha, M.P. Silva, P.d.N. Bezerra, W.B. S. Pinheiro, W. Gomes-Leal, A.S. Lopes, R.N. Carvalho Junior, Chemical composition, antioxidant activity, neuroprotective and anti-inflammatory effects of cipó-pucá (*Cissus sicyoides* L.) extracts obtained from supercritical extraction. J. Supercrit. Fluids 138 (2018) 36–45, https://doi.org/10.1016/j.supflu.2018.03.022.
[114] F. Pohl, M. Goua, G. Bermano, W.R. Russell, L. Scobbie, P. Maciel, P. Kong Thoo Lin, Revalorisation of rapeseed pomace extracts: an in vitro study into its anti-oxidant and DNA protective properties. Food Chem. 239 (2018) 323–332, https://doi.org/10.1016/j.foodchem.2017.06.129.
[115] M.D. Luque de Castro, F. Priego-Capote, Soxhlet extraction: past and present panacea. J. Chromatogr. A. 1217 (2010) 2383–2389, https://doi.org/10.1016/j.chroma.2009.11.027.
[116] F. Soxhlet, Die gewichtsanalytische Bestimmung des Milchfettes, Dinglers Polytech. J. 232 (1879) 461–465.
[117] S.A. Moreira, E.M.C. Alexandre, M. Pintado, J.A. Saraiva, Effect of emergent non-thermal extraction technologies on bioactive individual compounds profile from different plant materials. Food Res. Int. 115 (2019) 177–190, https://doi.org/10.1016/j.foodres.2018.08.046.
[118] K.A. Santos, E.J. Klein, C. da Silva, E.A. da Silva, L. Cardozo-Filho, Extraction of vetiver (*Chrysopogon zizanioides*) root oil by supercritical CO_2, pressurized-liquid, and ultrasound-assisted methods and modeling of supercritical extraction kinetics. J. Supercrit. Fluids 150 (2019) 30–39, https://doi.org/10.1016/j.supflu.2019.04.005.
[119] J.A. Arboleda Meija, G.P. Parpinello, A. Versari, C. Conidi, A. Cassano, Microwave-assisted extraction and membrane-based separation of biophenols from red wine lees. Food Bioprod. Process. 117 (2019) 74–83, https://doi.org/10.1016/j.fbp.2019.06.020.
[120] K.J. Shinwari, P.S. Rao, Thermal-assisted high hydrostatic pressure extraction of nutraceuticals from saffron (*Crocus sativus*): process optimization and cytotoxicity evaluation against cancer cells. Innov. Food Sci. Emerg. Technol. 48 (2018) 296–303, https://doi.org/10.1016/j.ifset.2018.07.003.
[121] S.F. Han, W. Jin, Q. Yang, A. El-Fatah Abomohra, X. Zhou, R. Tu, C. Chen, G.J. Xie, Q. Wang, Application of pulse electric field pretreatment for enhancing lipid extraction from *Chlorella*

pyrenoidosa grown in wastewater. Renew. Energy 133 (2019) 233–239, https://doi.org/10.1016/j.renene.2018.10.034.
[122] J. Zhang, C. Wen, H. Zhang, Y. Duan, H. Ma, Recent advances in the extraction of bioactive compounds with subcritical water: a review. Trends Food Sci. Technol. (2019) 1–58, https://doi.org/10.1016/j.tifs.2019.11.018.
[123] S. Roohinejad, M. Koubaa, F.J. Barba, R. Greiner, V. Orlien, N.I. Lebovka, Negative pressure cavitation extraction: a novel method for extraction of food bioactive compounds from plant materials. Trends Food Sci. Technol. 52 (2016) 98–108, https://doi.org/10.1016/j.tifs.2016.04.005.
[124] R. Mogwasi, D.K. Kariuki, M.Z. Getenga, V. Nischwitz, Comparison of aqueous and enzymatic extraction combination with sequential filtration for the profiling of selected trace elements in medicinal plants from Kenya. J. Trace Elem. Med. Biol. 54 (2019) 1–7, https://doi.org/10.1016/j.jtemb.2019.03.003.
[125] R.N. Cavalcanti, M.A.A. Meireles, Fundamentals of supercritical fluid extraction. in: Compr. Sampl. Sample Prep., second ed., Academic Press, Ontário, 2012, pp. 117–133, https://doi.org/10.1016/B978-0-12-381373-2.00039-9.
[126] G. Brunner, Applications of supercritical fluids. Annu. Rev. Chem. Biomol. Eng. 1 (2010) 321–342, https://doi.org/10.1146/annurev-chembioeng-073009-101311.
[127] M. Roodpeyma, S.E. Guigard, W.H. Stiver, Pressure control of a continuous pilot scale supercritical fluid extraction (SFE) process. J. Supercrit. Fluids 135 (2018) 120–129, https://doi.org/10.1016/j.supflu.2017.12.029.
[128] N. Hassim, M. Markom, M.I. Rosli, S. Harun, Scale-up criteria and economic analysis for supercritical fluid extraction of *Phyllanthus niruri*. Chem. Eng. Process. Process Intensif. 139 (2019) 14–22, https://doi.org/10.1016/j.cep.2019.03.011.
[129] A. Mouslim, S. Tavoularis, Direct tests of fluid-to-fluid scaling expressions for supercritical heat transfer in tubes, Int. J. Heat Mass Transf. (2019) 1–9.
[130] K.Y. Khaw, M.O. Parat, P.N. Shaw, J.R. Falconer, Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: a review. Molecules 22 (2017) 1–22, https://doi.org/10.3390/molecules22071186.
[131] G. Brunner, Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Process, first ed., Steinkopff, New York, 1994.
[132] M. Herrero, A. Cifuentes, E. Ibañez, Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae—a review. Food Chem. 98 (2006) 136–148, https://doi.org/10.1016/j.foodchem.2005.05.058.
[133] G. Brunner, Supercritical fluids: technology and application to food processing. J. Food Eng. 67 (2005) 21–33, https://doi.org/10.1016/j.jfoodeng.2004.05.060.
[134] I. Goodarznia, M.H. Eikani, Supercritical carbon dioxide extraction of essential oils: modeling and simulation. Chem. Eng. Sci. 53 (1998) 1387–1395, https://doi.org/10.1016/S0009-2509(97)90445-0.
[135] R. Marr, T. Gamse, Use of supercritical fluids for different processes including new developments—a review. Chem. Eng. Process. Process Intensif. 39 (2000) 19–28, https://doi.org/10.1016/S0255-2701(99)00070-7.
[136] M.J. Cocero, Á. Martín, F. Mattea, S. Varona, Encapsulation and co-precipitation processes with supercritical fluids: fundamentals and applications. J. Supercrit. Fluids 47 (2009) 546–555, https://doi.org/10.1016/j.supflu.2008.08.015.
[137] S.L. Wells, J. Desimone, CO_2 technology platform: an important tool for environmental problem solving. Angew. Chemie. Int. Ed. 40 (2001) 518–527, https://doi.org/10.1002/1521-3773(20010202)40:3<518::AID-ANIE518>3.0.CO;2-4.
[138] M. McHugh, V. Krukonis, Supercritical Fluid Extraction: Principles and Practice, second ed., (2013) Boston.
[139] V.M.B. Cunha, M.P. da Silva, S.H.B. de Sousa, P.d.N. Bezerra, E.G.O. Menezes, N.J.N. da Silva, D. A.D.d.S. Banna, M.E. Araújo, R.N. de Carvalho Júnior, Bacaba-de-leque (*Oenocarpus distichus* Mart.) oil extraction using supercritical CO_2 and bioactive compounds determination in the residual pulp. J. Supercrit. Fluids 144 (2019) 81–90, https://doi.org/10.1016/j.supflu.2018.10.010.

[140] A.A. Salvador, R. Podestá, J.M. Block, S.R.S. Ferreira, Increasing the value of pecan nut [*Carya illinoinensis* (Wangenh) C. Koch] cake by means of oil extraction and antioxidant activity evaluation. J. Supercrit. Fluids 116 (2016) 215–222, https://doi.org/10.1016/j.supflu.2016.05.046.
[141] C.E. Handford, M. Dean, M. Henchion, M. Spence, C.T. Elliott, K. Campbell, Implications of nanotechnology for the agri-food industry: opportunities, benefits and risks. Trends Food Sci. Technol. 40 (2014) 226–241, https://doi.org/10.1016/j.tifs.2014.09.007.
[142] E. Perez-Esteve, A. Bernardos, R. Martinez-Manez, J.M. Barat, Nanotechnology in the development of novel functional foods or their package. an overview based in patent analysis. Recent Pat. Food. Nutr. Agric. 5 (2013) 35–43, https://doi.org/10.2174/2212798411305010006.
[143] EFSA Scientific Committee, Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA J. 9 (2011) 1–95, https://doi.org/10.2903/j.efsa.2011.2140.
[144] B. Hu, X. Liu, C. Zhang, X. Zeng, Food macromolecule based nanodelivery systems for enhancing the bioavailability of polyphenols. J. Food Drug Anal. 25 (2017) 3–15, https://doi.org/10.1016/j.jfda.2016.11.004.
[145] S. Khorasani, M. Danaei, M.R. Mozafari, Nanoliposome technology for the food and nutraceutical industries. Trends Food Sci. Technol. 79 (2018) 106–115, https://doi.org/10.1016/j.tifs.2018.07.009.
[146] H.D. Silva, M.Â. Cerqueira, A.A. Vicente, Nanoemulsions for food applications: development and characterization. Food Bioprocess Technol. 5 (2012) 854–867, https://doi.org/10.1007/s11947-011-0683-7.
[147] C. Enrico, Nanotechnology-based drug delivery of natural compounds and phytochemicals for the treatment of cancer and other diseases. Stud. Nat. Prod. Chem. (2019) 91–123, https://doi.org/10.1016/B978-0-444-64185-4.00003-4.
[148] M. Henchion, M. McCarthy, E.J. Dillon, G. Greehy, S.N. McCarthy, Big issues for a small technology: consumer trade-offs in acceptance of nanotechnology in food. Innov. Food Sci. Emerg. Technol. 58 (2019) 1–9, https://doi.org/10.1016/j.ifset.2019.102210.
[149] D.J. McClements, J. Rao, Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit. Rev. Food Sci. Nutr. 51 (2011) 285–330, https://doi.org/10.1080/10408398.2011.559558.
[150] X. Li, Y. Liu, Y. Yu, W. Chen, Y. Liu, H. Yu, Nanoformulations of quercetin and cellulose nanofibers as healthcare supplements with sustained antioxidant activity. Carbohydr. Polym. 207 (2019) 160–168, https://doi.org/10.1016/j.carbpol.2018.11.084.
[151] The Royal Society, Nanoscience and nanotechnologies: opportunities and uncertainties. R. Soc. R. Acad. Eng. (2004) 127, https://doi.org/10.1007/s00234-004-1255-6.
[152] M.R. Mozafari, K. Khosravi-Darani, G.G. Borazan, J. Cui, A. Pardakhty, S. Yurdugul, Encapsulation of food ingredients using nanoliposome technology. Int. J. Food Prop. 11 (2008) 833–844, https://doi.org/10.1080/10942910701648115.
[153] A. Sorrentino, G. Gorrasi, V. Vittoria, Potential perspectives of bio-nanocomposites for food packaging applications. Trends Food Sci. Technol. 18 (2007) 84–95, https://doi.org/10.1016/J.TIFS.2006.09.004.
[154] Q. Huang, H. Yu, Q. Ru, Bioavailability and delivery of nutraceuticals using nanotechnology. J. Food Sci. 75 (2010) 50–57, https://doi.org/10.1111/j.1750-3841.2009.01457.x.
[155] D.J. McClements, E.A. Decker, Y. Park, Controlling lipid bioavailability through physicochemical and structural approaches. Crit. Rev. Food Sci. Nutr. 49 (2009) 48–67, https://doi.org/10.1080/10408390701764245.
[156] B.F. Gibbs, S. Kermasha, I. Alli, C.N. Mulligan, Encapsulation in the food industry: a review. Int. J. Food Sci. Nutr. 50 (1999) 213–224, https://doi.org/10.1080/096374899101256.
[157] T. Squillaro, A. Cimini, G. Peluso, A. Giordano, M.A.B. Melone, Nano-delivery systems for encapsulation of dietary polyphenols: an experimental approach for neurodegenerative diseases and brain tumors. Biochem. Pharmacol. 154 (2018) 303–317, https://doi.org/10.1016/j.bcp.2018.05.016.
[158] D.A. Campos, A.R. Madureira, B. Sarmento, M.M. Pintado, A.M. Gomes, Technological stability of solid lipid nanoparticles loaded with phenolic compounds: drying process and stability along storage. J. Food Eng. 196 (2017) 1–10, https://doi.org/10.1016/J.JFOODENG.2016.10.009.

[159] F.J. Gutiérrez, S.M. Albillos, E. Casas-Sanz, Z. Cruz, C. García-Estrada, A. García-Guerra, J. García-Reverter, M. García-Suárez, P. Gatón, C. González-Ferrero, I. Olabarrieta, M. Olasagasti, S. Rainieri, D. Rivera-Patiño, R. Rojo, A. Romo-Hualde, M.J. Sáiz-Abajo, M.L. Mussons, Methods for the nanoencapsulation of β-carotene in the food sector. Trends Food Sci. Technol. 32 (2013) 73–83, https://doi.org/10.1016/j.tifs.2013.05.007.
[160] P. Anand, H.B. Nair, B. Sung, A.B. Kunnumakkara, V.R. Yadav, R.R. Tekmal, B. B. Aggarwal, Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo. Biochem. Pharmacol. 79 (2010) 330–338, https://doi.org/10.1016/j.bcp.2009.09.003.
[161] A.H. Saberi, Y. Fang, D.J. McClements, Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. J. Colloid Interface Sci. 391 (2013) 95–102, https://doi.org/10.1016/j.jcis.2012.08.069.
[162] M. Gönen, R.B. Gupta, Developments in the encapsulation of bioactives using supercritical CO_2. in: Ref. Modul. Food Sci., Elsevier Inc., Richmond, VA, 2019, pp. 1–8, https://doi.org/10.1016/b978-0-08-100596-5.22674-7
[163] F. Temelli, Perspectives on the use of supercritical particle formation technologies for food ingredients. J. Supercrit. Fluids 134 (2018) 244–251, https://doi.org/10.1016/j.supflu.2017.11.010.
[164] N. Ventosa, S. Sala, J. Veciana, DELOS process: a crystallization technique using compressed fluids—1. Comparison to the GAS crystallization method. J. Supercrit. Fluids 26 (2003) 33–45, https://doi.org/10.1016/S0896-8446(02)00189-4.
[165] R. Thiering, F. Dehghani, N.R. Foster, Current issues relating to anti-solvent micronisation techniques and their extension to industrial scales. J. Supercrit. Fluids 21 (2001) 159–177, https://doi.org/10.1016/S0896-8446(01)00090-0.
[166] S. Varona, Á. Martín, M.J. Cocero, C.M.M. Duarte, Encapsulation of lavandin essential oil in poly-(ε{lunate}-caprolactones) by PGSS process. Chem. Eng. Technol. 36 (2013) 1187–1192, https://doi.org/10.1002/ceat.201200592.
[167] A.C. De Aguiar, L.P.S. Silva, C. Alves De Rezende, G.F. Barbero, J. Martínez, Encapsulation of pepper oleoresin by supercritical fluid extraction of emulsions. J. Supercrit. Fluids 112 (2016) 37–43, https://doi.org/10.1016/j.supflu.2016.02.009.
[168] P.M.C.L. Reis, N. Mezzomo, G.P.S. Aguiar, E.M.T.L. Senna, H. Hense, S.R.S. Ferreira, Ultrasound-assisted emulsion of laurel leaves essential oil (*Laurus nobilis* L.) encapsulated by SFEE. J. Supercrit. Fluids 147 (2019) 284–292, https://doi.org/10.1016/J.SUPFLU.2018.11.018.
[169] P. Becher, Emulsions: Theory and Practice, third ed., American Chemical Society, Wilmington, NC, 2001.
[170] S. Deen, S. Sajjadi, Dilute nanoemulsions via separation of satellite droplets. J. Colloid Interface Sci. 407 (2013) 354–360, https://doi.org/10.1016/j.jcis.2013.06.001.
[171] S.M. Jafari, E. Assadpoor, Y. He, B. Bhandari, Re-coalescence of emulsion droplets during high-energy emulsification. Food Hydrocoll. 22 (2008) 1191–1202, https://doi.org/10.1016/j.foodhyd.2007.09.006.
[172] E. Allemann, R. Gurny, E. Doelker, Drug-loaded nanoparticles: preparation methods drug targeting issues, Eur. J. Pharm. Biopharm. 39 (1993) 173–191.
[173] P. Couvreur, C. Dubernet, F. Puisieux, Controlled drug delivery with nanoparticles: current possibilities and future trends, Eur. J. Pharm. Biopharm. 41 (1995) 2–13.
[174] N. Sadurní, C. Solans, N. Azemar, M.J. García-Celma, Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications. Eur. J. Pharm. Sci. 26 (2005) 438–445, https://doi.org/10.1016/J.EJPS.2005.08.001.
[175] E.J. Windhab, M. Dressler, K. Feigl, P. Fischer, D. Megias-Alguacil, Emulsion processing—from single-drop deformation to design of complex processes and products. Chem. Eng. Sci. 60 (2005) 2101–2113, https://doi.org/10.1016/J.CES.2004.12.003.
[176] M.R. Mozafari, J. Flanagan, L. Matia-Merino, A. Awati, A. Omri, Z.E. Suntres, H. Singh, Recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. J. Sci. Food Agric. 86 (2006) 2038–2045, https://doi.org/10.1002/jsfa.2576.

[177] C. Prieto, C.M.M. Duarte, L. Calvo, Performance comparison of different supercritical fluid extraction equipments for the production of vitamin E in polycaprolactone nanocapsules by supercritical fluid extraction of emulsions. J. Supercrit. Fluids 122 (2017) 70–78, https://doi.org/10.1016/j.supflu.2016.11.015.
[178] D.J. McClements, Edible nanoemulsions: fabrication, properties, and functional performance. Soft Matter. 7 (2011) 2297–2316, https://doi.org/10.1039/c0sm00549e.
[179] S. Tamilvanan, Oil-in-water lipid emulsions: implications for parenteral and ocular delivering systems. Prog. Lipid Res. 43 (2004) 489–533, https://doi.org/10.1016/J.PLIPRES.2004.09.001.
[180] P. Chattopadhyay, B.Y. Shekunov, J.S. Seitzinger, R.W. Huff, Particles From Supercritical Fluid Extraction of Emulsion, US6998051(2006).
[181] C. Prieto, L. Calvo, The encapsulation of low viscosity omega-3 rich fish oil in polycaprolactone by supercritical fluid extraction of emulsions. J. Supercrit. Fluids 128 (2017) 227–234, https://doi.org/10.1016/j.supflu.2017.06.003.
[182] N. Mezzomo, E. de Paz, M. Maraschin, Á. Martín, M.J. Cocero, S.R.S. Ferreira, Supercritical anti-solvent precipitation of carotenoid fraction from pink shrimp residue: effect of operational conditions on encapsulation efficiency. J. Supercrit. Fluids 66 (2012) 342–349, https://doi.org/10.1016/J.SUPFLU.2011.08.006.
[183] D.T. Santos, Á. Martín, M.A.A. Meireles, M.J. Cocero, Production of stabilized sub-micrometric particles of carotenoids using supercritical fluid extraction of emulsions. J. Supercrit. Fluids 61 (2012) 167–174, https://doi.org/10.1016/J.SUPFLU.2011.09.011.
[184] M. Rotches-Ribalta, C. Andres-Lacueva, R. Estruch, E. Escribano, M. Urpi-Sarda, Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets. Pharmacol. Res. 66 (2012) 375–382, https://doi.org/10.1016/j.phrs.2012.08.001.
[185] J.L.P. Reátegui, F.M. Barrales, C.A. Rezende, C.L. Queiroga, J. Martínez, Production of Copaiba oleoresin particles from emulsions stabilized with modified starches. Ind. Crops Prod. 108 (2017) 128–139, https://doi.org/10.1016/j.indcrop.2017.06.027.
[186] G.D. Porta, N. Falco, E. Reverchon, Continuous supercritical emulsions extraction: a new technology for biopolymer microparticles production. Biotechnol. Bioeng. 108 (2011) 676–686, https://doi.org/10.1002/bit.22972.
[187] G. Della Porta, R. Campardelli, N. Falco, E. Reverchon, PLGA microdevices for retinoids sustained release produced by supercritical emulsion extraction: continuous versus batch operation layouts. J. Pharm. Sci. 100 (2011) 4357–4367, https://doi.org/10.1002/JPS.22647.
[188] M. Perrut, J. Jung, F. Leboeuf, Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes: part II: preparation of composite particles. Int. J. Pharm. 288 (2005) 11–16, https://doi.org/10.1016/j.ijpharm.2004.09.008.

CAPÍTULO II

Determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction

(Determinação de parâmetros de processo e das propriedades bioativas dos extratos da polpa de murici (*Byrsonima crassifolia*) obtidos por extração supercrítica)

Flávia Cristina Seabra Pires, Ana Paula de Souza e Silva, Marielba de los Angeles Rodriguez Salazar, Wanessa Almeida da Costa, Helber Samom Cardoso da Costa, Alessandra Santos Lopes, Herve Rogez, Raul Nunes de Carvalho Junior

Reprinted from The Journal of Supercritical Fluids, 2019:

PIRES, F. C. S.; SILVA, A. P. S.; SALAZAR, M. A. R.; COSTA, W. A.; COSTA, H. S. C.; LOPES, A. S.; ROGEZ, H.; CARVALHO JUNIOR, R. N. Determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction. **The Journal of Supercritical Fluids**, v. 146, p. 128–135, 2019. DOI: 10.1016/j.supflu.2019.01.014.

The Journal of Supercritical Fluids 146 (2019) 128–135

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

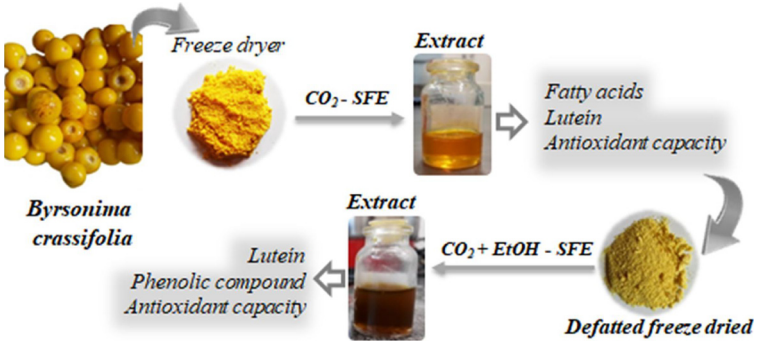
journal homepage: www.elsevier.com/locate/supflu

Determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction

Flávia Cristina Seabra Pires[a,*], Ana Paula de Souza e Silva[a], Marielba de los Angeles Rodriguez Salazar[a], Wanessa Almeida da Costa[a], Helber Samom Cardoso da Costa[b], Alessandra Santos Lopes[c], Herve Rogez[b], Raul Nunes de Carvalho Junior[a,*]

[a] LABEX (Extraction Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Para), Rua Augusto Corrêa S/N, 66075-900, Guamá, Belém, Pará, Brazil
[b] CVACBA (Center for Valorization of Amazonian Bioactive Compounds), UFPA (Federal University of Pará), Avenida Perimetral, 01, 66075-150, Guamá, Belém, Pará, Brazil
[c] LABIOTEC (Laboratory of Biotechnological Processes), ITEC (Institute of Technology), UFPA (Federal University of Para), Rua Augusto Corrêa S/N, 66075-900, Guamá, Belém, Pará, Brazil

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Byrsonima crassifolia
Supercritical fluid extraction
Dynamic extraction period
Phenolic compounds
Antioxidant capacity
Linear correlation

ABSTRACT

The obtaining of muruci extracts (*Byrsonima crassifolia*) was performed by CO_2-SFE and CO_2 + EtOH-SFE, which were conducted at 323.15 K–343.15 K, and at 15 MPa to 49 MPa. The increase of 50% in the solvent flow rates, and the reduction of 33% in the dynamic period increased the yields. For the CO_2-SFE, the highest values of global yield (9.12%), lutein (207.18 μg/g), and antioxidants (33.91 μmol TE/g) were obtained at 333.15 K. In the CO_2 + EtOH-SFE of the defatted freeze dried murici pulp, the highest values of lutein (209.73 μg/g), phenolics (43.69 mg GAE/g), and antioxidants (138.85 μmol TE/g) were obtained at 940 kg/m^3, with a strong positive phenolics/antioxidants correlation. The functional quality suggests hypercholesterolemic, antiatherosclerosis and antithrombogenic effects.

* Corresponding authors.
E-mail addresses: flaviapiress@gmail.com (F.C.S. Pires), raulncj@ufpa.br (R.N.d. Carvalho Junior).

https://doi.org/10.1016/j.supflu.2019.01.014
Received 20 November 2018; Received in revised form 16 January 2019; Accepted 22 January 2019
Available online 24 January 2019
0896-8446/

Nomenclature

ρ_a	Apparent density
ρCO_2	Supercritical CO_2 density
$\rho CO_2 + EtOH$	Density of supercritical CO_2 + ethanol
$\rho Solvent$	Solvent density
ρ_t	True density
ε	Bed porosity
A	Extraction with supercritical CO2 at $t_1$and $Q_{CO}2_1$
AI	Atherogenicity index
B	Extraction with supercritical CO2 at $t_2$and $Q_{CO}2_2$
CO_2	Carbon dioxide
CO_2-SFE	Supercritical fluid extraction with CO_2
$CO_2 + EtOH$-*SFE*	Supercritical fluid extraction with CO_2 + ethanol
d_{ap}	Average particle diameter
d.b.	Dry basis
DFDMP	defatted freeze dried murici pulp
EtOH	Ethanol
FDMP	Freeze dried murici pulp
HI	Hypocholesterolemic index
$m_{CO}2$	CO_2 mass
MUFA	Monounsaturated fatty acids
ORAC	Oxygen radical absorbance capacity
PUFA	Polyunsaturated fatty acids
Q_{CO_2}	Supercritical CO_2 mass flow
SFA	Saturated fatty acids
TI	Thrombogenicity index
UFA	Unsaturated fatty acids

1. Introduction

The murici (*Byrsonima crassifolia*) is a fruit that grows on a small tree native to tropical America and widespread throughout the Amazon region. The fruit is a drupe of 1 cm–2 cm, whose pulp is consumed in the form of nectars, candies, and ice creams due to its exotic flavor and odor [1,2]. The genus is known to present a pulp rich in carotenoids, phenolic compounds, and unsaturated fatty acids, thus constituting a source of antioxidants [3–6]. These components are generally attributed to beneficial health effects, which justify the use of murici in the treatment and/or prevention of diseases [7].

Lutein, the main carotenoid present in murici [5], is associated with a decreased risk of developing macular degeneration, as it is among the carotenoids commonly found in the retina, which is located in the back of the eye [8]. Quercetin, the major phenolic compound found in murici [9], may be attributed to the hypoglycemic and antiglycation effects of the fruit [10], since this compound was effective in diabetes mellitus treatment [11]. The presence of unsaturated fatty acids suggests that murici has therapeutic effects in the treatment and/or prevention of cardiovascular diseases such as atherosclerosis, thrombosis, and hypercholesterolemia, since this link was observed for other foods [12–14].

Several extraction techniques have been used to increase the content and bioavailability of bioactive compounds extracted from fruits [15–17]. Among these methods, the extraction with supercritical fluids stands out, especially with carbon dioxide (CO_2) [18,19]. This technology has been applied commercially in nutraceutical and pharmaceutical products for the extraction of free fatty acids and other bioactive compounds [20].

Extraction with supercritical CO_2 provides higher selectivity, purity, and yields, as well as lower extraction times. This is possible when process parameters are optimized, such as bed characteristics and process variables such as pressure, temperature, flow, among others [21,22]. However, extraction with supercritical CO_2 has limited ability to solubilize polar compounds, when it is necessary to use cosolvents, such as ethanol, to increase the solvation power on substances of high polarity during extraction [23].

Thus, the main objectives of this work were to study the extracting process of murici pulp (*B. crassifolia*) with supercritical CO_2, followed by re-extraction with CO_2 + ethanol to determine its best operating condition through the global yield isotherms, which includes temperature, pressure, and solvent flow; to obtain the bioactive compounds present in the murici pulp, such as: lutein, fatty acids, and phenolic compounds; and also to evaluate the functional quality and antioxidant capacity of extracts.

2. Materials and methods

2.1. Raw materials

The frozen murici pulp was obtained in the municipality of Terra Alta (Pará, Brasil) (01° 02′ 25. 9″ S and 47° 54′ 12. 3″ W). 30 kg of pulp were transported in a thermally insulated vessel and stored under freezing (255.15 K ± 1 K) until processing. The solvents used were CO_2 (99.9% purity, White Martins, Brazil) and EtOH (99.9% purity, PA, Merck, Brazil).

2.2. Freeze drying of murici pulp

The murici pulp was added in stainless steel trays, being frozen again. The sample was lyophilized in a semi-industrial lyophilizer (model LJI 015, JJ Científica, São Carlos, Brazil). The process was carried out at 233.15 K for two days. After lyophilization, approximately 2 kg of freeze dried muruci pulp (FDMP) was obtained. It presented (5.46 ± 0.30) g/100 g of moisture, (2.02 ± 0.02) g/100 g of ashes, (0.55 ± 0.02) g/100 g of proteins, (10.02 ± 0.15) g/100 g of lipids, and (21.60 ± 0.59) g/100 g of sugars. Such parameters were determined according to AOAC [24]. FDMP was vacuum packed and stored at 273.15 K.

2.3. Supercritical extractions: CO_2-SFE and CO_2 + EtOH-SFE

2.3.1. Operating conditions

The supercritical extractions were carried out at the Laboratory of Supercritical Extraction (LABEX/UFPA/Brazil) in a SPE-ED SFE unit (model 7071, Applied Separations, USA), coupled to a CO_2 cylinder, a compressor (model CSA 78, Schulz S/A, Brazil), a recirculator (model F08400796, Polyscience, USA) and a CO_2 flowmeter (model M 5SLPM, Alicat Scientific, USA). In the first stage, the extractions of the FDMP with 100% of supercritical CO_2 (CO_2-SFE) were carried out in order to obtain the most apolar extracts, and the defatted freeze dried murici pulp (DFDMP). In the second step, the DFDMP were extracted with 90% of supercritical CO_2 and 10% of ethanol (CO_2 + EtOH-SFE) to obtain the most polar extracts. The extraction cell used had internal diameter of 0.032 m and height of 0.126 m, constituting a total internal volume of 9.96×10^{-5} m^3. The operating conditions for both processes were: temperatures of 323.15 K and 333.15 K and pressures in the range of 15 MPa to 42 MPa. The densities were calculated using the Aspen Hysys software (Aspen One 8.6), which applies the Peng-Robinson [25] cubic equation of state with zero binary interaction parameters. The operating conditions of CO_2-SFE and CO_2 + EtOH-SFE are shown in Table 1.

The extracts global yields were calculated from the mathematical ratio between the masses of extract and the FDMP or DFDMP (w/w). To obtain the masses of the polar extracts, the residual ethanol was evaporated under vacuum at 313.15 K in CentriVap centrifuge (model

Table 1
Operating conditions of CO_2-SFE and CO_2 + EtOH-SFE of murici pulp (*B. crassifolia*).

Assay	Temperature (K)	Pressure (MPa)	ρCO_2 (kg/m^3)[a]	ρCO_2 + EtOH (kg/m^3)[a]
1	323.15	15	654	801
2	323.15	22	791	863
3	323.15	35	918	936
4	333.15	19	671	797
5	333.15	27	797	865
6	333.15	42	924	946

[a] ρCO_2: CO_2 density; ρCO_2 + EtOH: CO_2 + EtOH density.

78100, Labconco, USA) and the masses were weighed. The determinations were performed in duplicate and the results were expressed as % by mass (d.b.).

2.3.2. Characterization of beds before and after extractions

For the FDMP and DFDMP, the average particle diameter (d_{ap}), the true (ρ_t) and apparent densities (ρ_a), and the porosities (ε) of the extraction beds were determined. The DFDMP used was that which presented the highest yield in CO_2-SFE. Firstly, the samples were sieved with the aid of a vibrating shaker (Granutest) composed of 7 Tyler screens (WS Tyler, USA) from 8 to 42 mesh, for 900 s, at full speed. The d_{ap} was calculated according to ASAE method S319.4 [26], and the results were expressed as mm. The ρ_t of the particles were determined using automatic helium gas pycnometer (model Ultrapyc 1200e, Quantachrome, USA). For the beds preparation, 0.020 kg of FDMP and 0.015 kg of DFDMP were used, which formed beds with diameter/height ratios of 0.44 and 0.58, respectively. The ρ_a were determined from the mass/volume ratio of the sample (w/v) in the extraction cell, both expressed as kg/m^3. The ε were determined by the relationship between ρ_t and ρ_a. The determinations were performed in triplicate.

2.3.3. Evaluation of the solvent flow rates over the dynamic period of CO_2-SFE

The supercritical extraction process was performed in two stages: a static period (closed system) and a dynamic period (open system). In order to reduce the dynamic period, the bed-related parameters (ε, bed height/diameter ratio, feed mass, d_{ap}, ρ_t, and ρ_a), and process-related parameters (pressure of 49 MPa, temperature of 343.15 K, CO_2 density of 929.50 kg/m^3, static period of 1 800 s, and CO_2 mass of 0.96 kg) were kept constant. The procedure was performed in two steps: A and B. In A, the dynamic period was of 10 800 s (t_1) and the CO_2 flow was 8.85×10^{-5} kg/s ($Q_{CO}2_1$). In B, the dynamic period was 7 200 s (t_2) and the CO_2 flow ($Q_{CO}2_2$) was calculated through the mathematical Eq. (1), where $m_{CO}2_1 = m_{CO}2_2$. In both steps, the extracts were collected and weighed every 3 600 s to obtain the cumulative masses of extraction. From this study, all the extracts were obtained under the extraction conditions that obtained the highest cumulative yield and the shortest extraction time. The experiments were performed in duplicate.

$$Q_{CO_{2_2}} = \frac{m_{CO_{2_2}}}{t_2} \quad (1)$$

2.4. Lutein content

The lutein contents were determined for the FDMP, DFDMP, and for the extracts obtained by CO_2-SFE and CO_2 + EtOH-SFE, according to the methodology proposed by Rodriguez-Amaya and Kimura [27]. Acetone was used as the extraction solvent. 50% of ethyl ether and 50% of petroleum ether (v/v) were used in the partitioning. Quantifications were performed in ethanol using a spectrophotometer (model Evolution 60, Thermo Scientific, USA). The total lutein contents were calculated through absorbance measurements performed at 445 nm, and with the absorption coefficient of lutein in ethanol equal to 2550. Quantifications were performed in triplicate and the results were expressed as μg/g by mass (d.b.).

2.5. Phenolic compounds

For the quantification of the phenolic compounds of the FDMP, DFDMP and CO_2 + EtOH-SFE extracts, the Folin-Ciocalteu method was used [28,29]. In the extraction, the samples were homogenized for 120 s on a vortex mixer with an acidified ethanol solution (70% ethanol/29.5% water /0.5% acetic acid) at a concentration of 110 kg/m^3 (w/v). Then, the samples were centrifuged at 277.15 K per 1 200 s in a centrifuge (Model CT15RE, Hitachi, Japan). The supernatants were diluted in water until a concentration of 0.05 m^3/m^3 (v/v) and then homogenized. For the reaction, 5.00×10^{-7} m^3 of diluted sample, 1.25×10^{-6} m^3 of 7.5% sodium carbonate and 2.50×10^{-7} m^3 of Folin-Ciocalteu in a concentration of 1.00×10^3 mol/m^3 were added to the cuvettes, and subsequently homogenized. For the blank, water was used instead of the sample. The reaction was performed for 1 800 s in the absence of light. Quantification was performed on a UV-VIS spectrophotometer (Evolution Model 60, Thermo Scientific, USA), at 750 nm. The standard curve used was constructed using gallic acid at eight concentration points (1, 5, 10, 20, 40, 60, 80, 100 kg/m^3), according to the equation of a line $y = 0.1109\ x + 0.0087$, where y is the absorbance and x is the concentration (see the Supplementary material S1). The determination was performed in triplicate and the results were expressed as mg GAE/g by mass (d.b.).

2.6. Fatty acids and functional quality

The fatty acid profiles of the CO_2-SFE extracts were determined on gas chromatograph (model GC-2010, Shimadzu, Japan) equipped with a flame ionization detector, and a capillary column TG-WAX MS A (30 m·3.20 × 10^{-4} m·2.50 × 10^{-7} m). Conversions to fatty acid methyl esters were performed according to the Ce 2-66 AOCS method [30]. The operating conditions were: helium as carrier gas, flow rate of 8.33×10^{-7} m^3/s, FID detector at 523.15 K, injector (proportion 1:100), injection volume of 1.00×10^{-9} m^3. The programmed temperature of the column was 323.15 K for 300 s, with a subsequent increase until 523.15 K. The individual fatty acids peaks were identified by comparing their retention times with those of known mixtures of fatty acid standards (Nu-Check- Prep, USA), under the same operating conditions. The retention time and area of each peak were calculated using the software CG Software Solution. The determinations were performed in triplicate and the results were expressed as % by mass (d.b.). The atherogenicity (*AI*), thrombogenicity (*IT*) and hypocholesterolemia (*HI*) indexes were calculated from the fatty acid composition to obtain the functional profile of the extracts obtained by CO_2-SFE [31,32].

2.7. Oxygen radical absorbance capacity (ORAC) assay

The *ORAC* of the FDMP, DFDMP, and the extracts obtained by CO_2-SFE and CO_2 + EtOH-SFE were determined on a microplate fluorimeter, monitoring the effect of the sample on fluorescence decomposition, resulting from the oxidation induced by the peroxyl radical (ROO*) of fluorescein, according to Lai et al. [33] (see the Supplementary material S2). The extraction of the antioxidants was adapted to solubilize the antioxidants of all the samples, using acetone as the extracting solvent. The analysis was performed in triplicate. The results were expressed as μmol TE/g (d.b.).

2.8. Statistical analysis

The means and standard deviations were calculated for all results. Correlation analyses were performed using linear regression and

Pearson correlation coefficient (r), with $p < 0.05$. The softwares Excel 2000 SR-1 (Microsoft, Troy, USA) and Statistica Kernel Release 7.1 (StartSoft Inc., Tulsa, USA) were used to perform the analyses.

3. Results and discussion

3.1. Characterization of extraction beds

The d_{ap} values were 3.80×10^{-4} m ± 0.01 m for the FDMP and 3.00×10^{-4} m ± 0.01 m for the DFDMP. The low d_{ap} values obtained provided good accessibility to the solute, due to the increase in mass transfer area at the surface of the particles. These results were within the range obtained for natural products, which are from 2.50×10^{-4} m to 1.80×10^{-3} m [23]. The ρ_t of the FDMP and DFDMP beds were 1290 kg/m^3 ± 0.01 kg/m^3 and 1300 kg/m^3 ± 0.01 kg/m^3, respectively, whereas the ρ_a of the FDMP and DFDMP were 350.00 kg/m^3 ± 0.01 kg/m^3 and 284.80 kg/m^3 ± 0.01 kg/m^3, respectively. It can be observed that the values of ρ_t did not present great variations among themselves, being evidenced a greater difference in the values of ρ_a. This difference influenced the ε of the extraction beds: the ε of the FDMP (0.73 ± 0.01) was lower than that of the DFDMP (0.78 ± 0.01).

The lutein contents, phenolic compounds, and *ORAC* results of the FDMP and DFDMP are shown in Table 2. The lutein content of the FDMP was 56.42 μg/g (d.b.) ± 0.21 μg/g (d.b.). This value was higher than that found by Mariutti et al. [5], which was 20.89 μg/g (d.b.) ± 1.23 μg/g (d.b.). In DFDMP, the highest lutein content (41.92 μg/g d.b. ± 0.04 μg/g d.b.) was obtained at 333.15 K/19 MPa and the lowest lutein content (22.91 μg/g d.b. ± 0.10 μg/g d.b.) was obtained at 323.15 K/22 MPa. This may have been due to the increased exposure of the compounds under these conditions. The increase in pressure decreased the lutein content, except for the condition of 323.15 K/35 MPa, possibly due to the increased density of the solvent. It can be observed that the lutein contents of the DFDMP were smaller than those obtained for the FDMP. This occurred due to the extraction of part of these compounds in the 1 st extraction step. Although carotenoids are predominantly apolar compounds, xanthophylls, such as lutein, have medium polarity and may be present in free form or bound to fatty acid esters, thus modifying their solubility [34].

The value of phenolic compounds of the FDMP was 154.34 mg GAE/g (d.b.) ± 0.02 mg GAE/g (d.b.). This result was higher than that found by Souza et al. [35] (48.55 mg GAE/g d.b. ± 9.07 mg GAE/g d.b.). This value for the DFDMP ranged from 130.18 mg GAE/g (d.b.) ± 0.14 mg GAE/g (d.b.) to 189.75 mg GAE/g (d.b.) ± 0.13 mg GAE/g (d.b.), e.g., the lowest content in the most extreme condition (333.15 K/42 MPa) and the highest content in the mildest condition (323.15 K/15 MPa). These values demonstrate that these compounds are practically not present in the extracts obtained by CO_2-SFE due to the polarity of the supercritical CO_2, which solubilized and extracted the fatty acid/lipid fraction from the matrix and other apolar bioactive compounds [36,23,37,38]. A similar behavior was observed by Batista et al. [19] for the defatted freeze dried açaí pulp obtained by CO_2-SFE. This facilitated the subsequent extraction of phenolic compounds from the DFDMP.

The *ORAC* of the FDMP was 6.62 μmol TE/g (d.b.) ± 0.36 μmol TE/g (d.b.). This value was higher than that found by Mariutti et al. [9] (6.60×10^{-4} μmol TE/g d.b. ± 0.04 μmol TE/g d.b.). After the CO_2-SFE, the *ORAC* of the DFDMP were reduced, with the highest value (2.41 μmol TE/g d.b. ± 0.10 μmol TE/g d.b.) obtained at 323.15 K/15 MPa and the lowest value (1.97 μmol TE/g d.b. ± 0.04 μmol TE/g d.b.) obtained at 333.15 K/27 MPa. It can be observed that the increase in temperature decreased the *ORAC*, possibly due to the thermal degradation of the antioxidant compounds. The variation between the *ORAC* values of the FDMP and of the DFDMP occurred due to the extraction of some antioxidant compounds in the first step, such as carotenoids, fatty acids, esters, etc. These results demonstrate that the DFDMPs obtained after CO_2-SFE can be applied to obtain extracts rich in bioactive antioxidant compounds and free of toxic residues.

3.2. Evaluation of solvent flow rates over the dynamic extraction period

The $Q_{CO}2_2$ calculated for t_2 = 7 200 s was 1.33×10^{-4} kg/s. In Fig. 1, the cumulative yields of A and B are presented. It can be seen that the yields increased with increasing time for both extractions. The yield of A (9.57% d.b. ± 0.36% d.b.) was lower than that of B (10.01% d.b. ± 0.39% d.b.). This occurred due to the increase in the superficial velocity of the solvent over the particles, which consequently increased the convective and diffusive rate of extraction. Therefore, for a 33% lower dynamic period and a CO_2 flow rate 50% higher than that used in A, it was possible to obtain a higher yield of the murici (*B. crassifolia*) pulp extract. In the work performed by Shan et al. [39], it was also observed that the increase in the CO_2 flow rate increased the rate of extraction of bitter melon pulp (*Momordica charantia* L.) by supercritical extraction. Although the flow calculation was done for 7 200 s, the

Table 2
Lutein content, phenolics compounds, and ORAC of the freeze dried murici pulp, defatted freeze dried murici pulp, and of the different extracts of the murici pulp (*B. crassifolia*) obtained by supercritical extraction.

Samples	Lutein content (μg/g d.b.)*	Phenolic compounds (mg GAE/g d.b.)*	ORAC[c] (μmol TE/g d.b.)*
FDMP[a]	56.42 ± 0.21	154.34 ± 0.02	6.62 ± 0.36
323.15K/15MPa			
1st step			
CO_2-SFE extract	83.36 ± 0.68	–	29.36 ± 1.10
2nd step			
DFDMP[b]	27.75 ± 0.02	189.75 ± 0.13	2.41 ± 0.10
CO_2 + EtOH-SFE extract	144.21 ± 0.29	22.50 ± 0.11	66.20 ± 0.81
323.15 K/22 MPa			
1 st step			
CO_2-SFE extract	146.07 ± 0.83	–	32.30 ± 1.15
2nd step			
DFDMP	22.91 ± 0.10	142.31 ± 0.25	2.30 ± 0.14
CO_2 + EtOH-SFE extract	143.21 ± 0.30	31.33 ± 0.17	59.85 ± 1.29
323.15 K/35 MPa			
1 st step			
CO_2-SFE extract	112.18 ± 0.68	–	30.55 ± 0.69
2nd step			
DFDMP	30.02 ± 0.10	161.52 ± 0.25	2.37 ± 0.12
CO_2 + EtOH-SFE extract	209.73 ± 0.69	34.08 ± 0.06	69.53 ± 0.68
333.15 K/19 MPa			
1 st step			
CO_2-SFE extract	22.65 ± 0.11	–	32.81 ± 0.42
2nd step			
DFDMP	41.92 ± 0.04	147.36 ± 0.14	2.03 ± 0.10
CO_2 + EtOH-SFE extract	180.75 ± 0.15	25.27 ± 0.06	87.47 ± 2.62
333.15 K/27 MPa			
1 st step			
CO_2-SFE extract	173.40 ± 0.85	–	33.91 ± 0.63
2nd step			
DFDMP	34.88 ± 0.07	146.32 ± 0.19	1.97 ± 0.04
CO_2 + EtOH-SFE extract	79.04 ± 0.38	33.40 ± 0.15	84.84 ± 0.81
333.15 K/42 MPa			
1 st step			
CO_2-SFE extract	207.18 ± 0.18	–	30.96 ± 0.47
2nd step			
DFDMP	29.87 ± 0.06	130.18 ± 0.14	2.07 ± 0.09
CO_2 + EtOH-SFE extract	172.59 ± 0.70	43.69 ± 0.33	138.85 ± 3.03

The results were obtained on mass basis.
[a] FDMP = freeze dried murici pulp.
[b] DFDMP = defatted freeze dried murici pulp.
[c] ORAC = oxygen radical absorbance capacity.

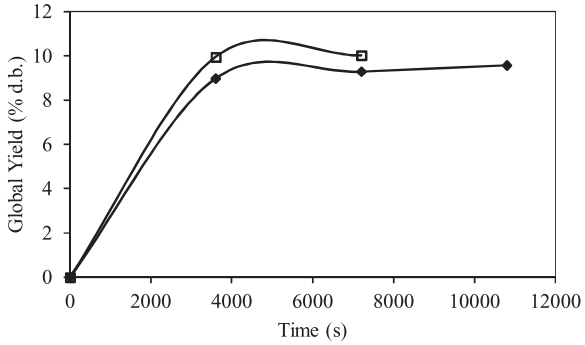


Fig. 1. Cumulative yields for different solvent flow rates and dynamic periods of supercritical CO_2 extraction of the murici pulp (*B. crassifolia*). Steps: A (—◆—) and B (—□—).

other extractions (CO_2-SFE and CO_2 + EtOH-SFE) were performed using the dynamic period of 3 600 s, since at this time it was possible to obtain experimentally about 98.76% of the total mass extract, in addition to using a mass of CO_2 (mCO_2 = 0.474 kg) 50.41% lower than that used in the process for 7 200 s. Thus, the reduction of the dynamic period starting from the increase of the solvent flow rate provided the improvement of the process performance, as it allowed to obtain a larger amount of extract per fraction of time, and a lower exposure of the thermosensitive bioactive compounds, which minimized their degradation and increased their stability [40].

3.3. Global yield isotherms

In Fig. 2a and b, the global yield obtained in CO_2-SFE (1 st step) and in CO_2 + EtOH-SFE (2nd step) are presented. In CO_2-SFE, it is observed that the highest global yield (9.12% d.b. ± 0.08% d.b.) was obtained in the condition of 333.15 K/27 MPa, and the lowest global yield (5.97% d.b. ± 0.04% d.b.) was obtained at 323.15 K/15 MPa. These resuls were also achieved by Cunha et al. [18] for bacaba-de-leque pulp extract (*Oenocarpus distichus* Mart.) obtained by CO_2-SFE. For both isotherms obtained by CO_2-SFE, increases in temperature and pressure raised the global yield until a pressure of 27 MPa, where the solvent density prevailed over the solubilization of the solutes. After this point, an inflection point occurred in the isotherms, where the vapor pressure of the solute prevailed over the global yield. This behavior was also observed by Espinosa-Pardo et al. [41] for the peach palm pulp extract (*Bactris gasipaes*) obtained by CO_2-SFE. In CO_2 + EtOH-SFE, the highest global yield (8.81% d.b. ± 0.12% d.b.) was obtained at 323.15 K/15 MPa and the lowest global yield (3.70% d.b. ± 0.40% d.b.) was obtained at 333.15 K/42 MPa. The isotherms obtained by CO_2 + EtOH-SFE presented an inverse behavior in relation to the isotherms obtained by CO_2-SFE, where the highest global yield were obtained by reducing temperature and pressure. This behavior demonstrates that CO_2 + EtOH-SFE solubilized the remaining compounds that had not been extracted by CO_2-SFE. It can be seen that both supercritical extractions had higher global yield at densities of approximately 800 kg/m^3 (Fig. 2b). This suggests that the murici pulp compounds are more soluble in the supercritical fluids under these conditions, which confirmed the selectivity of the different solvents used in supercritical conditions.

3.4. Characterization of the extracts

The lutein contents, phenolic compounds, and *ORAC* of the extracts obtained by CO_2-SFE (1 st step) and CO_2 + EtOH-SFE (2nd step) are presented in Table 2. The highest lutein content of the extracts obtained by CO_2-SFE (207.08 μg/g d.b. ± 0.18 μg/g d.b.) was reached at 333.15 K/42 MPa and the lowest one (22.65 μg/g d.b. ± 0.11 μg/g d.b.) was obtained at 333.15 K/19 MPa. It can be observed that the temperature had no definite effect on the response. However, the increased pressure increased the lutein content of the extracts, due to the increased density of CO_2. The highest lutein content of the extracts obtained by CO_2 + EtOH-SFE (209.73 μg/g d.b. ± 0.69 μg/g d.b.) was achieved at 323.15 K/35 MPa and the lowest lutein content (79.04 μg/g d.b. ± 0.38 μg/g d.b.) was obtained at 333.15 K/27 MPa. In general, it can be observed that the extracts collected by CO_2 + EtOH-SFE presented high lutein content levels, except when the densities of the CO_2 + EtOH mixture were approximately 860 kg/m^3. This demonstrates that, in this condition, the solute/solvent interactions are weaker and less solubilization of lutein occurs. From this point, lutein contents on the 2nd step increase with the reduction in temperature, along with the increase in pressure and density of the CO_2 + EtOH mixture. This was possibly due to the decrease in thermal degradation and the increase in the solvating power of lutein, with the co-solvent addition. When comparing the two extraction steps, the highest lutein content were obtained with the use of the co-solvent. This behavior was also observed by Cobb et al. [42], while optimizing the supercritical fluid extraction of lutein from corn gluten meal. Therefore, the use of ethanol in supercritical extraction is ideal for extraction of lutein because of its GRAS (generally recognized as safe) status, and because it is used in low concentrations in relation to conventional ethanol extractions that use large amounts of solvent [43]. In addition, obtaining lutein as an extract increases its bioavailability [44], mainly because it is associated with the presence of other lipids [45]. From these results, it can be inferred that both extracts of murici pulp (*B. crassifolia*) obtained by supercritical extraction are great sources of lutein.

The highest phenolic compounds of the extracts obtained by CO_2 + EtOH-SFE (43.69 mg GAE/g d.b. ± 0.33 mg GAE/g d.b.) was reached at 333.15 K/42 MPa and the lowest (22.50 mg GAE/g d.b. ± 0.11 mg GAE/g d.b.) was reached at 323.15 K/15 MPa. The phenolic compounds increased with the use of higher temperature and pressure, with consequent increase in the density of the CO_2 + EtOH mixture. The same behavior was observed by Shan et al. [39]. The phenolic compounds values show that the CO_2 + EtOH-SFE of the DFDMP was effective in obtaining extracts with phenolic compounds, since the extract had total phenolic equal to 28.31%, with the use of 90% less alcohol than what is generally used in conventional extractions. In addition, the phenolic compounds of the murici pulp may be associated with the hypoglycemic activity of the fruit [10], since several studies have suggested that ingestion of these compounds reduce glycemic levels in the body [11,46].

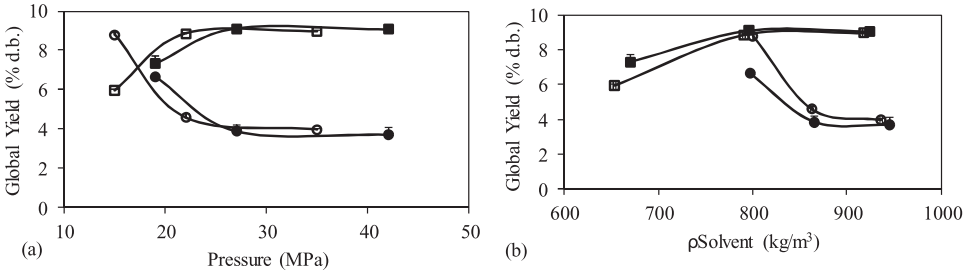


Fig. 2. Global yield isotherms of the extracts of murici pulp (*B. crassifolia*) obtained by CO_2-SFE at 323.15 K (—□—), and at 333.15 K (—■—); and by CO_2 + EtOH-SFE at 323.15 K (—○—), and at 333.15 K (—●—). The standard deviations for all results were ≤ 0.4%.

The highest *ORAC* value of the extracts obtained by CO_2-SFE (33.91 μmol TE/g d.b. ± 0.63 μmol TE/g d.b.) was reached at 333.15 K/27 MPa, whereas the lowest value (29.36 μmol TE/g d.b. ± 1.10 μmol TE/g d.b.) was obtained at 323.15 K/15 MPa. It can be observed that the *ORAC* increased with increasing temperature. After the CO_2 + EtOH-SFE of the DFDMP, there was a substantial increase in the antioxidant capacity of the extracts. Sato et al. [47] also observed the increase in the antioxidant capacity with the use of ethanol as co-solvent in the extraction of strawberry pulp. The highest *ORAC* value of CO_2 + EtOH-SFE (138.85 μmol TE/g d.b. ± 3.03 μmol TE/g d.b.) was obtained at 333.15 K/42 MPa, whereas the lowest value (59.85 μmol TE/g d.b. ± 1.29 μmol TE/g d.b.) was obtained at 323.15 K/22 MPa. It was possible to observe that the *ORAC* increased with increasing temperature, pressure and density of the solvent. It is important to emphasize that the *ORAC* method is considered to be one of the best models of antioxidant reactions in food and in vivo, when lipid oxidation radicals and reactive oxygen species (ROS) are present [48]. Therefore, according to the *ORAC* values, the extracts of murici pulp obtained by CO_2-SFE and by CO_2 + EtOH-SFE can be considered good sources of antioxidant compounds, since they present, respectively, about 5.12 and 20.97 times more antioxidant capacity than the FDMP.

The total fatty acids of the extracts of murici pulp (*B. crassifolia*) obtained by CO_2-SFE are presented in Table 3. The mass-based contents of the different fatty acids identified ranged from 0.10% to 40.04% ± 1.8% (Table 3), and there were no large variations in the different extraction conditions. A similar behavior was observed by Barrales et al. [49] for the extraction of passion fruit pulp and seed (*Passiflora edulis sp.*) obtained in different conditions. In general, oleic acid was the major compound, followed by palmitic and linoleic acids. These results are similar to those obtained by Santos et al. [12]. The high content of palmitic acid is due to the chemical bond between this fatty acid and lutein in natural products [50]. These extracts are rich in unsaturated fatty acids (63%), with mean values of 41% *MUFA* and 22% *PUFA*. In the nutritional evaluation, the low *AI* (0.57) and *TI* (1.09) mean values and the high *HI* mean values (1.82) demonstrated the functional potential of the murici extract obtained by CO_2-SFE [51–53], being similar to the behavior observed by Pinto et al. [13] for bacaba (*Oenocarpus bacaba*) extract obtained by CO_2-SFE. The functional character is due to the fact that *AI*, *TI* and *HI* allow the quantification of the fat capacity to cause atherosclerosis, thrombosis and cholesterol production, respectively, being useful for the diagnosis and monitoring of lipid-lowering treatment, and the alteration of these values may be associated with cardiac, cerebrovascular, and peripheral-vascular problems [54]. From these results, it can be inferred that the murici fatty acid composition is responsible for its antihyperlipidemic effect, verified in the in vivo study performed by Perez-Gutierrez et al. [10].

3.5. Linear correlations

Correlations were tested to evaluate the linear statistical influence of all variables on responses and responses among themselves in all trials ($n = 6/p < 0.05$). The Pearson coefficients (r) between the parameters presented large amplitudes, which ranged from −0.97 to 0.95 (Table 4). The positive r values indicated that there was a directly proportional linear relationship between the variables, whereas the negative values showed the inverse relationship. For values close to zero, it is possible to conclude that there were no linear relationships among variables, whereas for extreme values (−1 or +1), there were strong relationships [55]. For the CO_2-SFE extracts, all the strong correlations were positive; the temperature had moderate effect on the *ORAC*, whereas the pressure and density presented effect on global yield and lutein content. This pattern was also observed by Hsu et al. [56], who correlated the lutein extraction in daylily flowers by CO_2-SFE. Regarding the responses, the best correlations were observed for Global yield × Lutein content and Global yield × *ORAC*. This indicates that it was possible to obtain a larger mass of extract and bioactive compounds in conditions of higher temperature, pressure and density, which was caused by the increase in the solubility of the supercritical CO_2.

For the DFDMP, the temperature correlations on all responses, while the pressure and density had only negative effect on phenolic compounds. The negative values of all phenolic compounds correlations suggest that they are sensitive to extreme process conditions, and are inversely proportional to the increase in the parameters. Between the responses, it can be observed that there was a negative correlation for Lutein content × *ORAC* and a positive one for Phenolic compounds × *ORAC*. For the CO_2 + EtOH-SFE extracts, only the pressure and the density showed a positive correlation on Global yield and *ORAC*, and negative on lutein content; for phenolic compounds, no strongly linear effects were observed. Correlations between Phenolic compounds × Global yield (negative) and Phenolic compounds × *ORAC* (positive) were observed among the responses. The negative correlation between phenolic compounds × global yield was also observed by Reis et al. [57] for tamarind seed extracts (*Tamarindus indica*) obtained by CO_2 + EtOH-SFE. This correlation indicates that the extract with the highest mass does not present the highest contents of bioactive compounds, which confirms the selectivity of the supercritical CO_2 + EtOH under the different extraction conditions. The positive linear correlations between phenolic compounds × *ORAC* was also observed by Viganó et al. [58] for the passion bagasse extract obtained by sequential high pressure extractions, using ethanol as cossolvent. This correlation suggests that the phenolic compounds in the DFDMP and in the CO_2 + EtOH-SFE extracts are the main responsible for the antioxidant activity in the samples.

4. Conclusion

In the supercritical extraction of murici pulp (*B. crassifolia*), it was possible to reduce the dynamic extraction period from 7 200 s to 3 600 s, for in this period a yield of 98.76% was reached, based on

Table 3
Total fatty acids and functional quality of the extracts of murici pulp (*B. crassifolia*) obtained by CO_2-SFE.

Fatty acids (% d.b.)	323.15 K/ 15 MPa	323.15 K/ 22 MPa	323.15 K/ 35 MPa	333.15 K/ 19 MPa	333.15 K/ 27 MPa	333.15 K/ 42 MPa
C14:0	0.48	0.51	0.51	0.53	0.52	0.52
C16:0	33.04	34.30	34.36	33.69	34.33	34.37
C16:1	1.47	1.41	1.40	1.52	1.41	1.41
C18:0	1.31	1.64	1.65	1.36	1.65	1.66
C18:1	40.04	39.08	39.08	39.01	38.98	39.03
C18:2	22.48	21.70	21.61	22.66	21.67	21.56
C18:3	0.71	0.72	0.72	0.74	0.74	0.74
C20:0	0.18	0.35	0.36	0.21	0.36	0.36
C22:0	0.12	0.20	0.21	0.11	0.23	0.24
C24:0	0.17	0.10	0.11	0.17	0.11	0.12
SFA	35.30	37.10	37.20	36.07	37.20	37.27
UFA	64.70	62.91	62.81	63.93	62.80	62.74
MUFA	41.51	40.49	40.48	40.53	40.39	40.44
PUFA	23.19	22.42	22.33	23.40	22.41	22.30
AI	0.54	0.58	0.58	0.56	0.58	0.58
TI	1.02	1.09	1.10	1.05	1.10	1.10
HI	1.93	1.81	1.80	1.87	1.80	1.80

The results were obtained by mass basis; C14:0 (myristic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C18:0 (stearic acid); C18:1 (oleic acid); C18:2 (linoleic acid); C18:3 (linolenic acid); C20:0 (arachidic acid); C22:0 (behenic acid); C24:0 (lignoceric acid); SFA (saturated fatty acids); UFA (unsaturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); AI (atherogenicity index); TI (thrombogenicity index); HI (hypocholesterolemic index). The standard deviations for all fatty acids were lower than 1.8%.

Table 4
Pearson correlation coefficients (r) between the variables (n = 6) of the supercritical extraction of the murici pulp (*B. crassifolia*).

		Temperature	Pressure	ρSolvent[a]	Global Yield	Lutein content	Phenolic compounds	ORAC[b]
CO_2-SFE extract	Global yield	0.24	0.75	0.84	1.00			
	Luteín content	0.17	0.69	0.72	0.70	1.00		
	Phenolic compounds	–	–	–	–	–	–	
	ORAC	0.60	−0.05	−0.05	0.48	0.10	–	1.00
DFDMP	Global yield	–	–	–	–			
	Luteín content	0.73	−0.10	−0.30	–	1.00		
	Phenolic compounds	−0.62	−0.58	−0.53	–	−0.15	1.00	
	ORAC	−0.97	−0.24	−0.06	–	−0.68	0.70	1.00
CO_2 + EtOH-SFE extract	Global yield	0.24	0.75	0.84	1.00			
	Luteín content	−0.28	−0.80	−0.83	0.03	1.00		
	Phenolic compounds	−0.26	0.30	0.30	−0.86	0.09	1.00	
	ORAC	0.35	0.95	0.91	−0.37	0.11	0.72	1.00

[a] ρSolvent = solvent density.
[b] ORAC = oxygen radical absorbance capacity.

increases in solvent flow rates, with consequent reducing in the CO_2 mass used in the process.

The highest values of global yield isotherms were obtained at 333.15 K/27 MPa and 323.15 K/15 MPa, for CO_2-SFE and CO_2 + EtOH-SFE, respectively. The highest lutein content was obtained at 333.15 K/19 MPa for CO_2-SFE, whereas for CO_2 + EtOH-SFE, it was obtained at 323.15 K/35 MPa. The maximum phenolic compounds of CO_2 + EtOH-SFE extracts was obtained at 333.15 K/42 MPa. The highest *ORAC* were obtained at 333.15 K; for CO_2-SFE extracts was at 27 MPa, and for CO_2 + EtOH-SFE extracts was at 42 MPa.

The extracts of murici pulp (*B. crassifolia*) obtained by CO_2-SFE presented high levels of lutein, unsaturated fatty acids and high antioxidant capacity. Their low *AI* and *TI*, and high *HI* values suggest that the extract may have an effect on the treatment and/or prevention of hypercholesterolemia, atherosclerosis, and thrombogenesis.

Obtaining extracts of the murici pulp (*B. crassifolia*) by CO_2 + EtOH-SFE from the DFDMP was efficient, since the extracts proved to be with lutein, phenolic compounds and presented high antioxidant capacity. This procedure also allowed a better use of the matrix, reduced the waste of organic material in the environment and added value to an extraction by-product.

Therefore, both extracts of the murici pulp (*B. crassifolia*) obtained by supercritical extraction can be used due to their functional properties associated to the content of bioactive compounds and antioxidants, besides being good alternatives to the use of extracts obtained with toxic solvents.

Acknowledgements

Flávia Cristina Seabra Pires thanks CAPES - PROPESP/UFPA, process number 1662244/2016, and the laboratories LABEX/UFPA, LABIOTEC/UFPA, and CVACBA/UFPA for the scientific and structural contributions.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.supflu.2019.01.014.

References

[1] E. Béjar, M.H. Malone, Pharmacological and chemical screening of *Byrsonima crassifolia*, a medicinal tree from Mexico. Part I, J. Ethnopharmacol. 39 (1993) 141–158, https://doi.org/10.1016/0378-8741(93)90029-5.
[2] G.L. Alves, M.R.B. Franco, Headspace gas chromatography–mass spectrometry of volatile compounds in murici (*Byrsonima crassifolia* L. Rich), J. Chromatogr. A 985 (2003) 297–301, https://doi.org/10.1016/S0021-9673(02)01398-5.
[3] C.M. Rezende, S.R.G. Fraga, Chemical and aroma determination of the pulp and seeds of murici (*Byrsonima crassifolia* L.), J. Braz. Chem. Soc. 14 (2003) 425–428, https://doi.org/10.1590/S0103-50532003000300014.
[4] V.R. De Souza, P.A.P. Pereira, F. Queiroz, S.V. Borges, J.D.D.S. Carneiro, Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits, Food Chem. 134 (2012) 381–386, https://doi.org/10.1016/j.foodchem.2012.02.191.
[5] L.R.B. Mariutti, E. Rodrigues, A.Z. Mercadante, Carotenoids from *Byrsonima crassifolia*: identification, quantification and in vitro scavenging capacity against peroxyl radicals, J. Food Anal. 31 (2013) 155–160, https://doi.org/10.1016/j.jfca.2013.05.005.
[6] L.C. Neves, P.M.C. Da Silva, C.G.B. Lima, V.J. Bastos, S.R. Roberto, Study to determine the optimum harvest date of murici (*Byrsonima coccolobifolia* Kunth.) from quality and functional attributes, Sci. Hortic. 188 (2015) 49–56, https://doi.org/10.1016/j.scienta.2015.02.039.
[7] M. Martínez-Vázquez, A.R. González-Esquinca, L. Cazares Luna, M.N. Moreno Gutiérrez, A.N. García-Argáez, Antimicrobial activity of *Byrsonima crassifolia* (L.) H.B.K, J. Ethnopharmacol. 66 (1999) 79–82, https://doi.org/10.1016/S0378-8741(98)00155-X.
[8] H.M. Rasmussen, E.J. Johnson, Nutrients for the aging eye, Clin. Interv. Aging 8 (2013) 741–748, https://doi.org/10.2147/CIA.S45399.
[9] L.R.B. Mariutti, E. Rodrigues, R.C. Chisté, E. Fernandes, A.Z. Mercadante, The Amazonian fruit *Byrsonima crassifolia* effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage, Food Res. Int. 64 (2014) 618–625, https://doi.org/10.1016/j.foodres.2014.07.032.
[10] R.M. Perez-Gutierrez, A. Muñiz-Ramirez, Y.G. Gomez, E.B. Ramírez, Antihyperglycemic, antihyperlipidemic and antiglycation effects of *Byrsonima crassifolia* fruit and seed in normal and streptozotocin-induced diabetic rats, Plant Foods Hum. Nutr. 65 (2010) 350–357, https://doi.org/10.1007/s11130-010-0181-5.
[11] C.P. Braga, A.C. Momentti, F.B. Peixoto, R.D.F.F. Baptista, F.A. Santos, F.H. Fava, A.A.H. Fernandes, Influence of treatment with quercetin on lipid parameters and oxidative stress of pregnant diabetic rats, J. Physiol. Pharmacol. 91 (2013) 171–177, https://doi.org/10.1139/cjpp-2012-0173.
[12] O.V. Santos, N.C.F. Correa, R.N. Carvalho Junior, C.E.F. Costa, J.F.C. Moraes, S.C.S. Lannes, Quality parameters and thermogravimetric and oxidative profile of Muruci oil (*Byrsonima crassifolia* L.) obtained by supercritical CO_2, J. Food Sci. Technol. 38 (2018) 172–179, https://doi.org/10.1590/1678-457x.30616.
[13] R.H.H. Pinto, C. Sena, O.V. Santos, W.A. Costa, A.M.C. Rodrigues, R.N. Carvalho, Extraction of bacaba (*Oenocarpus bacaba*) oil with supercritical CO_2: global yield isotherms, fatty acid composition, functional quality, oxidative stability, spectroscopic profile and antioxidant activity, Grasas Aceites 69 (2018) 1–8, https://doi.org/10.3989/gya.0883171.
[14] Y.A. Attia, M.A. Al-Harthi, M.A. Korish, M.M. Shiboob, Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market, Lipids Health Dis. 14 (2015) 1–8, https://doi.org/10.1186/s12944-015-0133-z.
[15] S. Chen, Z. Zeng, N. Hu, B. Bai, H. Wang, Y. Suo, Simultaneous optimization of the ultrasound-assisted extraction for phenolic compounds content and antioxidant activity of *Lycium ruthenicum* Murr. fruit using response surface methodology, Food Chem. 242 (2018) 1–8, https://doi.org/10.1016/j.foodchem.2017.08.105.
[16] N. Seifzadeh, M. Ali Sahari, M. Barzegar, H.A. Gavlighi, L. Calani, D. Del Rio, G. Galaverna, Evaluation of polyphenolic compounds in membrane concentrated pistachio hull extract, Food Chem. 277 (2019) 398–406, https://doi.org/10.1016/j.foodchem.2018.10.001.
[17] A. Masci, A. Coccia, E. Lendaro, L. Mosca, P. Paolicelli, S. Cesa, Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction, Food Chem. 202 (2016) 59–69, https://doi.org/10.1016/j.foodchem.2016.01.106.
[18] V.M.B. Cunha, M.P. Silva, S.H.B. Sousa, P.N. Bezerra, E.G.O. Menezes, N.J.N. Silva, D.A.D.S. Banna, M.E. Araújo, R.N. Carvalho Jr., Bacaba-de-leque (*Oenocarpus distichus* Mart.) oil extraction using supercritical CO_2 and bioactive compounds determination in the residual pulp, J. Supercrit. Fluids 144 (2018) 81–90, https://doi.org/10.1016/j.supflu.2018.10.010.
[19] C.C.R. Batista, M.S. Oliveira, M.E. Araújo, A.M.C. Rodrigues, J.R.S. Botelho, A.P.S. Souza Filho, N.T. Machado, R.N. Carvalho Jr., Supercritical CO_2 extraction of

açaí (*Euterpe oleracea*) berry oil: global yield, fatty acids, allelopathic activities, and determination of phenolic and anthocyanins total compounds in the residual pulp, J. Supercrit. Fluids 107 (2016) 364–369, https://doi.org/10.1016/j.supflu.2015.10.006.
[20] O. Wrona, K. Rafinska, C. Mozenski, B. Buszewski, Supercritical fluid extraction of bioactive compounds from plant materials, J. AOAC Int. 100 (2017) 1624–1635, https://doi.org/10.5740/jaoacint.17-0232.
[21] A. Berna, A. Tárrega, M. Blasco, S. Subirats, Supercritical CO_2 extraction of essential oil from orange peel; effect of the height of the bed, J. Supercrit. Fluids 18 (2000) 227–237, https://doi.org/10.1016/S0896-8446(00)00082-6.
[22] A.T. Serra, I.J. Seabra, M.E.M. Braga, M.R. Bronze, H.C. de Sousa, C.M.M. Duarte, Processing cherries (*Prunus avium*) using supercritical fluid technology. Part1: recovery of extract fractions rich in bioactive compounds, J. Supercrit. Fluids 55 (2010) 184–191, https://doi.org/10.1016/j.supflu.2010.06.005.
[23] C.G. Pereira, M.A.A. Meireles, Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives, Food Bioprocess. Technol. 3 (2010) 340–372, https://doi.org/10.1007/s11947-009-0263-2.
[24] AOAC, Association of Official Analytical Chemists, 16th ed., International Official Methods of Analysis, Arlington, 1997.
[25] D.-Y. Peng, D. Robinson, A new two-constant equation of state, Ind. Eng. Chem. Fundam. 15 (1976) 59–64, https://doi.org/10.1021/i160057a011.
[26] ASAE, Method of determining and expressing fineness of feed materials by sieving: revision of ASAE standard: ASAE S319.4, ASAE Standards: Standards, Engineering Practices and Data, American society of agricultural engineers, St. Joseph, Miguigan, 2008, pp. 1–4.
[27] D.B. Rodriguez-Amaya, M. Kimura, Harvestplus Handbook for Carotenoid Analysis, 1st ed., Harvest Plus, Washington, 2004.
[28] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymol. 109 (1999) 152–178, https://doi.org/10.1016/S0076-6879(99)99017-1.
[29] S. Georgé, P. Brat, P. Alter, M.J. Amiot, Rapid determination of polyphenols and vitamin C in plant-derived products, J. Agric. Food Chem. 53 (2005) 1370–1373, https://doi.org/10.1021/ jf048396b.
[30] AOCS, AOCS Official Method Ce 2-66: Preparation of Methyl Esters of Long Chain Fatty Acids, 7th ed., Official methods and recommended practices of the American Oil Chemists Society, Ill, 1997.
[31] T.L.V. Ulbricht, D.A.T. Southgate, Coronary heart disease: seven dietary factors, Lancet 338 (1991) 985–992, https://doi.org/10.1016/0140-6736(91)91846-M.
[32] J. Santos-Silva, R.J.B. Bessa, F. Santos-Silva, Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat, Livest. Prod. Sci. 77 (2002) 187–194, https://doi.org/10.1016/S0301-6226(02)00059-3.
[33] T.N.H. Lai, C. André, H. Rogez, E. Mignolet, T.B.T. Nguyen, Y. Larondelle, Nutritional composition and antioxidant properties of the sim fruit (*Rhodomyrtus tomentosa*), Food Chem. 168 (2015) 410–416, https://doi.org/10.1016/j.foodchem.2014.07.081.
[34] C. Nimalaratne, J. Wu, A. Schieber, Egg yolk carotenoids: composition, analysis, and effects of processing on their stability, in carotenoid cleavage products, in: P. Winterhalter, S.E. Ebeler (Eds.), Carotenoid Cleavage Products, American Chemical Society: ACS Symposium Series, Washington, 2013, pp. 219–225.
[35] V.R. Souza, P.A.P. Pereira, F. Queiroz, S.V. Borges, J.D.D.S. Carneiro, Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits, Food Chem. 134 (2012) 381–386, https://doi.org/10.1016/j.foodchem.2012.02.191.
[36] F. Sahena, I.S.M. Zaidul, S. Jinap, A.A. Karim, K.A. Abbas, N.A.N. Norulaini, A.K.M. Omar, Application of supercritical CO_2 in lipid extraction – a review, J. Food Eng. 95 (2009) 240–253, https://doi.org/10.1016/j.jfoodeng.2009.06.026.
[37] M. Herrero, M. Castro-Puyana, J.A. Mendiola, E. Ibañez, Compressed fluids for the extraction of bioactive compounds, Trends Analyt. Chem. 43 (2013) 67–83, https://doi.org/10.1016/j.trac.2012.12.008.
[38] R.P.F.F. Silva, T.A.P. Rocha-Santos, A. Duarte, Supercritical fluid extraction of bioactive compounds, Trends Analyt. Chem. 76 (2016) 40–51, https://doi.org/10.1016/j.trac.2015.11.013.
[39] B. Shan, J.-H. Xie, J.-H. Zhu, Y. Peng, Ethanol modified supercritical carbon dioxide extraction of flavonoids from *Momordica charantia* L. and its antioxidant activity, Food Bioprod. Process. 90 (2012) 579–587, https://doi.org/10.1016/j.fbp.2011.09.004.
[40] M.A. Summen, H.S. Erge, Thermal degradation kinetics of bioactive compounds and visual color in raspberry pulp, J. Food Process. Pres. 38 (2014) 551–557, https://doi.org/10.1111/jfpp.12002.
[41] F.A. Espinosa-Pardo, J. Martinez, H.A. Martinez-Correa, Extraction of bioactive compounds from peach palm pulp (*Bactris gasipaes*) using supercritical CO_2, J. Supercrit. Fluids 93 (2014) 2–6, https://doi.org/10.1016/j.supflu.2014.05.010.
[42] B. Cobb, J. Kallenbach, C. Hall, S. Pryor, Optimizing the supercritical fluid extraction of lutein from corn gluten meal, Food Bioprocess. Technol. 11 (2018) 757–764, https://doi.org/10.1007/s11947-017-2052-7.
[43] I.D. Nwachukwu, C.C. Udenigwe, R.E. Aluko, Lutein and zeaxanthin: production technology, bioavailability, mechanisms of action, visual function, and health claim status, Trends Food Sci. Technol. 49 (2016) 74–84, https://doi.org/10.1016/j.tifs.2015.12.005.
[44] J.K. Aschoff, S. Kaufmann, O. Kalkan, S. Neidhart, R. Carle, R.M. Schweiggert, In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices *Citrus sinensis* (L.) Osbeck, J. Agric. Food Chem. 63 (2015) 578–587, https://doi.org/10.1021/jf505297t.
[45] R.M. Schweiggert, R. Carle, Carotenoid deposition in plant and animal foods and its impact on bioavailability, Crit. Rev. Food Sci. Nutr. 57 (2017) 1807–1830, https://doi.org/10.1080/10408398.2015.1012756.
[46] Y.-X. Zou, W.-Z. Shen, S.-T. Liao, F. Liu, S.-Q. Zheng, J.B. Blumberg, O.C.Y. Chen, Mulberry leaf phenolics ameliorate hyperglycemia-induced oxidative stress and stabilize mitochondrial membrane potential in HepG2 cells, Int. J. Food Sci. Nutr. 65 (2014) 960–966, https://doi.org/10.3109/09637486.2014.940285.
[47] T. Sato, F. Fukuda, K.-I. Nihei, N. Itoh, Effect of temperature and pressure on the extraction of strawberry receptacles with a mixture of supercritical carbon dioxide and entrainers, J. Supercrit. Fluids 130 (2017) 23–29, https://doi.org/10.1016/j.supflu.2017.07.011.
[48] K.M. Schaich, X. Tian, J. Xie, Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays, J. Funct. Foods 14 (2015) 111–125, https://doi.org/10.1016/j.jff.2015.01.043.
[49] F.M. Barrales, C.A. Rezende, J. Martínez, Supercritical CO_2 extraction of passion fruit (*Passiflora edulis* sp.) seed oil assisted by ultrasound, J. Supercrit. Fluids 104 (2015) 183–192, https://doi.org/10.1016/j.supflu.2015.06.006.
[50] M. Khalil, J. Raila, M. Ali, K.M.S. Islam, R. Schenk, J.-P. Krause, F.J. Schweigert, H. Rawel, Stability and bioavailability of lutein ester supplements from Tagetes flower prepared under food processing conditions, J. Funct. Foods 4 (2012) 602–610, https://doi.org/10.1016/j.jff.2012.03.006.
[51] V. Laudadio, E. Ceci, N.M.B. Lastella, V. Tufarelli, Dietary high-polyphenols extra-virgin olive oil is effective in reducing cholesterol content in eggs, Lipids Health Dis. 14 (2015) 1–7, https://doi.org/10.1186/s12944-015-0001-x.
[52] G. Mutungi, J. Ratliff, M. Puglisi, M. Torres-Gonzalez, U. Vaishnav, J. Leite, E. Quann, J.S. Volek, M.L. Fernandez, Dietary cholesterol from eggs increases plasma HDL cholesterol in overweight men consuming a carbohydrate-restricted diet, J. Nutr. 138 (2008) 272–276, https://doi.org/10.1093/jn/138.2.272.
[53] E.R. Grela, K. Ognik, A. Czeck, J. Matras, Quality assessment of eggs from laying hens fed a mixture with Lucerne protein concentrate, J. Anim. Feed. Sci. Technol. 23 (2014) 236–243, https://doi.org/10.22358/jafs/65686/2014.
[54] S.J. Hosseini-Vashan, H. Sarir, N. Afzali, M. Mallekaneh, A. Allahressani, Influence of different layer rations on atherogenesis and thrombogenesis indices in egg yolks, J. Birjand Univ. Med. Sci 17 (2010) 265–273 http://journal.bums.ac.ir/article-1-842-en.html.
[55] J. Cohen, Statistical Power Analysis for the Behavioral Sciences, 2nd ed., Routledge, New York, 1988.
[56] Y.-W. Hsu, C.-F. Tsai, W.-K. Chen, Y.-C. Ho, F.-J. Lu, Determination of lutein and zeaxanthin and antioxidant capacity of supercritical carbon dioxide extract from daylily (*Hemerocallis disticha*), Food Chem. 129 (2011) 1813–1818, https://doi.org/10.1016/j.foodchem.2011.05.116.
[57] P.M.C.L. Reis, C. Dariva, G.Â.B. Vieira, H. Hense, Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (*Tamarindus indica*), sweet variety, J. Food Eng. 173 (2016) 116–123, https://doi.org/10.1016/j.jfoodeng.2015.11.001.
[58] J. Viganó, A.C. Aguiar, D.R. Moraes, J.L.P. Jara, M.N. Eberlin, C.B.B. Cazarin, M.R. Maróstica, J. Martínez, Sequential high pressure extractions applied to recover piceatannol and scirpusin B from passion fruit bagasse, Food Res. Int. 85 (2016) 51–58, https://doi.org/10.1016/j.foodres.2016.04.015.

CAPÍTULO III

Bioactive Compounds and Evaluation of Antioxidant, Cytotoxic and Cytoprotective Effects of Murici Pulp Extracts (*Byrsonima crassifolia*) Obtained by Supercritical Extraction in HepG2 Cells Treated with H_2O_2

(Compostos bioativos e avaliação dos efeitos antioxidante, citotóxico e citoprotetor de extratos de polpa de murici (*Byrsonima crassifolia*) obtidos por extração supercrítica em células HepG2 tratadas com H_2O_2)

Flávia Cristina Seabra Pires, Joicy Corrêa de Oliveira, Eduardo Gama Ortiz Menezes, Ana Paula de Souza e Silva, Maria Caroline Rodrigues Ferreira, Leticia Maria Martins Siqueira, Andryo Orfi Almada-Vilhena, Julio Cesar Pieczarka, Cleusa Yoshiko Nagamachi, Raul Nunes de Carvalho Junior

Reprinted from Foods, 2021:
PIRES, F. C. S.; OLIVEIRA, J. C.; MENEZES, E. G. O.; SILVA, A. P. S.; FERREIRA, M. C. R.; SIQUEIRA, L. M. M.; ALMADA-VILHENA, A. O.; PIECZARKA, J. C.; NAGAMACHI, C. Y.; CARVALHO JUNIOR, R. N. Bioactive Compounds and Evaluation of Antioxidant, Cytotoxic and Cytoprotective Effects of Murici Pulp Extracts (*Byrsonima crassifolia*) Obtained by Supercritical Extraction in HepG2 Cells Treated with H_2O_2. **Foods**, v. 10, n. 737, p. 1-22, 2021. DOI: 10.3390/foods10040737.

foods

MDPI

Article

Bioactive Compounds and Evaluation of Antioxidant, Cytotoxic and Cytoprotective Effects of Murici Pulp Extracts (*Byrsonima crassifolia*) Obtained by Supercritical Extraction in HepG2 Cells Treated with H_2O_2

Flávia Cristina Seabra Pires [1], Joicy Corrêa de Oliveira [2], Eduardo Gama Ortiz Menezes [3], Ana Paula de Souza e Silva [1], Maria Caroline Rodrigues Ferreira [1], Leticia Maria Martins Siqueira [3], Andryo Orfi Almada-Vilhena [4], Julio Cesar Pieczarka [4], Cleusa Yoshiko Nagamachi [4] and Raul Nunes de Carvalho Junior [5,*]

[1] LABEX (Extraction Laboratory), LABTECS (Supercritical Technology Laboratory), PPGCTA (PostGraduate Program in Food Science and Technology), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, Guamá, Belém, PA 66075-900, Brazil; flaviapiress@gmail.com (F.C.S.P.); anapaula-eng@hotmail.com (A.P.d.S.eS.); carolinerof@gmail.com (M.C.R.F.)

[2] LABEX (Extraction Laboratory), FEA (College of Food Engineering), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, Guamá, Belém, PA 66075-900, Brazil; joicyo90@gmail.com

[3] LABEX (Extraction Laboratory), PRODERNA (Postgraduate Program in Natural Resources Engineering in the Amazon), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, Guamá, Belém, PA 66075-900, Brazil; ortizegom@hotmail.com (E.G.O.M.); leticiammsiqueira@outlook.pt (L.M.M.S.)

[4] CEABIO (Center for Advanced Studies of the Biodiversity and Cell Culture Laboratory), PCT-Guamá (Guamá Science and Technology Park), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, Guamá, Belém, PA 66075-900, Brazil; andryoorfi@hotmail.com (A.O.A.-V.); julio@ufpa.br (J.C.P.); cleusa@ufpa.br (C.Y.N.)

[5] LABEX (Extraction Laboratory), LABTECS (Supercritical Technology Laboratory), FEA (College of Food Engineering), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, Guamá, Belém, PA 66075-900, Brazil

* Correspondence: raulncj@ufpa.br; Tel.: +55-91-98742-0195

Citation: Pires, F.C.S.; Oliveira, J.C.d.; Menezes, E.G.O.; Silva, A.P.d.S.e; Ferreira, M.C.R.; Siqueira, L.M.M.; Almada-Vilhena, A.O.; Pieczarka, J.C.; Nagamachi, C.Y.; Carvalho Junior, R.N.d. Bioactive Compounds and Evaluation of Antioxidant, Cytotoxic and Cytoprotective Effects of Murici Pulp Extracts (*Byrsonima crassifolia*) Obtained by Supercritical Extraction in HepG2 Cells Treated with H_2O_2. *Foods* **2021**, *10*, 737. https://doi.org/10.3390/foods10040737

Academic Editors: Elisabete M. C. Alexandre, Jorge Manuel Alexandre Saraiva and Manuela Pintado

Received: 30 January 2021
Accepted: 25 February 2021
Published: 30 March 2021

Abstract: The use of clean technologies in the development of bioactive plant extracts has been encouraged, but it is necessary to verify the cytotoxicity and cytoprotection for food and pharmaceutical applications. Therefore, the objective of this work was to obtain the experimental data of the supercritical sequential extraction of murici pulp, to determine the main bioactive compounds obtained and to evaluate the possible cytotoxicity and cytoprotection of the extracts in models of HepG2 cells treated with H_2O_2. The murici pulp was subjected to sequential extraction with supercritical CO_2 and CO_2+ethanol, at 343.15 K, and 22, 32, and 49 MPa. Higher extraction yields were obtained at 49 MPa. The oil presented lutein (224.77 μg/g), oleic, palmitic, and linoleic, as the main fatty acids, and POLi (17.63%), POO (15.84%), PPO (13.63%), and LiOO (10.26%), as the main triglycerides. The ethanolic extract presented lutein (242.16 μg/g), phenolic compounds (20.63 mg GAE/g), and flavonoids (0.65 mg QE/g). The ethanolic extract showed greater antioxidant activity (122.61 and 17.14 μmol TE/g) than oil (43.48 and 6.04 μmol TE/g). Both extracts did not show cytotoxicity and only murici oil showed a cytoprotective effect. Despite this, the results qualify both extracts for food/pharmaceutical applications.

Keywords: supercritical CO_2; supercritical CO_2+ethanol; global yield; lutein; phenolic compounds; flavonoids; fatty acids; triglycerides; ORAC; DPPH

1. Introduction

Murici (*Byrsonima crassifolia*) originates from Central and South Americas, where a tropical and temperate climate prevails. The fruit has been used for production of pulps, ice creams, etc. [1]. It has also been used in folk medicine, since the pre-Hispanic era, due to its therapeutic effects attributed to its bioactive compounds, such as antihyperglycemic, antihyperlipidemic, and antioxidant activities [2,3].

About these compounds contained in murici pulp, some studies have evaluated their phytochemical profile. In the carotenoid class, Rodrigues et al. [4] identified as major carotenoids (all-E)-lutein, (all-E)-zeaxanthin, and (all-E)-lutein-3-O-myristate coeluted with (all-E)-β-carotene, where the free and esterified lutein corresponded to 48% total carotenoid content. In the study by Irías-Mata et al. [5], the main carotenoids were (all-E)-lutein, (all-E)-zeaxanthin, and (all-E)-β-carotene, where (all-E)-lutein contributed 80–89% of total carotenoids. In the study carried out by Mariutti et al. [6], the majorities were (all-E)-lutein and (all-E)-zeaxanthin, where lutein represented about 67.39% of total carotenoids.

Another class that stands out is the phenolic compounds, where in the study developed by Gordon et al. [7] quercetin hexoside, quercetin and tetragalloylquinic acid were identified as the main carotenoids in murici pulp. In the work of Mariutti et al. [3], the major phenolic compounds were quercetin, gallic acid, quercetin hexoside, and quercetin pentoside, where quercetin represented about 66% of total phenolic compounds. Among the unsaturated fatty acids present in murici oil, the main compounds identified were oleic, linoleic, and linolenic acids, which make up about 64% of the lipid profile [8].

Due to the presence of several bioactive compounds in plants, its use has been increasingly explored due to the numerous benefits of its use on human health. One of the ways to verticalize the consumption of plant products is extract manufacturing. Some studies have addressed the obtaining of murici pulp extracts obtained by different extraction techniques and solvents such as exhaustive maceration with acetone [6], agitation and sonication on ultrasound with a methanol/ethyl acetate/petroleum ether extraction solution (1:1:1, $v/v/v$) [5], consecutive centrifugation with methanol extraction solution at 50% (v/v), acetone extraction solution at 70% (v/v), and water [9], agitation, and centrifugation with methanol/water extracting solution (8:2, v/v) [3], among others.

It is important to note that in recent years, there has been a growth in global interest in the use of clean technologies for the development of food and pharmaceutical products. Therefore, the use of supercritical technology appears as a potential alternative to obtain 100% pure plant extracts, free of solvents, and environmentally "green" [10–12], where in the scientific literature can be found two works that addressed the extraction of murici pulp by extraction The work developed by Santos et al. [8] addressed the quality parameters and thermogravimetric and oxidative profile of murici oil (*Byrsonima crassifolia* L.) obtained by supercritical CO_2, and the work developed by Pires et al. [13] addresses the determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction, the latter being the scientific basis of the present study.

Many studies have approached the use of supercritical CO_2 to obtain nonpolar plant extracts. Among the main compounds present in such extracts, unsaturated fatty acids and carotenoids stand out due to their beneficial health effects [14–17]. In addition, other studies have addressed their applications due to the characteristics of their medium and long-chain triglycerides [18–21]. Thus, it is important to know the type of triglyceride present in the extracts, since the prediction through the composition of fatty acids have showed a triglyceride profile quite similar to that obtained experimentally [22].

Although extraction with supercritical CO_2 is used on large scale, some studies have found that the residual extraction bed concentrates polar bioactive compounds, which allows defatted pulps to be better utilized in the industry, and minimizes the waste of high-value industrial residues in the environment [23–25]. For polarity modification, it is necessary to use a co-solvent, such as ethanol, because of its GRAS status, generally recognized as safe for use in food and pharmaceutical products, in small quantities [26].

Due to the potential application of plant extracts in the food and pharmaceutical area, several studies have been developed with the objective of verifying the possible cytotoxicity and cytoprotection of these extracts in cell models, where the human hepatoma cell line (HepG2) has been widely applied [27–30]. This strain has been used for demonstrating to be more sensitive to cytotoxic compounds compared to other cell lines (HeLa, ECC-1 and CHO-K1). In addition, HepG2 cells exhibit genotypic and phenotypic characteristics of normal liver cells, allowing for a wide variety of liver-specific metabolic responses, thus increasing the likelihood of predicting human susceptibility to the biological effects of plant extracts [30–33].

Therefore, the objective of this work was to obtain the experimental data of the supercritical sequential extraction of murici pulp, to determine the main bioactive compounds obtained and to evaluate the possible cytotoxicity and cytoprotection of the extracts in models of HepG2 cells treated with H_2O_2.

2. Materials and Methods

2.1. Raw Materials and Sample Preparation

Murici pulp was obtained in Terra Alta town (Pará, Brazil) (01°02′25.9″ S and 47°54′12.3″ W) (Accession Number: 199530/ Barcode: IAN199530/ Sisgen: A3DB8EB). Approximately 30 kg of pulp were lyophilized in a semi-industrial lyophilizer (model LJI 015, JJ Científica, São Carlos, Brazil), at 233.15 K, for two days. The freeze-dried pulp obtained presented 5.46 ± 0.30 g/100 g of moisture, 2.02 ± 0.02 g/100 g of ashes, 0.55 ± 0.02 g/100 g of proteins, 10.02 ± 0.15 g/100 g of lipids, 21.60 ± 0.59 g/100 g of sugars, 56.42 ± 0.21 µg/g (d.b.) of lutein, 15.43 ± 0.02 mg GAE/g (d.b.) of phenolic compounds, and 6.62 ± 0.36 µmol TE/g (d.b.) of antioxidant capacity (ORAC) [13]. The freeze-dried pulp was vacuum-packed, protected from light, and stored under freezing until extraction (253.15 K ± 1 K).

2.2. Sequential Supercritical Extractions: CO_2–SFE (Extraction with Supercritical CO_2) and CO_2+EtOH–SFE (Extraction with Supercritical CO_2 and Ethanol)

The sequential supercritical extractions were performed in the Extraction Laboratory (LABEX/UFPA/Brazil) in a SPE-ED SFE unit (model 7071, Applied Separations, Allentown, PA, USA), coupled to a CO_2 cylinder, a compressor (model CSA 78, Schulz S/A, Joinville, Brazil), a recirculator (model F08400796, Polyscience, Niles, IL, USA), and a CO_2 flowmeter (model M 5SLPM, Alicat Scientific, New York, USA). Based on the results of the evaluation of the solvent flow rates over the dynamic period of CO_2-SFE in the supercritical extraction of murici pulp, published in an article by our research group [13], the global yield isotherms of the present work were obtained in 343.15 K, under CO_2 densities of approximately 700, 800, and 900 kg/m^3, and CO_2+Ethanol densities of approximately 775, 858, and 944 kg/m^3. For the calculation of these densities at this temperature, pressures of 22, 32, and 49 MPa were considered, using the Aspen Hysys software (Aspen One 8.6), which applies the cubic state equation of Peng-Robinson [34] with binary interaction parameters zero.

The beds were formed by 0.02 kg of sample, corresponding to a bed height of 0.078 m, with porosity of 0.7. The supercritical extraction process was performed in a static period (closed system) of 1800 s, and a dynamic period (open system) of 3600 s, at a flow rate of 1.33×10^{-4} kg/s. In the first stage, the extractions of the freeze-dried pulp with supercritical CO_2 (CO_2-SFE) were carried out in order to obtain the most apolar extracts (Oils), and the defatted freeze dried murici pulp. In the second step, the defatted pulps were extracted with supercritical CO_2 and ethanol (CO_2+EtOH-SFE) (90:10, v/v) to obtain the most polar extracts (Ethanolic extracts). The co-solvent feeding was carried out only in the static period. The operating conditions of CO_2-SFE and CO_2+EtOH-SFE are shown in Table 1. After these procedures, the global yields were calculated on a dry basis (d.b.), from the mathematical ratio between the extract mass and the dry sample mass (freeze dried pulp or defatted pulps). For ethanolic extracts, the residual solvents were evaporated in a CentriVap centrifuge (model 78100, Labconco, Kansas, EUA), under vacuum, at 313.15 K.

The determinations were performed in triplicate and the results were expressed in in percentage on dry basis (% d.b.).

Table 1. Operating conditions of CO_2–SFE and CO_2+EtOH–SFE of murici pulp (*B. crassifolia*) and characterization of the different defatted pulps and extracts.

Samples	Pressure (MPa)	ϱSolvent (kg/m^3)	Luteín Content (μg/g d.b.) *	Phenolic Compounds (mg GAE/g d.b.) *	Flavonoids Content (mg QE/g d.b.) *	ORAC (μmol TE/g d.b.) *	DPPH (μmol TE/g d.b.) *
Oils (CO_2 –SFE)	22	695	62.38 ± 0.67 ^{c}	n.d.	n.d.	34.44 ± 0.21 ^{b}	6.01 ± 0.31 ^{a}
	32	804	196.18 ± 0.90 ^{b}	n.d.	n.d.	43.48 ± 0.88 ^{a}	6.04 ± 0.19 ^{a}
	49	900	224.77 ± 0.67 ^{a}	n.d.	n.d.	32.83 ± 0.27 ^{c}	6.01 ± 0.19 ^{a}
Defatted pulps	22	695	30.31 ± 0.06 ^{a}	24.58 ± 0.86 ^{a}	0.43 ± 0.01 ^{b}	1.90 ± 0.06 ^{a}	2.47 ± 0.10 ^{a}
	32	804	21.93 ± 0.10 ^{c}	19.67 ± 0.27 ^{b}	0.52 ± 0.01 ^{a}	1.45 ± 0.03 ^{c}	1.69 ± 0.15 ^{b}
	49	900	22.58 ± 0.10 ^{b}	12.02 ± 0.42 ^{c}	0.34 ± 0.01 ^{c}	1.58 ± 0.04 ^{b}	1.37 ± 0.13 ^{c}
Ethanolic extracts (CO_2+EtOH–SFE)	22	775	242.16 ± 0.55 ^{a}	6.73 ± 0.15 ^{c}	0.65 ± 0.07 ^{a}	100.88 ± 1.41 ^{b}	12.87 ± 0.38 ^{c}
	32	858	163.76 ± 0.94 ^{b}	7.93 ± 0.27 ^{b}	0.59 ± 0.02 ^{a}	117.45 ± 2.40 ^{a}	15.01 ± 0.19 ^{b}
	49	944	88.46 ± 0.58 ^{c}	20.63 ± 0.76 ^{a}	0.64 ± 0.01 ^{a}	122.61 ± 3.79 ^{a}	17.14 ± 0.45 ^{a}

* Different letters in the same column, per sample, showed a difference in significance level of 5% ($p < 0.05$); ORAC: oxygen radical absorbance capacity; DPPH: Antioxidant Activity; CO_2 –SFE: extraction with supercritical CO_2; CO_2+EtOH–SFE: extraction with supercritical CO_2 and ethanol; n.d: not detected; LOD of lutein = 6.17 × 10^{-4} kg/m^3; LOD of phenolic compounds = 1.22 × 10^{-4} kg/m^3; LOD of flavonoids = 1.28 × 10^{-4} kg/m^3; LOD of ORAC = 5.92 × 10^{-4} mol/m^3; LOD of DPPH = 6.46 × 10^{-4} mol/m^3.

2.3. Characterization of the Defatted Pulps and Extracts

2.3.1. Lutein Content

The lutein levels of the defatted pulps, oils and ethanolic extracts were determined according to Rodriguez-Amaya and Kimura method [35]. Acetone P.A. was used in the extraction, while a mixture of 50% ethyl ether and 50% petroleum ether (*v*/*v*) was used in the partition. The readings were made in a spectrophotometer (model IL-592, Kasuaki, Araucária, Brazil), at 445 nm. Further, the lutein absorption coefficient in ethanol (2550) was used. The analyses were performed in triplicate and the results were expressed in microgram of lutein per gram of sample on a dry basis (μg/g d.b.).

2.3.2. Phenolic Compounds

The phenolic compounds content of the defatted pulps and ethanolic extracts was determined by Folin–Ciocalteu method [36,37]. About 1.00 × 10^{-4} kg of sample was subjected to extraction (1:19) with 1.90 × 10^{-6} m^3 of an acidified ethanolic solution (70% ethanol, 29.5% distilled water and 0.5% acetic acid) (*m*/*v*). For the reactions, 1.00 × 10^{-8} m^3 of the extracted samples were diluted 1.59 × 10^{-6} m^3 of distilled water (1:159) (*v*/*v*). The reactions were performed using 5.00 × 10^{-7} m^3 of diluted sample, 1.25 × 10^{-5} m^3 of sodium carbonate solution at 7.5% and 2.50 × 10^{-7} m^3 of Folin at 1 N (*v*/*v*/*v*). The readings were performed in a spectrophotometer (model IL−592, Kasuaki, Araucária, Brazil), at 750 nm. The standard curve of gallic acid was made starting from a 0.50 kg/m^3 (*m*/*v*) stock solution, where five concentration points were used (0.9 to 8.3 mg/L), according to the equation of a straight line y = 0.0994x + 0.0108, where y is the absorbance and x is the concentration (See Appendix A). The determinations were performed in triplicate and the results were expressed in milligram of gallic acid equivalent per gram of sample on a dry basis (mg GAE/g d.b.).

2.3.3. Flavonoid Content

The determination of total flavonoid content of the defatted pulps and ethanolic extracts was performed by the spectrophotometric method [38,39]. About 1.00 × 10^{-4} kg of sample was subjected to extraction (1:19) with 1.90 × 10^{-6} m^3 of an acidified ethanolic solution (70% ethanol, 29.5% distilled water and 0.5% acetic acid) (*m*/*v*). For the reactions,

3.00×10^{-7} m^3 of the extracted samples were diluted 2.70×10^{-6} m^3 of distilled water (1:9) (v/v). The reactions were performed using 1.00×10^{-6} m^3 of diluted sample and 1.00×10^{-6} m^3 of aluminum chloride ethanolic solution at 2% (v/v). The readings were made in a spectrophotometer (model IL-592, Kasuaki, Araucária, Brazil), at 430 nm. The standard quercetin curve was made from a 0.10 kg/m^3 (m/v) ethanolic stock solution, where nine concentration points were used (0.2 to 13 mg/L), according to the equation of a straight line y = 0.0715x + 0.0689, where y is the absorbance and x is the concentration (See Appendix B). The determinations were performed in triplicate and the results were expressed in milligram of quercetin equivalent per gram of sample on a dry basis (mg QE/g d.b.).

2.3.4. Fatty Acids and Functional Potential

The fatty acid profile of the oils was determined by Gas Chromatography (model GC-2010, Shimadzu, Tokyo, Japan). The methylation was carried out according to the method Ce-2-66 of AOCS [40]. About 2.00×10^{-5} kg of murici oil was saponified with 4.00×10^{-7} m^3 of methanolic sodium hydroxide solution at 0.5 N, which was subjected to a heating block at 373.15 K for 300 s. The mixture was cooled and esterified with addition of 4.00×10^{-7} m^3 of boron trifluoride methanolic solution at 14%, being submitted again to heating at 373.15 K for 300 s. Then, the mixture was cooled and 8.50×10^{-6} m^3 of saturated sodium chloride solution and 1.00×10^{-6} m^3 of n-heptane UV-HPLC 99% were added. The mixture was stirred and left to rest until complete phase separation, where the supernatant was analyzed. The operating conditions were: helium as carrier gas, with 8.33×10^{-7} m^3/s flow rate, FID detector at 523.15 K, injector with 1:100 split ratio, and injection volume of 1.00×10^{-9} m^3. The column programmed temperature (TG-WAX MS A/30 m $\times$ 3.20×10^{-4} m $\times$ 2.50×10^{-7} m) was 323.15 K during 300 s, with a subsequent increase to 523.15 K. Fatty acids individual peaks were identified by comparing their retention times with known fatty acid patterns (Nu-Check-Prep, Inc., Elysian, MN, USA), under the same operating conditions. The retention times and each peak area were calculated using GC Software Solution. The determinations were done in triplicate and the results were expressed in percentage on dry basis (% d.b.). Functional potential was evaluated by atherogenicity (AI), thrombogenicity (TI), and hypocholesterolemic (HI) indexes, calculated from the polyunsaturated/saturated ratio of fatty acids [41,42].

2.3.5. Probable Triglyceride Compositions

By definition, triglycerides are made up of one molecule of glycerol and three molecules of fatty acids. In the process of determining the composition of fatty acids, the oil is transesterified and the triglyceride molecules are broken down allowing the release of fatty acids [43]. Thus, the possible composition of triglycerides can be predicted from the experimental composition of fatty acids using knowledge about combinatorial analysis. The triglyceride composition of murici oil was predicted based on the statistical technique of distribution of random variables (random theory 1,2,3) proposed by Norris and Mattil [44], which applies the knowledge of combinatorial analysis associated with an algorithm, which uses the concept of simple binomial tree. In other words, theory 1, 2, and 3 assumes that each fatty acid can be randomly distributed in any of the three positions of the glycerol molecule, without distinguishing the isomers and without mischaracterizing the concept of the triglyceride molecule. According to this theory, it is considered that from an amount of X of fatty acids, it is possible to obtain an amount of X^3 of triglycerides that will be formed. After obtaining the result of the possible combinations, each group was classified according to the theory of casual distribution through the carbon equivalent number (EC), calculated by the relationship between the carbon number (N°c) and the number of double bonds (N°db), disregarding the groups that presented concentration below 1% (EC = N°c − 2.N°db). The Visual Basic for Applications in Excel (VBA) spreadsheet used in the calculations was developed by the Laboratory of Separation Processes and Applied Thermodynamics (TERM@/UFPA).

2.3.6. Oxygen Radical Absorbance Capacity (ORAC)

The antioxidant capacities of the defatted pulps, oils, and ethanolic extracts were determined by ORAC method, according to Lai et al. [45]. For the extraction, about 2.00×10^{-4} kg of sample was homogenized in 1.80×10^{-6} m^3 of acetone P.A. (1:9) (m/v). For each sample, three dilutions were made in phosphate buffer solution pH 7.4, 800, 1200, and 1600× for oils, 60, 120, and 240× for defatted pulps and 1000, 2000, and 3000× for ethanolic extracts. About 2.50×10^{-8} m^3 of each dilution was added in 96-well microplates. The standard Trolox curve was made from a 0.10 mol/m^3 (mol/v) stock solution, where five concentrations points were used (0 to 8 µmol TE/L), according to the equation of a straight line y = 2.5162x + 1.0071, where y is the absorbance and x is the concentration (see Appendix C). A fluorescence solution in phosphate buffer solution pH 7.4 at 1.04×10^{-8} kg/m^3 (m/v) was used. The readings were performed on a Fluorescence Microplate Reader (model FLx800, BioTek, Winooski, VT, USA), monitoring the effect of the samples on the fluorescence decomposition, resulting from the oxidations induced by the peroxyl radical (ROO*), produced through the thermal decomposition (310.15 K) of the AAPH solution (2.2′-Azobis (2-amidinopropane) dihydrochloride) in phosphate buffer solution pH 7.4 at 41.40 kg/m^3 (m/v), in the presence of oxygen. The analysis was done in triplicate. The results were expressed in micromol of Trolox equivalent per gram of sample on a dry basis (µmol TE/g d.b.).

2.3.7. Antioxidant Activity (DPPH)

The defatted pulps, oils, and ethanolic extracts were subjected to the determination of antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hidrate) spectrophotometric method, according to Brand-Williams et al. [46], with modifications. Acetone P.A. was used as extractive solvent, where about 2.00×10^{-4} kg of sample was homogenized in 1.80×10^{-6} m^3 of acetone P.A. (1:9) (m/v). The procedure was carried out on a spectrophotometer (model IL-592, Kasuaki, Araucária, Brazil), at 517 nm, with readings monitored every 300 s until the reaction reached a plateau in 1800 s. The standard Trolox curve was performed starting from a 5 mol/m^3 (mol/v) stock solution, where six concentrations were used (0 to 800 µM), according to the equation of a straight line y = 0.0006x + 0.0033, where y is the absorbance and x is the concentration (See Appendix D). The reactions were performed using 5.00×10^{-8} m^3 of sample and 1.95×10^{-6} m^3 of DPPH solution at 0.06 mol/m^3 (mol/v) (v/v). The DPPH remaining at the end of the reaction was determined and quantified, using the standard Trolox curve. The antioxidant activity of the DPPH method was expressed in micromol of Trolox equivalent per gram of sample on a dry basis (µmol TE/g d.b.).

2.4. Cell Culture

HepG2 cells (liver hepatocellular carcinoma) were cultured for the cytotoxicity and cytoprotection assays of the extracts. The HepG2 cell lines were stored under cryogenics in liquid nitrogen, added with fetal bovine serum and DMSO. The cells were thawed, centrifuged, and the supernatant was discarded (fetal bovine serum+DMSO). Then, the HepG2 cells were added with DMEM medium and fetal bovine serum, transferred to T75 flasks and incubated at 310.15 K and 5% CO_2, under saturated humidity. The HepG2 cells contained in T75 flasks were washed twice with Hanks' balanced salt solution to remove possible cellular metabolites. Then, the cells were added with trypsin and incubated for 300 s at 310.15 K and 5% CO_2, under saturated humidity, to remove the cells adhered to the walls of the flask. DMEM was added to resolubilize the cells. For counting, 5.00×10^{-8} m^3 of trypan blue and 5.00×10^{-8} m^3 of cells in DMEM in eppendorf were added, homogenized and placed on a microscope slide. Cell counting was performed with the aid of an Inverted Microscope (Axiovert 200, Zeiss, Jena, Germany) and a manual cell counter (GT-08HM, Global Trade, Jaboticabal, Brazil).

2.5. In Vitro Evaluation of Cytotoxicity and Cytoprotection

The oil and ethanolic extract that had the highest levels lutein content were subjected to cytotoxicity and cytoprotection assay with HepG2 cells. The samples were solubilized in ethanol to a concentration of 8 kg/m^3 (m/v). For cytotoxicity tests, the samples were solubilized in DMEM medium at five concentration points (0.20, 0.10, 0.05, 0.025, and 0.01 kg/m^3) (m/v). For cytoprotection assays, the samples were solubilized in DMEM medium at a concentration of 0.05 kg/m^3 and 1.00×10^{-8} m^3 of hydrogen peroxide was added (v/v). The exposure was performed in 96-well microplates, where 1.00×10^{-7} m^3 of HepG2 cells solubilized in DMEM medium (13:87) (v/v) were added, which was equivalent to a concentration of 6.70×10^3 CFU/well. The microplates were incubated at 310.15 K and 5% CO_2 under saturated humidity for 24 h. Then, 1.00×10^{-7} m^3 of each sample concentration was added to the plates. The incubation times were 24 and 48 h for cytotoxicity tests and 24, 48 and 72 h for cytoprotection tests. For the colorimetric reaction, the MTT Assay Kit was used, using the Mosman protocol [47], where 1.00×10^{-7} m^3 of the MTT solution were added to the wells and the microplates were incubated for 3 h at 310.15 K and 5% CO_2, under saturated moisture in the absence of light. The MTT solution was removed and 1.00×10^{-7} m^3 of DMSO was added, incubating the plates again for 1 h, under the same conditions. After the reaction, the microplates were read in an Absorbance Microplate Reader (Elx800, BioTek, Winooski, USA) at 570 nm, in duplicate. Cytotoxicity and cytoprotection were determined by the average percentage of cell survival in relation to the unexposed control.

2.6. Statistical Analysis

The means and standard deviations were calculated for all analyses. The results of the physical–chemical analyses were submitted to Tukey test, at a significance level of 5% ($p < 0.05$). The results of the cytotoxicity and cytoprotection tests were normalized and subjected to the ANOVA test and Tukey test, at a significance level of 5% ($p < 0.05$). Excel 2000 SR-1 (Microsoft, Troy, NY, USA), Statistica Kernel Release 7.1 (StartSoft Inc., Tulsa, OK, USA) and Bioestat (version 5.3) programs were used as tools.

3. Results and Discussion

3.1. Global Yield Isotherms

Global yield isotherms of extracts of *B. crassifolia* pulp obtained by CO_2-SFE and CO_2+EtOH-SFE at 343.15 K can be seen in Figure 1. The global yields of CO_2-SFE ranged from 8.38% d.b. ± 0.13% d.b. (22 MPa-695 kg/m^3) to 9.83% d.b. ± 0.36% d.b. (49 MPa-900 kg/m^3). It can be seen that the increase in pressure and solvent density also increased the extraction yields. According to Silva et al. [48], pressure variations in isothermal conditions define the performance of an SFE system, since this parameter has a great influence on fluid hydrodynamics, solubility, and mass transfer. In the study by Pires et al. [13], for 323.15 K and 333.15 K isotherms of CO_2-SFE of murici pulp, such behavior was also observed. When comparing the influence of temperature on this response obtained in both studies, it can be observed that global yields also increased with increasing temperature. This can be attributed to the increase in the solvation power of the solvents in the most drastic extraction conditions, which allowed a greater solubilization of the murici pulp compounds [49]. This behavior has also been observed by other authors [22].

Global yields of CO_2+EtOH-SFE ranged from 5.47% d.b. ± 0.40% d.b. (22 MPa-775 kg/m^3) to 9.77% d.b. ± 0.10% d.b. (49 MPa-944 kg/m^3). It was possible to observe that the increase in pressure and density also increased the extraction yields. An inverse behavior was observed for 323.15 and 333.15 K isotherms, using the co-solvent in the dynamic period of CO_2+EtOH-SFE of murici pulp [13]. This demonstrates that the use of co-solvent only in the static extraction period, at higher temperatures, made it possible to obtain an extract with high yield. This is due to the longer contact time between the solvent mixture and the solutes, which caused an increase in the solubilization of these compounds, with a consequent increase in these yields. Thus, it can be said that applying

ethanol as a co-solvent (10% *v*/*v*), in the static period, increases the solubilization of polar bioactive compounds.

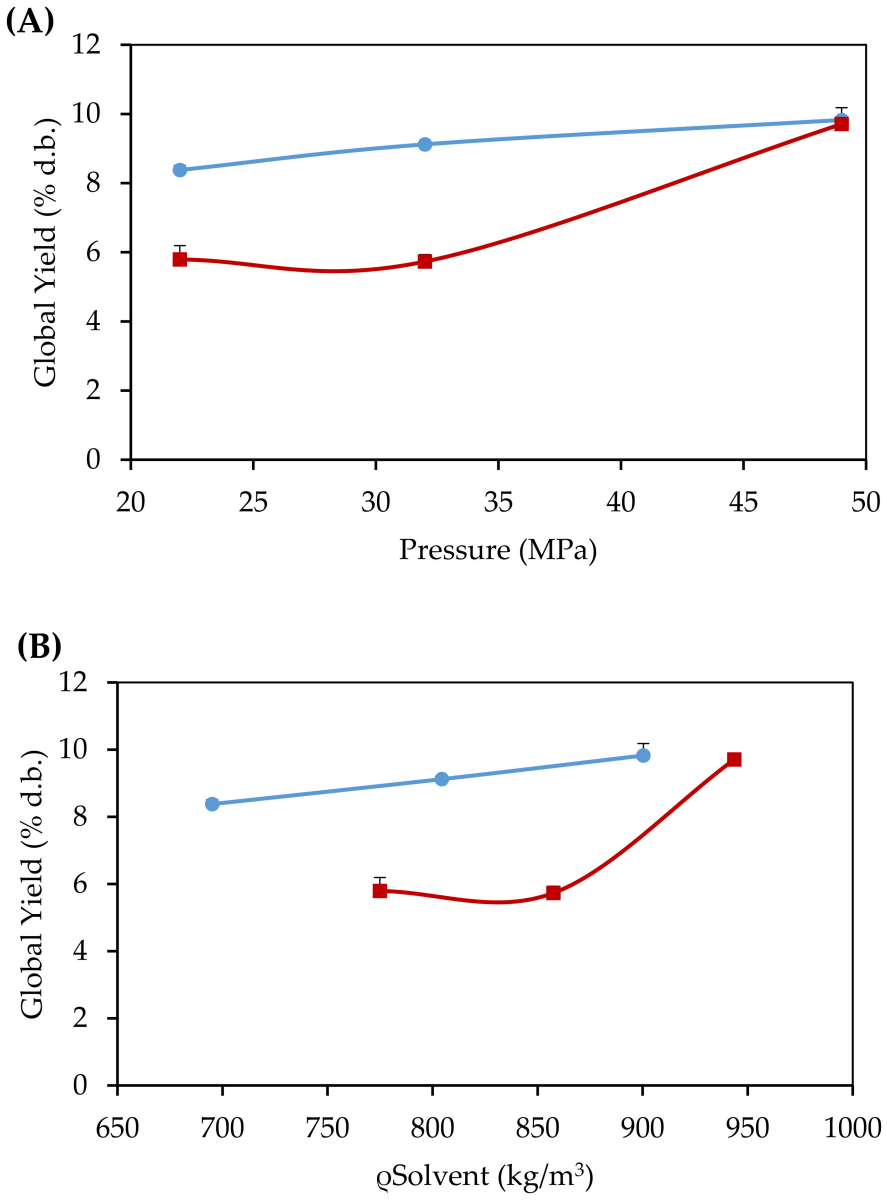


Figure 1. Global yield isotherms of murici pulp extracts (*B. crassifolia*) obtained by CO_2-SFE (—●—) (**A**) and CO_2+EtOH-SFE (—■—) (**B**), at 343.15 K (Standard deviations ≤ 0.4%).

3.2. Lutein Content

Lutein content of the defatted pulps, oils, and ethanolic extracts of *B. crassifolia* pulp can be seen in Table 1. Lutein contents of oils ranged from 62.38 µg/g d.b. ± 0.67 µg/g d.b. (22 MPa-695 kg/m^3) to 224.77 µg/g d.b. ± 0.67 µg/g d.b. (49 MPa-900 kg/m^3). These values were higher than those obtained for the murici pulp extract obtained by maceration with acetone (43.90 µg/g) [4]. The increase in pressure increased the lutein levels in the oils. This behavior was also observed by Yen et al. [50], for lutein extraction with supercritical CO_2. The high content of lutein in oils was mainly due to lutein being linked to fatty acids present in the plant matrices [51]. Thus, the nonpolar extract which presented the highest global yield consequently had the highest lutein content. The lutein composition of defatted pulps ranged from 21.93 µg/g d.b ± 0.10 µg/g d.b. (32 MPa-804 kg/m^3) to 30.31 µg/g d.b. ± 0.06 µg/g d.b. (22 MPa-695 kg/m^3). Pressure did not present a defined

behavior for these results; however, the fact that the higher content of lutein in the defatted matrix was obtained in the condition of lower content in the oils is justified, since the residual lutein remained in the ethanolic extracts ranged from 88.46 μg/g d.b. ± 0.58 μg/g d.b. (49 MPa-944 kg/m^3) to 242.16 μg/g d.b. ± 0.55 μg/g d.b. (22 MPa-775 kg/m^3). These results were superior to that found for the aqueous extract of murici pulp (23.39 μg/g) [52]. In this case, the increase in pressure reduced the levels of lutein, presenting an opposite behavior to that of CO_2-SFE. This shows that lutein was not obtained in CO_2-SFE, but in CO_2+EtOH-SFE. It was also possible to observe that the use of ethanol as a co-solvent enabled a good recovery of the remaining lutein in defatted pulp. This behavior was also evidenced by Cobb et al. [53]. Although carotenoids are mostly nonpolar compounds, lutein has an intermediate polarity [26]. This explains the presence of this compound in similar amounts in both extracts. When comparing the levels of lutein in the oil and ethanol extract of murici pulp obtained at 343.15 K in the present study with those found in the study by Pires et al. [13] obtained at 323.15 and 333.15 K, it is possible to observe that the increase in the process temperature increased the lutein levels in supercritical extractions with CO_2 and with CO_2+ethanol. This may have occurred due to the increase in the solute vapor pressure, which increased the diffusion rate and promoted an increase in the transfer rate of mass of lutein contained in the plant matrix for supercritical solvents [49]. This demonstrates that better recovery of lutein from murici pulp occurs in supercritical extraction at 343.15 K.

3.3. Phenolic Compounds

The contents of phenolic compounds in the defatted pulps and ethanolic extracts of *B. crassifolia* pulp can be seen in Table 1. For the recovery of phenolic compounds, it is important to emphasize that a pretreatment with supercritical CO_2 is necessary to obtain an extraction bed more concentrated in polar bioactive compounds [54]. This explains the fact that phenolic compounds from murici oils were not detected, containing results below the detection limit of the analysis (LOD = 1.22 × 10^{-4} kg/m^3), which indicates that these compounds were concentrated in defatted beds. This behavior is quite consistent, since phenolic compounds are polar substances and supercritical CO_2 is a nonpolar solvent [55]. Therefore, defatted beds were used to extract these compounds. The levels of phenolic compounds in the defatted pulps were in the range of 12.02 mg GAE/g d.b. ± 0.42 mg GAE/g d.b. (49 MPa-900 kg/m^3) and 24.58 mg GAE/g d.b. ± 0.86 mg GAE/g d.b. (22 MPa-695 kg/m^3). The increase in pressure decreased the levels of phenolic compounds in defatted pulps. This behavior was also observed by Batista et al. [23] for defatted açaí pulp (*Euterpe oleracea*), obtained under the same extraction conditions as the present study. This was possibly due to the fact that the increased pressure facilitated the enzymatic oxidation of defatted pulps [56]. The levels of phenolic compounds in ethanolic extracts ranged from 6.73 mg GAE/g d.b. ± 0.15 mg GAE/g d.b. (22 MPa-775 kg/m^3) to 20.63 mg GAE/g d.b. ± 0.76 mg GAE/g d.b. (49 MPa-944 kg/m^3). These values were higher than those found for the murici pulp extract obtained with an extracting solution of methanol, ethanol, distilled water, and hydrochloric acid (69:20:10:1, *v*/*v*/*v*/*v*) (0.80 mg GAE/g) [57]. The increase in pressure also increased the levels of phenolic compounds in ethanolic extracts. Some studies have also reported this behavior [58,59]. This may have occurred because the increased pressure promoted the rupture of plant tissues, cell walls, and organelles, improving the mass transfer of the solvent to the sample, and of compounds to the solvent [60,61].

3.4. Flavonoid Content

Flavonoid contents in the defatted pulps and ethanolic extracts of *B. crassifolia* pulp are shown in Table 1. The flavonoid contents of murici oils were not detected, being below the detection limit of the analysis (LOD = 1.28 × 10^{-4} kg/m^3). This behavior was similar to that obtained for the content of phenolic compounds, since flavonoids are one of the classes belonging to the group of polyphenols [62]. Flavonoid levels in defatted

pulps ranged from 0.34 mg QE/g d.b. ± 0.01 mg QE/g d.b. (49 MPa-900 kg/m^3) to 0.52 mg QE/g d.b. ± 0.01 mg QE/g d.b. (32 MPa-804 kg/m^3). The flavonoid content did not show a defined linear behavior with increasing pressure. This behavior demonstrates that the selectivity of these compounds is not defined mainly by pressure, but by the vapor pressure of solutes. The levels of flavonoids in ethanolic extracts were in the range of 0.59 mg QE/g d.b. ± 0.02 mg QE/g d.b. (22 MPa-775 kg/m^3) to 0.65 mg QE/g d.b. ± 0.07 mg QE/g d.b. (32 MPa-858 kg/m^3); however, there was no significant difference between these values. These results were higher than that found for the methanolic extract of murici pulp (0.2 mg QE/g) [63]. Although there was no difference between the results, it was possible to observe that the levels of recovery/concentration of flavonoids in the extracts were different, being 1.45, 1.01, and 1.85 times for pressures of 22, 32, and 49 MPa, respectively. It can be seen that up to 32 MPa, the increase in pressure reduced the recovery of flavonoid content. This may have been due to repulsive solute-solvent interactions. This behavior was also observed by Chauhan et al. [64] for the extraction of flavonoids from black grape juice (*Vitis vinifera*), at high pressures. However, ethanolic extract obtained at 49 MPa was the one that showed the best recovery/concentration of these compounds in comparison to the other extraction conditions, since its flavonoid content almost doubled (0.64 mg QE/g d.b.) in relation to the defatted pulps content used in the CO_2+EtOH-SFE, in this same condition (0.34 mg QE/g d.b.). This was possibly due to the increase in density of the solvent mixture that allowed a greater transfer of these compounds to the extract. Therefore, it can be said that 49 MPa pressure facilitated the extraction of flavonoids of murici pulp.

3.5. Fatty Acids and Functional Quality

The fatty acid profile of oils of *B. crassifolia* pulp is shown in Figure 2 and Table 2. In all pressures evaluated, it was possible to observe that there was no significant difference among the fatty acid profiles, with the majority being oleic acid, followed by palmitic and linoleic acids. This behavior and fatty acid profile were also obtained for the extraction of murici pulp conducted at temperatures from 323.15 K to 333.15 K, and pressures from 15 MPa to 42 MPa [13]. This allows us to affirm that temperature and pressure did not influence the composition of fatty acids present in the oils of murici pulp. In addition, the fatty acid profiles of the oils presented about 63% of unsaturated fatty acids (UFA), being predominantly monounsaturated with about 40% (MUFA). This was confirmed by the results obtained from the saturated fatty acids/unsaturated fatty acids (SFA/UFA) relation, which was 0.60. However, the fatty acid profile of murici pulp oil obtained with chloroform-methanol 2:1 (v/v) was slightly different from that observed, with palmitic, oleic, and linoleic acid as major factors [65]. These results indicate that the use of conventional extractions makes it possible to obtain murici pulp oils that are richer in saturated fatty acids, which reduces their functional potential. It can be observed that the atherogenicity index (AI) and thrombogenicity index (TI) were relatively low, whereas the hypocholesterolemic index (HI) was high. This demonstrates that the extracts have functional potential since the values of these indices indicate anti-atherosclerogenic, antithrombogenic, and antihypercholesterolemic effects [11,14]. These same functional potentials were observed in uxi oil [66] and bacaba oil [14] obtained by supercritical extraction. Therefore, this confirms that in any reported supercritical extraction condition, it is possible to obtain a relatively stable nonpolar extract of murici pulp with antithrombogenic, antihypercholesterolemic, and anti-atherosclerogenic activities, properties, constituting a product with functional quality.

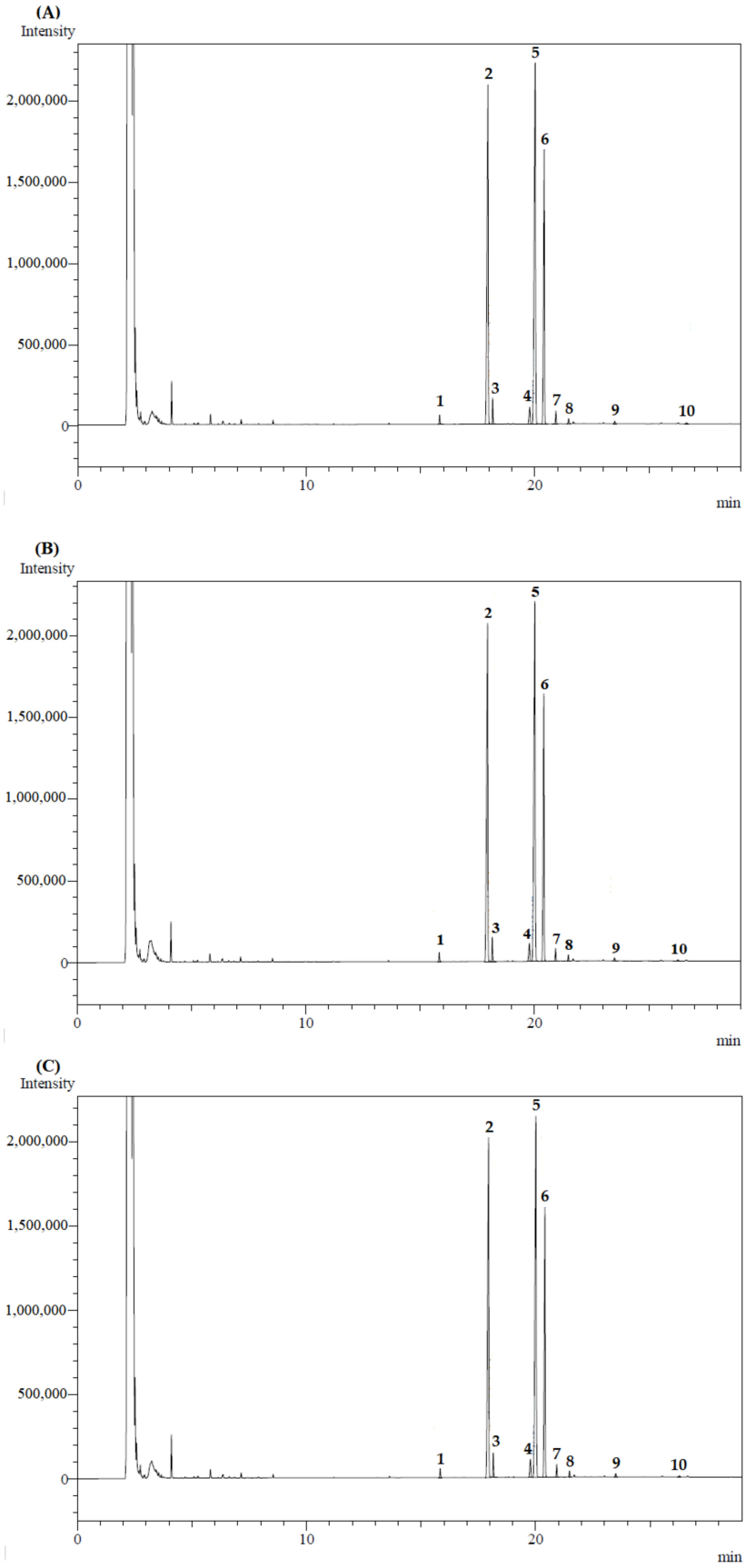


Figure 2. Total ion chromatogram of oils of murici pulp extracts (*B. crassifolia*) obtained by CO_2-SFE, at 343.15 K and 22 MPa (**A**), 32 MPa (**B**), and 49 MPa (**C**), with the peak numbers corresponding to the compounds identification cited in Table 2.

Table 2. Total fatty acids and functional potential of the oils of murici pulp (*B. crassifolia*) obtained by CO_2-SFE at 343.15 K.

Peak Number	Fatty Acids	Relative Area (% d.b.) *		
		22 MPa-695 kg/m^3	32 MPa-804 kg/m^3	49 MPa-900 kg/m^3
1	C14:0	0.51 ^{a}	0.51 ^{a}	0.52 ^{a}
2	C16:0	34.18 ^{a}	34.38 ^{a}	34.39 ^{a}
3	C16:1	1.43 ^{a}	1.39 ^{a}	1.39 ^{a}
4	C18:0	1.57 ^{a}	1.66 ^{a}	1.67 ^{a}
5	C18:1	39.21 ^{a}	39.04 ^{a}	39.04 ^{a}
6	C18:2	21.78 ^{a}	21.58 ^{a}	21.53 ^{a}
7	C18:3	0.72 ^{a}	0.72 ^{a}	0.74 ^{a}
8	C20:0	0.30 ^{a}	0.36 ^{a}	0.37 ^{a}
9	C22:0	0.18 ^{a}	0.23 ^{a}	0.24 ^{a}
10	C24:0	0.11 ^{a}	0.12 ^{a}	0.13 ^{a}
-	SFA	36.85 ^{a}	37.26 ^{a}	37.32 ^{a}
-	UFA	63.14 ^{a}	62.73 ^{a}	62.70 ^{a}
-	MUFA	40.64 ^{a}	40.43 ^{a}	40.43 ^{a}
-	PUFA	22.50 ^{a}	22.30 ^{a}	22.27 ^{a}
-	S/U	0.58 ^{a}	0.59 ^{a}	0.60 ^{a}
-	AI	0.57 ^{a}	0.58 ^{a}	0.58 ^{a}
-	IT	1.09 ^{a}	1.10 ^{a}	1.10 ^{a}
-	HH	1.82 ^{a}	1.80 ^{a}	1.80 ^{a}

* The results were obtained by mass basis; C14:0 (myristic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C18:0 (stearic acid); C18:1 (oleic acid); C18:2 (linoleic acid); C18:3 (linolenic acid); C20:0 (arachidic acid); C22:0 (behenic acid); C24:0 (lignoceric acid); SFA (saturated fatty acids); UFA (unsaturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); AI (atherogenicity index); TI (thrombogenicity index); HI (hypocholesterolemic index). The standard deviations for all fatty acids were lower than 1.8%. Different letters in the same line showed a difference in significance level of 5% ($p < 0.05$).

3.6. Probable Triglyceride Composition

The composition of triglycerides of oils of *B. crassifolia* pulp is shown in Table 3. For the prediction of this composition, palmitic, oleic, linoleic, palmitoleic, and stearic acids were used, since they represented about 99% of the total fatty acid composition of oils of *B. crassifolia* pulp. From these acids, it was possible to obtain 89% of the triglyceride composition present in oils. It can be seen that for all extraction conditions, the major triglycerides were POLi (palmitic, oleic, and linoleic), POO (palmitic, oleic, and oleic), PPO (palmitic, palmitic, and oleic), and LiOO (linoleic, oleic, and oleic), constituting 58% of the triglyceride profile. These results are in accordance with the fatty acid profiles obtained in the present study, since the major triglycerides obtained were formed by palmitic, oleic, and linoleic acids. The use of triglyceride profile prediction has also been reported by other authors and the compositions obtained are quite similar to those obtained experimentally [22,66,67]. All triglycerides identified can be classified as long-chain triglycerides (LCT), since they have more than 36 carbon atoms. It is important to highlight that LCT have been used in several applications, such as in short and long-term treatments with ketogenic diet on cortical spreading depression [68], to increase bioavailability and reduce hepatic metabolism of neurotropic agents [69], to reduce the release of inflammatory mediators from the gastrointestinal tract, and exert a protective effect on the inflammatory response of liver during sepsis [18]. Therefore, the knowledge of the triglyceride composition in the extracts is of great importance for its use in food and pharmaceutical industries.

Table 3. Prediction of triglyceride composition of the oils of murici pulp (*B. crassifolia*) obtained by CO_2-SFE at 343.15 K.

Triglycerides	X:Y *	MM (g/mol)	Fração Molar (% d.b.)		
			22 MPa-695 kg/m^3	32 MPa-804 kg/m^3	49 MPa-900 kg/m^3
PPP	48:0	806	3.63	4.05	4.07
OOO	54:3	884	6.45	5.98	5.99
LiLiLi	54:6	878	1.14	1.02	1.01
PPO	50:1	832	13.18	13.83	13.88
POO	52:2	858	15.97	15.76	15.79
LiPP	50:2	830	7.40	7.68	7.68
PLiLi	52:4	854	5.03	4.86	4.83
LiOO	54:4	882	10.87	9.97	9.93
OLiLi	54:5	880	6.10	5.54	5.49
POLi	52:3	856	17.93	17.50	17.46
OPaP	50:2	830	1.17	1.14	1.13
PSO	52:1	860	1.05	1.32	1.33

* X = Number of carbons; Y = Number of double bonds; MM = molar mass; P (palmitic acid); O (oleic acid); Li (linoleic acid); Pa (palmitoleic acid); S (stearic acid).

3.7. Oxygen Radical Absorbance Capacity (ORAC)

ORACs of the defatted pulps, oils and ethanolic extracts of *B. crassifolia* pulp are shown in Table 1. ORAC values of oils ranged from 32.83 µmol TE/g d.b. ± 0.27 µmol TE/g d.b. (49 MPa-900 kg/m^3) to 43.48 µmol TE/g d.b. ± 0.88 µmol TE/g d.b. (32 MPa-804 kg/m^3). It can be observed that the increase in pressure also increased the antioxidant capacity up to 32 MPa, reducing it again at 49 MPa. This behavior was also observed for oils of murici pulp obtained under the same CO_2 densities, but different conditions of temperature and pressure [13]. This allows us to say that the bioactive compounds that confer the antioxidant capacity to these extracts are more soluble at 800 kg/m^3, which confirms the selectivity of supercritical CO_2. The ORAC results of defatted pulps ranged from 1.45 µmol TE/g d.b. ± 0.03 µmol TE/g d.b. (32 MPa-804 kg/m^3) to 1.90 µmol TE/g d.b. ± 0.06 µmol TE/g d.b. (22 MPa-695 kg/m^3). The condition of highest ORAC of CO_2-SFE extracts, consequently, was the one with the lowest ORAC of the defatted pulps, since more compounds were removed from the extraction bed. The ORAC results of ethanolic extracts ranged from 100.88 µmol TE/g d.b. ± 1.41 µmol TE/g d.b. (22 MPa-775 kg/m^3) to 122.61 µmol TE/g d.b. ± 3.79 µmol TE/g d.b. (49 MPa-944 kg/m^3). These results were superior to that found for the murici pulp extract obtained with methanol/water solution (80:20, *v*/*v*) (26.50 µmol TE/g d.b.) [63]. It was possible to observe that the increase in pressure and, consequently, in the mixture density, also increased the antioxidant capacity of extracts. It can also be noted that the antioxidant capacities of the extracts obtained with ethanol as co-solvent were much higher than those that used only CO_2. This behavior was also observed by Serra et al. [54] when using ethanol as co-solvent (proportion 90:10, *v*/*v*). This occurred due to the change in polarity of the solvent, which allowed the extraction of polar bioactive substances, such as phenolic compounds and flavonoids.

3.8. Antioxidant Capacity (DPPH)

The results of DPPH of the defatted pulps, oils and ethanolic extracts of *B. crassifolia* pulp can be seen in Table 1. DPPH values of oils ranged from 6.01 µmol TE/g d.b. ± 0.31 µmol TE/g d.b. (22 MPa-695 kg/m^3 and 49 MPa-994 kg/m^3) to 6.04 µmol TE/g d.b. ± 0.19 µmol TE/g d.b. (32 MPa-804 kg/m^3), but there was no significant difference between them, suggesting that the pressure did not influence the antioxidant activity of nonpolar extracts. The values of DPPH of the defatted pulps ranged from 1.37 µmol TE/g d.b. ± 0.13 µmol TE/g d.b. (49 MPa-900 kg/m^3) to 2.47 µmol TE/g d.b. ± 0.10 µmol TE/g d.b. (22 MPa-695 kg/m^3). It can be seen that the increase in pressure decreased the antioxidant activity of the defatted pulps. DPPH values of ethanolic extracts ranged from

12.87 μmol TE/g d.b. ± 0.38 μmol TE/g d.b. (22 MPa-775 kg/m^3) to 17.14 μmol TE/g d.b. ± 0.45 μmol TE/g d.b. (49 MPa-994 kg/m^3). These results were superior to those found for the hydrophilic extracts of murici pulp obtained with acetone/water/acetic acid (70:29:5:0.5, *v*/*v*/*v*) (3.73 μmol TE/g) [70]. Then, it was observed, one more time, that the increase in pressure also increased the antioxidant capacity of these extracts. This was similar to the behavior observed for ORAC. It is important to note that the methods used to determine the antioxidant action of murici extracts, in the present study, were obtained by different techniques and, therefore, cannot be compared with each other [71]. However, both presented satisfactory values, which allows us to say that the extracts are good sources of antioxidant compounds.

3.9. In Vitro Evaluation of Cytotoxicity and Cytoprotection

The oil and ethanolic extract of the *B. crassifolia* pulp used in the cytotoxicity and cytoprotection tests were obtained at 49 MPa-900 kg/m^3 and 22 MPa-775 kg/m^3, respectively. The percentages of cytotoxicity and cytoprotection are shown in Figure 3. It can be observed in the cytotoxicity tests of the oil that the levels survival rates ranged from 91.72% to 103.32% (±8.95%) in 24 h, and 101.08% to 113.57% (±8.26%) in 48 h. For the ethanolic extract, the ranges from 97.66% to 120.68% (±20.47%) were obtained in 24 h, and 94.27% to 111.60% (±10.79%) in 48 h. For both samples, it was observed that regardless of the concentration and incubation time, no significant differences were observed in the levels of cell viability in relation to the negative controls and ethanol, which demonstrates that no extract showed cytotoxicity in the concentration conditions addressed.

Murici oil cytoprotection assays showed survival values in the 90.34% to 112.81% (±9.60%) range in 24 h, 95.29% to 109.77% (±6.28%) in 48 h, and 95.21% to 106.18% (±4.73%) in 72 h. Significant differences were observed only at 72 h in relation to the levels of survival between the oil and the H_2O_2 control, where the survival levels of cells exposed with murici oil increased 19.60% in relation to the H_2O_2 control. This allows us to say that the oil presented a cytoprotective effect in 72 h of incubation, where the increased exposure time stimulated proliferation and inhibited cell apoptosis, being effective in neutralizing the oxidative stress induced by H_2O_2 in HepG2 cells. The cytoprotection result of murici oil suggests that compounds such as unsaturated fatty acids, present in the oil, were responsible for increasing the cytoprotection time under the studied conditions. According to Bak, Jun and Jeong [27], non-polar bioactive compounds have stood out for biological roles due to their greater bioavailability compared to polar bioactive compounds. This may have occurred due to the induction of significant changes in the composition of cellular fatty acids, with an increase in the levels of essential fatty acids, indicating a process of absorption of these important bioactive components [72]. The cytoprotection tests of the ethanolic extract showed survival values in the ranges of 96.30% to 103.44% (±3.12%) in 24 h, 79.74% to 85.25% (±2.45%) in 48 h and 85.79% to 97.21% (±5.08%) in 72 h. It was observed that regardless of the concentration and the incubation time, no significant differences were observed in the levels of cell viability in relation to the controls, which demonstrates that the ethanolic extract did not present cytoprotection using the sample concentration of 0.05 kg/m^3 and the H_2O_2 concentration of 1.00×10^{-8} m^3. These results indicate that, although the ethanolic extract has shown higher ORAC and DPPH results, attributed to the phenolic compounds, flavonoids and lutein, the sample concentration and the hydrogen peroxide concentration used was not sufficient to cause a significant increase in the levels of survival. An opposite behavior was reported by Barbosa et al. [28] when using the same concentrations of H_2O_2 and sample to evaluate the cytoprotective effect of aqueous extracts of *Pleurotus ostreatus* in HepG2 cells, where significant differences in survival levels were found. Therefore, it is suggested that future work be carried out exploring the gradients of sample concentration and hydrogen peroxide to adapt the cellular model of oxidative stress to ethanol extracts of murici. Therefore, the non-toxic effects of both extracts and the cytoprotective effect of the oil suggest that both the oil and

the ethanolic extract of the *B. crassifolia* pulp can be used for the development of food, cosmetic and pharmaceutical products.

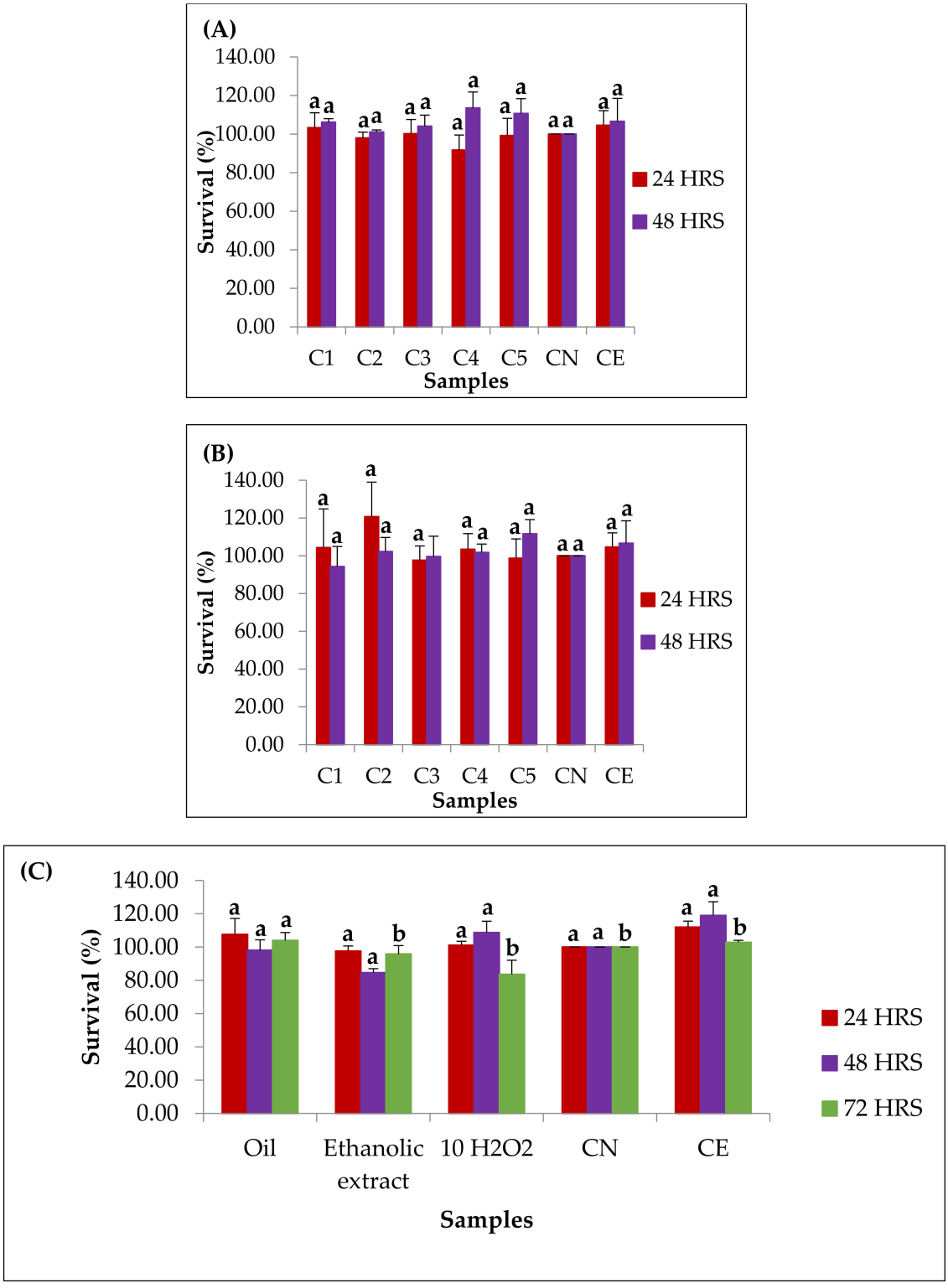


Figure 3. Cytotoxicity data of oil (**A**) and ethanolic extract (**B**) of murici pulp (*B. crassifolia*) obtained by CO_2-SFE and CO_2+EtOH-SFE, together with cytoprotection data using 0.05 kg/m^3 of samples in HepG2 cells treated with H_2O_2 (**C**). * Different letters for the same incubation time showed a difference in significance level of 5% ($p < 0.05$) (ANOVA; Tukey test for multiple comparisons); C1: concentration 0.20 kg/m^3; C2: concentration 0.10 kg/m^3; C3: concentration 0.05 kg/m^3; C4: concentration 0.025 kg/m^3; C5: concentration 0.01 kg/m^3; CN: negative control; CE: ethanol control; 10 H_2O_2: peroxide control.

4. Conclusions

Oils showed good levels of lutein, antioxidant capacity, and unsaturated fatty acids.

It was observed that the fatty acid compositions did not vary with the operating conditions of extraction. Therefore, in any condition of supercritical extraction reported, it was possible to obtain relatively stable oils of murici with antithrombogenic, antihypercholesterolemic, and anti-atherosclerogenic activities, constituting a product with functional quality.

The fatty acid profile allowed the prediction of triglyceride composition and demonstrated that oils are constituted of long-chain triglycerides, which can guide possible applications in food and pharmaceuticals sectors.

The use of co-solvent only in the static period made it possible to obtain polar extracts containing lutein, phenolic compounds, flavonoids, and antioxidant activity.

Obtaining the sequential extraction of murici pulp (*B. crassifolia*) by supercritical extraction at 343.15 K enabled the valuation and better use of the plant matrix for the production of high added-value extracts, with different compositions, and wide industrial applications.

Both extracts did not show cytotoxicity and only murici oil showed a cytoprotective effect in 72 h of exposure, where the increased exposure time stimulated proliferation and inhibited cell apoptosis, being effective in neutralizing the oxidative stress induced by H_2O_2 in HepG2 cells.

The results of this study showed remarkable nutritional/nutraceutical value of murici pulp extracts (*B. crassifolia*), and qualify it as a potential resource for use as food and in the development of dietary supplements.

Author Contributions: Conceptualization, F.C.S.P. and R.N.d.C.J.; methodology, F.C.S.P., E.G.O.M., J.C.P., C.Y.N., and R.N.d.C.J.; software, F.C.S.P., E.G.O.M., A.O.A.-V., M.C.R.F., and L.M.M.S.; validation, F.C.S.P., E.G.O.M., A.O.A.-V., and R.N.d.C.J.; formal analysis, F.C.S.P., J.C.d.O., and A.P.d.S.eS.; investigation, F.C.S.P., M.C.R.F., and L.M.M.S.; resources, J.C.P., C.Y.N., and R.N.d.C.J.; data curation, F.C.S.P., E.G.O.M., A.O.A.-V., M.C.R.F., and L.M.M.S.; writing—original draft preparation, F.C.S.P. and R.N.d.C.J.; writing—review and editing, F.C.S.P. and R.N.d.C.J.; visualization, F.C.S.P., A.P.d.S.eS., and R.N.d.C.J.; supervision, M.C.R.F. and L.M.M.S.; project administration, F.C.S.P. and R.N.d.C.J.; funding acquisition, J.C.P., C.Y.N., and R.N.d.C.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES)-Finance Code 001, and by the Pró-Reitoria de Pesquisa e Pós-Graduação (PROPESP/UFPA).

Acknowledgments: The authors also thank LABEX/UFPA, LABTECS/UFPA, and CEABIO/UFPA for their scientific and structural contributions.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. Calibration curve for phenolic compounds.

Gallic Acid Concentration (mg/L)	Absorbance *
White	0.094
0.9	0.093
1.8	0.188
2.5	0.262
6.3	0.647
8.3	0.828

* Absorbance = mean absorbance − white absorbance.

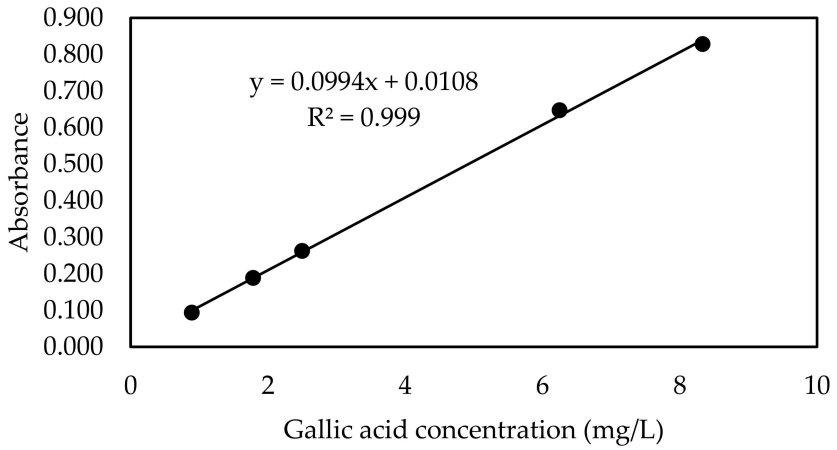


Figure A1. Calibration curve for phenolic compounds.

Appendix B

Table A2. Calibration curve for flavonoids content.

Quercetin Concentration (mg/L)	Absorbance *
White	0.087
0.2	0.098
0.5	0.233
2.4	0.400
4.5	0.537
6.5	0.669
8.3	0.803
10.0	0.896
11.5	0.973
13.0	0.087

* Absorbance = mean absorbance − white absorbance.

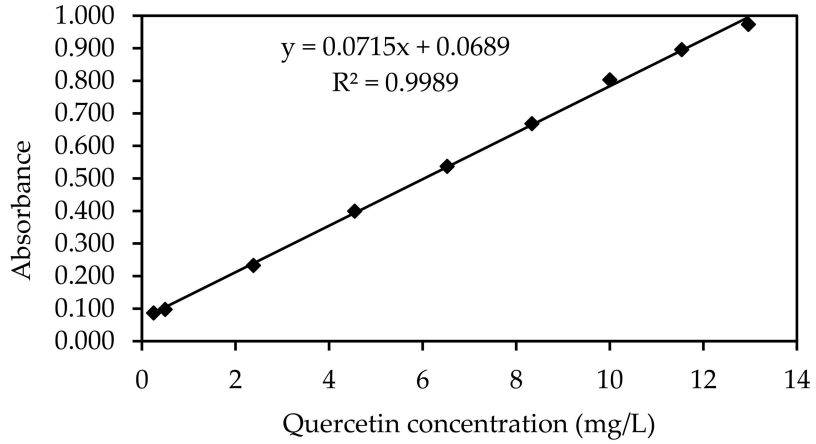


Figure A2. Calibration curve for flavonoids content.

Appendix C

Table A3. Calibration curve for ORAC.

Trolox Concentration (μmol TE/L)	NET AUC *
0	0.921 0.631 0.204 0.253 1.400
1	3.774 3.939 3.470 3.456
2	6.449 6.407 5.987 5.963 6.388 5.611
4	11.491 11.145 11.440 11.291
8	20.761 19.823 21.661 20.461

* NET AUC = AUC (Antioxidant) − AUC (blank); AUC = Area under the fluorescence decay curve.

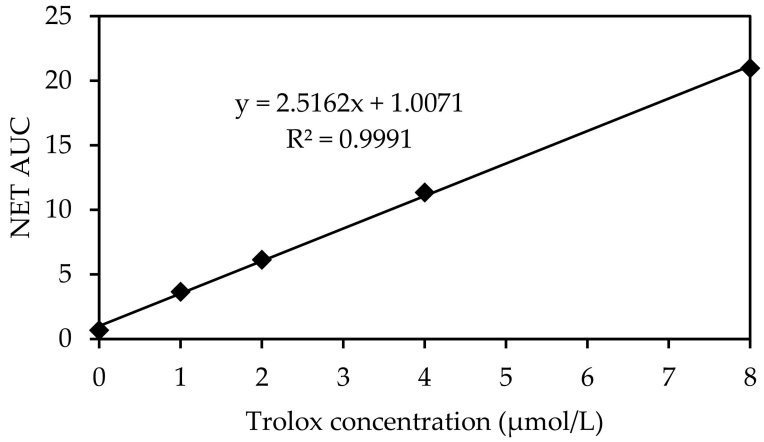


Figure A3. Calibration curve for ORAC.

Appendix D

Table A4. Calibration curve for antioxidant activity (DPPH).

Trolox Concentration (μmol/L)	Absorbance *
20.00	0.460
12.50	0.296
6.25	0.151
2.50	0.056
0.63	0.021
0	0.000

* Absorbance = mean absorbance − white absorbance.

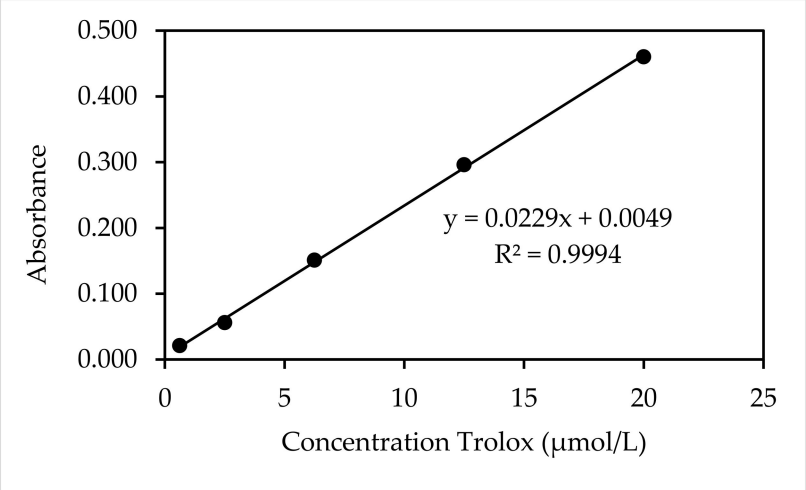


Figure A4. Calibration curve for antioxidant activity (DPPH).

References

1. Rezende, C.M.; Fraga, S.R.G. Chemical and aroma determination of the pulp and seeds of murici (*Byrsonima crassifolia* L.). *J. Braz. Chem. Soc.* **2003**, *14*, 425–428. [CrossRef]
2. Perez-Gutierrez, R.M.; Muñiz-Ramirez, A.; Gomez, Y.G.; Ramírez, E.B. Antihyperglycemic, antihyperlipidemic and antiglycation effects of *Byrsonima crassifolia* fruit and seed in normal and streptozotocin-induced diabetic rats. *Plant Foods Hum. Nutr.* **2010**, *65*, 350–357. [CrossRef]
3. Mariutti, L.R.B.; Rodrigues, E.; Chisté, R.C.; Fernandes, E.; Mercadante, A.Z. The Amazonian fruit *Byrsonima crassifolia* effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage. *Food Res. Int.* **2014**, *64*, 618–625. [CrossRef]
4. Rodrigues, D.B.; Mariutti, L.R.B.; Mercadante, A.Z. An In vitro digestion method adapted for carotenoids and carotenoid esters: Moving forward towards standardization. *Food Funct.* **2016**, *7*, 4992–5001. [CrossRef] [PubMed]
5. Irías-Mata, A.; Jiménez, V.M.; Steingass, C.B.; Schweiggert, R.M.; Carle, R.; Esquivel, P. Carotenoids and xanthophyll esters of yellow and red nance fruits (*Byrsonima crassifolia* (L.) Kunth) from Costa Rica. *Food Res. Int.* **2018**, *111*, 708–714. [CrossRef]
6. Mariutti, L.R.B.; Rodrigues, E.; Mercadante, A.Z. Carotenoids from *Byrsonima crassifolia*: Identification, quantification and in vitro scavenging capacity against peroxyl radicals. *J. Food Compos. Anal.* **2013**, *31*, 155–160. [CrossRef]
7. Gordon, A.; Jungfer, E.; Silva, B.A.; Maia, J.G.S.; Marx, F. Phenolic constituents and antioxidant capacity of four underutilized fruits from the Amazon region. *J. Agric. Food Chem.* **2011**, *59*, 7688–7699. [CrossRef] [PubMed]
8. Santos, O.V.; Correa, N.C.F.; Carvalho Junior, R.; Costa, C.E.F.; Moraes, J.D.F.C.; da Lannes, S.C.S. Quality parameters and thermogravimetric and oxidative profile of muruci oil (*Byrsonima crassifolia* L.) obtained by supercritical CO_2. *Food Sci. Technol.* **2018**, *38*, 172–179. [CrossRef]
9. Moreira-Araújo, R.S.D.R.; Barros, N.V.D.A.; Porto, R.G.C.L.; de Brandão, A.C.A.S.; de Lima, A.; Fett, R. Bioactive compounds and antioxidant activity three fruit species from the Brazilian Cerrado. *Rev. Bras. Frutic.* **2019**, *41*, 1–8. [CrossRef]
10. Cvjetko Bubalo, M.; Vidović, S.; Radojčić Redovniković, I.; Jokić, S. New perspective in extraction of plant biologically active compounds by green solvents. *Food Bioprod. Process.* **2018**, *109*, 52–73. [CrossRef]
11. Machida, H.; Takesue, M.; Smith, R.L. Green chemical processes with supercritical fluids: Properties, materials, separations and energy. *J. Supercrit. Fluids* **2011**, *60*, 2–15. [CrossRef]
12. Herrero, M.; Castro-Puyana, M.; Mendiola, J.A.; Ibañez, E. Compressed fluids for the extraction of bioactive compounds. *Trends Anal. Chem.* **2013**, *43*, 67–83. [CrossRef]
13. Pires, F.C.S.; de e Silva, A.P.S.; de los Salazar, M.A.R.; da Costa, W.A.; da Costa, H.S.C.; Lopes, A.S.; Rogez, H.; de Carvalho Junior, R.N. Determination of process parameters and bioactive properties of the murici pulp (*Byrsonima crassifolia*) extracts obtained by supercritical extraction. *J. Supercrit. Fluids* **2019**, *146*, 128–135. [CrossRef]
14. Pinto, R.H.H.; Sena, C.; Santos, O.V.; Da Costa, W.A.; Rodrigues, A.M.C.; Carvalho Junior, R.N. Extraction of bacaba (*Oenocarpus bacaba*) oil with supercritical CO_2: Global yield isotherms, fatty acid composition, functional quality, oxidative stability, spectroscopic profile and antioxidant activity. *Grasas Aceites* **2018**, *69*, 246. [CrossRef]
15. Salvador, A.A.; Podestá, R.; Block, J.M.; Ferreira, S.R.S. Increasing the value of pecan nut [*Carya illinoinensis* (Wangenh) C. Koch] cake by means of oil extraction and antioxidant activity evaluation. *J. Supercrit. Fluids* **2016**, *116*, 215–222. [CrossRef]
16. Carvalho, R.H.R.; Galvão, E.L.; Barros, J.A.C.; Conceição, M.M.; Sousa, E.M.B.D. Extraction, fatty acid profile and antioxidant activity of sesame extract: (*Sesamum indicum* L.). *Braz. J. Chem. Eng.* **2012**, *29*, 409–420. [CrossRef]

17. Pires, F.C.S.; e de Silva, A.P.S.; Silva, I.Q.; de Oliveira, J.C.; Gama, E.M.O.; Costa, W.A.; de Carvalho Junior, R.N. Application of supercritical technology in the production of dietary supplement based on plant extracts. In *Green Sustainable Process for Chemical and Environmental Engineering and Science*; Inamuddin, R., Boddula, M.I., Ahamed, A.M., Asiri, Eds.; Solventes; Elsevier: Amsterdam, The Netherlands, 2021; pp. 161–183. [CrossRef]
18. Feilitzsch, M.; Maier, T.; Junginger, J.; Meile, T.; Kueper, M.A.; Koenigsrainer, A.; Glatzle, J. W1834 Enteral Immunonutrition with Long Chain Triglycerides Significantly Reduces the Recruitment of Immune Cells Into the Liver During Experimental Sepsis. *Gastroenterology* **2008**, *134*, 911. [CrossRef]
19. Lira, C.R.G.; Zucco, F.; Negrão, A.N.; Silva, M.A.S.; Murakami, F.S. Nutraceuticals: An overview on safety, quality control and legislation. *Rev. Bras. Farmácia* **2009**, *90*, 45–49.
20. St-Onge, M.P.; Mayrsohn, B.; O'keeffe, M.; Kissileff, H.R.; Choudhury, A.R.; Laferrère, B. Impact of medium and long chain triglycerides consumption on appetite and food intake in overweight men. *Eur. J. Clin. Nutr.* **2014**, *68*, 1134–1140. [CrossRef] [PubMed]
21. Daar, J.; Khan, A.; Khan, J.; Khan, A.; Khan, G.M. Studies on self-nanoemulsifying drug delivery system of flurbiprofen employing long, medium and short chain triglycerides. *Pak. J. Pharm. Sci.* **2017**, *30*, 601–606.
22. Cunha, V.M.B.; da Silva, M.P.; de Sousa, S.H.B.; do Bezerra, P.N.; Menezes, E.G.O.; da Silva, N.J.N.; da Banna, D.A.D.S.; Araújo, M.E.; de Carvalho Júnior, R.N. The Journal of Supercritical Fluids Bacaba-de-leque (*Oenocarpus distichus* Mart.) oil extraction using supercritical CO_2 and bioactive compounds determination in the residual pulp. *J. Supercrit. Fluids* **2019**, *144*, 81–90. [CrossRef]
23. de Batista, C.C.R.; de Oliveira, M.S.; Araújo, M.E.; Rodrigues, A.M.C.; Botelho, J.R.S.; da Souza Filho, A.P.S.; Machado, N.T.; de Carvalho Junior, R.N. Supercritical CO_2 extraction of açaí (*Euterpe oleracea*) berry oil: Global yield, fatty acids, allelopathic activities, and determination of phenolic and anthocyanins total compounds in the residual pulp. *J. Supercrit. Fluids* **2016**, *107*, 364–369. [CrossRef]
24. Santos, O.V.; Corrêa, N.C.F.; Carvalho, R.N.; Costa, C.E.F.; França, L.F.F.; Lannes, S.C.S. Comparative parameters of the nutritional contribution and functional claims of Brazil nut kernels, oil and defatted cake. *Food Res. Int.* **2013**, *51*, 841–847. [CrossRef]
25. Pohl, F.; Goua, M.; Bermano, G.; Russell, W.R.; Scobbie, L.; Maciel, P.; Kong Thoo Lin, P. Revalorisation of rapeseed pomace extracts: An in vitro study into its anti-oxidant and DNA protective properties. *Food Chem.* **2018**, *239*, 323–332. [CrossRef]
26. Nwachukwu, I.D.; Udenigwe, C.C.; Aluko, R.E. Lutein and zeaxanthin: Production technology, bioavailability, mechanisms of action, visual function, and health claim status. *Trends Food Sci. Technol.* **2016**, *49*, 74–84. [CrossRef]
27. Bak, M.J.; Jun, M.; Jeong, W.S. Antioxidant and hepatoprotective effects of the red ginseng essential oil in H_2O_2-treated HepG2 cells and CCL 4-treated mice. *Int. J. Mol. Sci.* **2012**, *13*, 2314–2330. [CrossRef] [PubMed]
28. Barbosa, J.R.; Maurício, M.M.; Oliveira, L.C.; Luiza, L.H.; Almada-Vilhena, A.O.; Oliveira, R.M.; Pieczarka, J.C.; Davi do Socorro, B.; Carvalho Junior, R.N. Obtaining extracts rich in antioxidant polysaccharides from the edible mushroom *Pleurotus ostreatus* using binary system with hot water and supercritical CO_2. *Food Chem.* **2020**, *330*, 127173. [CrossRef] [PubMed]
29. Hong, S.; Sowndhararajan, K.; Joo, T.; Lim, C.; Cho, H.; Kim, S.; Kim, G.Y.; Jhoo, J.W. Ethanol and supercritical fluid extracts of hemp seed (*Cannabis sativa* L.) increase gene expression of antioxidant enzymes in HepG2 cells. *Asian Pac. J. Reprod.* **2015**, *4*, 147–152. [CrossRef]
30. Jondeau, A.; Dahbi, L.; Bani-Estivals, M.H.; Chagnon, M.C. Evaluation of the sensitivity of three sublethal cytotoxicity assays in human HepG2 cell line using water contaminants. *Toxicology* **2006**, *226*, 218–228. [CrossRef] [PubMed]
31. Schoonen, W.G.E.J.; De Roos, J.A.D.M.; Westerink, W.M.A.; Débiton, E. Cytotoxic effects of 110 reference compounds on HepG2 cells and for 60 compounds on HeLa, ECC-1 and CHO cells. II Mechanistic assays on NAD(P)H, ATP and DNA contents. *Toxicol. Vitr.* **2005**, *19*, 491–503. [CrossRef] [PubMed]
32. Darroudi, F.; Natarajan, A.T. Metabolic activation of chemicals to mutagenic carcinogens by human hepatoma microsomal extracts in Chinese hamster ovary cells (in vitro). *Mutagenesis* **1993**, *8*, 11–15. [CrossRef] [PubMed]
33. Natarajan, A.T.; Darroudi, F. Use of human hepatoma cells for in vitro metabolic activation of chemical mutagens/carcinogens. *Mutagenesis* **1991**, *6*, 399–403. [CrossRef]
34. Peng, D.Y.; Robinson, D.B. A New Two-Constant Equation of State. *Ind. Eng. Chem. Fundam.* **1976**, *15*, 59–64. [CrossRef]
35. Rodriguez-Amaya, D.; Kimura, M. *HarvestPlus Handbook for Carotenoid Analysis*, 1st ed.; Breeding Crops for Better Nutrition: Washington, DC, USA, 2004.
36. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1998**, *299*, 152–178. [CrossRef]
37. Georgé, S.; Brat, P.; Alter, P.; Amiot, M.J. Rapid determination of polyphenols and vitamin C in plant-derived products. *J. Agric. Food Chem.* **2005**, *53*, 1370–1373. [CrossRef]
38. Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J.; Nacoulma, O.G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem.* **2005**, *91*, 571–577. [CrossRef]
39. Dowd, L.E. Spectrophotometric determination of quercetin. *Anal. Chem.* **1959**, *31*, 1184–1187. [CrossRef]
40. AOCS. Official Methods and Recommended Practices of the American Oil Chemists' Society (method Ce 2-66): Preparation of methyl esters of fatty acids. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 7th ed.; The American Oil Chemists' Society: Boulder, CO, USA, 2017.
41. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary heart disease: Seven dietary factors. *Lancet* **1991**, *338*, 985–992. [CrossRef]

42. Bessa, R.J.B. Effect of genotype, feeding system and slaughter weight on the quality of light lambs II. Fatty acid composition of meat. *Livest. Prod. Sci.* **2002**, *77*, 187–194.
43. Gunstone, F.D.; Norris, F.A. *Lipids in Food Chemistry, Biochemistry and Technology*, 1st ed.; Elsevier: Pergamon, Turkey, 1983.
44. Norris, F.A.; Mattil, K.F. A new approach to the glyceride structure of natural fats. *J. Am. Oil Chem. Soc.* **1947**, *24*, 274–275. [CrossRef]
45. Lai, T.N.H.; André, C.; Rogez, H.; Mignolet, E.; Nguyen, T.B.T.; Larondelle, Y. Nutritional composition and antioxidant properties of the sim fruit (*Rhodomyrtus tomentosa*). *Food Chem.* **2015**, *168*, 410–416. [CrossRef]
46. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
47. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [CrossRef]
48. da Silva, R.P.F.F.; Rocha-Santos, T.A.P.; Duarte, A.C. Supercritical fluid extraction of bioactive compounds. *Trends Anal. Chem.* **2016**, *76*, 40–51. [CrossRef]
49. Pereira, C.G.; Meireles, M.A.A. Supercritical fluid extraction of bioactive compounds: Fundamentals, applications and economic perspectives. *Food Bioprocess Technol.* **2010**, *3*, 340–372. [CrossRef]
50. Yen, H.W.; Chiang, W.C.; Sun, C.H. Supercritical fluid extraction of lutein from Scenedesmus cultured in an autotrophical photobioreactor. *J. Taiwan Inst. Chem. Eng.* **2012**, *43*, 53–57. [CrossRef]
51. Schweiggert, R.M.; Carle, R. Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1807–1830. [CrossRef] [PubMed]
52. De Souza, V.R.; Brum, M.C.M.; Guimarães, I.D.S.; De Freitas Dos Santos, P.; Do Amaral, T.O.; Abreu, J.P.; Passos, T.; Freitas-Silva, O.; Gimba, E.R.P.; Teodoro, A.J. Amazon fruits inhibit growth and promote pro-apoptotic effects on human ovarian carcinoma cell lines. *Biomolecules* **2019**, *9*, 707. [CrossRef]
53. Cobb, B.F.; Kallenbach, J.; Hall, C.A., III; Pryor, S.W. Optimizing the Supercritical Fluid Extraction of Lutein from Corn Gluten Meal. *Food Bioprocess Technol.* **2018**, *11*, 757–764. [CrossRef]
54. Serra, A.T.; Seabra, I.J.; Braga, M.E.M.; Bronze, M.R.; de Sousa, H.C.; Duarte, C.M.M. Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: Recovery of extract fractions rich in bioactive compounds. *J. Supercrit. Fluids* **2010**, *55*, 184–191. [CrossRef]
55. Cunha, V.M.B.; da Silva, M.P.; da Costa, W.A.; de Oliveira, M.S.; Bezerra, F.W.F.; de Melo, A.C.; Pinto, R.H.H.; Machado, N.T.; Araujo, M.E.; de Carvalho Junior, R.N. Carbon Dioxide Use in High-Pressure Extraction Processes. In *Carbon Dioxide Chemistry, Capture and Oil Recovery*; IntechOpen: London, UK, 2018; pp. 211–240.
56. Terefe, N.S.; Kleintschek, T.; Gamage, T.; Fanning, K.J.; Netzel, G.; Versteeg, C.; Netzel, M. Comparative effects of thermal and high pressure processing on phenolic phytochemicals in different strawberry cultivars. *Innov. Food Sci. Emerg. Technol.* **2013**, *19*, 57–65. [CrossRef]
57. Silva, E.M.; Souza, J.N.S.; Rogez, H.; Rees, J.F.; Larondelle, Y. Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chem.* **2007**, *101*, 1012–1018. [CrossRef]
58. Casquete, R.; Castro, S.M.; Villalobos, M.C.; Serradilla, M.J.; Queirós, R.P.; Saraiva, J.A.; Córdoba, M.G.; Teixeira, P. High pressure extraction of phenolic compounds from citrus peels†. *High Press. Res.* **2014**, *34*, 447–451. [CrossRef]
59. Pinela, J.; Prieto, M.A.; Barros, L.; Carvalho, A.M.; Oliveira, M.B.P.P.; Saraiva, J.A.; Ferreira, I.C.F.R. Cold extraction of phenolic compounds from watercress by high hydrostatic pressure: Process modelling and optimization. *Sep. Purif. Technol.* **2018**, *192*, 501–512. [CrossRef]
60. Patras, A.; Brunton, N.P.; Da Pieve, S.; Butler, F. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 308–313. [CrossRef]
61. Huang, H.W.; Hsu, C.P.; Yang, B.B.; Wang, C.Y. Advances in the extraction of natural ingredients by high pressure extraction technology. *Trends Food Sci. Technol.* **2013**, *33*, 54–62. [CrossRef]
62. Chen, Z.; Zheng, S.; Li, L.; Jiang, H. Metabolism of Flavonoids in Human: A Comprehensive Review. *Curr. Drug Metab.* **2014**, *15*, 48–61. [CrossRef] [PubMed]
63. Souza, J.N.S.; Silva, E.M.; Loir, A.; ßois Rees, J.-F.; Rogez, H.; Larondelle, Y. Antioxidant capacity of four polyphenol-rich Amazonian plant extracts: A correlation study using chemical and biological in vitro assays. *Food Chem.* **2008**, *106*, 331–339. [CrossRef]
64. Chauhan, O.P.; Raju, P.S.; Ravi, N.; Roopa, N.; Bawa, A.S. Studies on retention of antioxidant activity, phenolics and flavonoids in high pressure processed black grape juice and their modelling. *Int. J. Food Sci. Technol.* **2011**, *46*, 2562–2568. [CrossRef]
65. Rodrigues, D.B.; Mariutti, L.R.B.; Mercadante, A.Z. Two-step cleanup procedure for the identification of carotenoid esters by liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. *J. Chromatogr. A* **2016**, *1457*, 116–124. [CrossRef]
66. Pinto, R.H.H.; Menezes, E.G.O.; Freitas, L.C.; de Andrade, E.H.A.; Ribeiro-Costa, R.M.; Silva Júnior, J.O.C.; Carvalho Junior, R.N. Supercritical CO_2 extraction of uxi (*Endopleura uchi*) oil: Global yield isotherms, fatty acid profile, functional quality and thermal stability. *J. Supercrit. Fluids* **2020**, *165*, 104932. [CrossRef]

67. Silva, M.P.; Cunha, V.M.B.; Sousa, S.H.B.; Menezes, E.G.O.; Bezerra, P.D.N.; De Farias Neto, J.T.; Filho, G.N.R.; Araújo, M.E.; De Carvalho, R.N. Supercritical CO_2 extraction of lyophilized Açaí (*Euterpe oleracea* Mart.) pulp oil from three municipalities in the state of Pará, Brazil. *J. CO2 Util.* **2019**, *31*, 226–234. [CrossRef]
68. de Almeida Rabello Oliveira, M.; da Rocha Ataíde, T.; de Oliveira, S.L.; de Melo Lucena, A.L.; de Lira, C.E.P.R.; Soares, A.A.; de Almeida, C.B.S.; Ximenes-da-Silva, A. Effects of short-term and long-term treatment with medium- and long-chain triglycerides ketogenic diet on cortical spreading depression in young rats. *Neurosci. Lett.* **2008**, *434*, 66–70. [CrossRef] [PubMed]
69. Aburahma, M.H.; El-Laithy, H.M.; Hamza, Y.E.-S. Oral bioavailability enhancement of vinpocetine using self-microemulsifying drug delivery system containing long chain triglycerides: Preparation and in vitro/in vivo evaluation. *Clin. Res. Regul. Aff.* **2010**, *27*, 97–107. [CrossRef]
70. Moo-Huchin, V.M.; Estrada-Mota, I.; Estrada-León, R.; Cuevas-Glory, L.; Ortiz-Vázquez, E.; Vargas, M.L.V.; Betancur-Ancona, D.; Sauri-Duch, E. Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chem.* **2014**, *152*, 508–515. [CrossRef]
71. Schaich, K.M.; Tian, X.; Xie, J. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. *J. Funct. Foods* **2015**, *14*, 111–125. [CrossRef]
72. Rosa, A.; Maxia, A.; Putzu, D.; Atzeri, A.; Era, B.; Fais, A.; Sanna, C.; Piras, A. Chemical composition of Lycium europaeum fruit oil obtained by supercritical CO_2 extraction and evaluation of its antioxidant activity, cytotoxicity and cell absorption. *Food Chem.* **2017**, *230*, 82–90. [CrossRef]

CAPÍTULO IV

Particle production of murici oil (*Byrsonima crassifolia*) by SFEE: Study of formulation, stability, particle size and concentration of lutein

(Produção de partículas de óleo de murici (*Byrsonima crassifolia*) por SFEE: Estudo da formulação, estabilidade, tamanho de partícula e concentração de luteína)

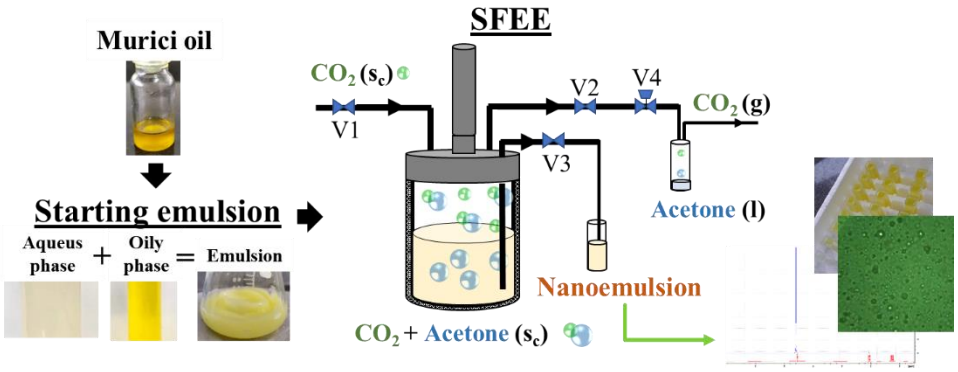
Flávia Cristina Seabra Pires, Joicy Corrêa de Oliveira, Cintya Cordovil Rodrigues, Ana Paula de Souza e Silva, Maria Caroline Rodrigues Ferreira, Leticia Maria Martins Siqueira, José Otávio Carrera Silva Junior, Roseane Maria Ribeiro Costa, Davi do Socorro Barros Brasil, Raul Nunes de Carvalho Junior

Submitted from The Journal of Supercritical Fluids, 2021:
PIRES, F. C. S.; OLIVEIRA, J. C.; RODRIGUES, C. C.; SILVA, A. P. S.; FERREIRA, M. C. R.; SIQUEIRA, L. M. M.; SILVA JUNIOR, J. O. C; COSTA, R. M. R.; BRASIL, D. S. B.; CARVALHO JUNIOR, R. N. Particle production of murici oil (*Byrsonima crassifolia*) by SFEE: Study of formulation, stability, particle size and concentration of lutein.

Abstract

The murici oil particles were obtained by Supercritical fluid extraction of emulsions at 313.15 K and 8 MPa, where the effects of different emulsifiers (csgel, stargel, tween 20 and tween 80), thickening agents (purity gum 1773 and xanthan gum), oil concentrations (6, 9 and 12 mg/mL), static periods (0, 1800 and 3600 s) and dynamic periods (3600, 10800 and 18000 s) on lutein content/retention, particle size and emulsion stability (creaming index-CI, polydispersity-Pdi and Zeta potential-ZP). More stable emulsions (CI=0/Pdi=0.68/ZP=-49.77 mV) and smaller (726.10 nm) were obtained using an oil concentration of 6 mg/mL, csgel and xanthan gum. The increase in the static and dynamic periods increased the lutein content/retention (214.71 µg/g/96.53%) and the stability (ZP=-51.73 mV) and decreased the particle size (364.03 nm). The images showed non-aggregated spheres and capsules, proving the encapsulation. Therefore, it was possible to produce murici oil nanoemulsions with good concentration/retention of lutein and high stability.

Keywords: Murici oil, lutein, emulsion, supercritical, encapsulation, nanoparticles.


Murici oil
Starting emulsion
Aqueus phase
+
Oily phase
=
Emulsion
SFEE
CO_2 (s_c)
V1
V2
V3
V4
CO_2 (g)
Acetone (l)
Nanoemulsion
CO_2 + Acetone (s_c)

Particle production of murici oil (*Byrsonima crassifolia*) by SFEE: Study of formulation, stability, particle size and concentration of lutein

Flávia Cristina Seabra Pires[a], Joicy Corrêa de Oliveira[a], Cintya Cordovil Rodrigues[a], Ana Paula de Souza e Silva[a], Maria Caroline Rodrigues Ferreira[a], Leticia Maria Martins Siqueira[a], José Otávio Carrera Silva Junior[b], Roseane Maria Ribeiro Costa[c], Davi do Socorro Barros Brasil[d], Raul Nunes de Carvalho Junior[a,*]

[a] LABEX (Extraction Laboratory), LABTECS (Supercritical Technology Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, 66075-900, Guamá, Belém, Pará, Brazil.

[b] Laboratory P&D Pharmaceutical and Cosmetic, ICS (Institute of Health Sciences), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, 66075-900, Guamá, Belém, Pará, Brazil.

[c] Laboratory of Pharmaceutical Nanotechnology, ICS (Institute of Health Sciences), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, 66075-900, Guamá, Belém, Pará, Brazil.

[d] LACOS (Cosmetics Laboratory), ITEC (Institute of Technology), UFPA (Federal University of Pará), Augusto Corrêa Street S/N, 66075-900, Guamá, Belém, Pará, Brazil.

*Correspondence: raulncj@ufpa.br; Tel.: +55-91-98742-0195.

1 Introduction

Murici (*Byrsonima crassifolia*) is a native fruit of South America that produces oil with antioxidant, antihyperlidemic, antithrombogenic and anti-atherogenic properties [1,2]. Murici pulp oil is yellow in color and has lutein as the major carotenoid [3], which is a compound widely associated with the treatment and prevention of age-related macular degenerative diseases, since this carotenoid is part of the retina of the eyes [4]. Studies suggest that the continuous ingestion of lutein regenerates the macula of the eyes, where currently on the market it is possible to find several dietary supplements based on this compound [5,6].

Due to the worldwide appeal for the use of clean technologies in the pharmaceutical and food industries, as well as the search for products of natural origin in order to minimize possible side effects, the implementation of supercritical technology in the product production chain has revolutionized this scenario [7]. Many studies have been carried out on the extraction of vegetable oils with therapeutic potential [8–10], as well as on the production of particles using supercritical CO_2 [11–13].

The use of supercritical CO_2 in the extraction of vegetable oils rich in bioactive compounds is widely used due to its several benefits as a moderate critical point, which allows the use of moderate temperatures, non-toxicity, where it is separated from the oil/extract as soon as it reaches ambient temperature and pressure conditions, and environmental safety, since it is not flammable and does not pollute the ozone layer [14,15].

The production of particles using supercritical CO_2 has been the subject of several studies, such as the techniques RESS (rapid expansion of supercritical solutions) [16], SAS (supercritical anti-solvent) [11] e SEDS (solution enhanced dispersion of solids) [17],

although SFEE (supercritical fluid extraction of emulsions) has been largely addressed in recent studies involving the production of particles from oil-in-water (O/W) emulsions [12,18,19].

SFEE represents an innovative and versatile method, which combines conventional emulsion preparation processes with supercritical antisolvent process (SAS) for the production of particles [20]. Among the conventional emulsion preparation techniques, the ultrasound technique has been widely used due to this process allowing the obtaining of particles at the nano and micrometric scale [19,21,22]. At SFEE, the organic solvent added to the emulsions is removed by supercritical CO_2, which acts as an anti-solvent, and a suspension of oil particles in water with a characteristic size and morphology is obtained, where particle agglomeration is avoided due to the use of emulsifier [12,20].

Therefore, the objective of this work was to produce particles of murici oil through the SFEE process and to evaluate the effects of the formulation of starting emulsions using different emulsifiers, thickening agents and oil concentrations, in addition to evaluate the effects of using different static and dynamic periods of SFEE on stability, lutein content, lutein retention, particle size, polydispersity (Pdi) and Zeta potential (ZP) of emulsions.

2 Materials and Methods

2.1 Materials

The lyophilized murici pulp (01° 02' 25. 9" S and 47° 54' 12. 3" W) (Accession number: 199530/ Barcode: IAN199530/ Sisgen: A3DB8EB) was used as raw material for obtaining the oil. Carbon dioxide (CO_2) with 99.9 % purity (White Martins, Campinas, Brazil) was used. In emulsions, the nonionic emulsifiers used were those derived from glycol

and glycerol esters: Csgel (INS 1520/471/475/470) (DuPorto, Recife, Brazil) and Stargel (INS 471/475/470) (Kerry, Três Corações, Brazil); and Polysorbates: Tween 20 (INS 432) (Dynamics, Indaiatuba, Brazil) and Tween 80 (INS 433) (Neon, Suzano, Brazil). The natural thickeners used were hydrophilic colloids: Purity gum 1773 (Ingredion, Mogi Guaçu, Brazil) and Xanthan gum (INS 415) (Cargill, São Paulo, Brazil). Acetone was used as an organic solvent for emulsions (Vetec, Rio de Janeiro, Brazil). Deuterium oxide (D_2O) with 99 % purity (Sigma-Aldrich, São Paulo, Brazil) was used in the determination of residual solvent.

2.2 Supercritical CO_2 extraction of murici oil

The supercritical extraction of murici oil was carried out in a SPE-ED SFE unit (model 7071, Applied Separations, USA). The equipment went into operation with the aid of a CO_2 cylinder, a compressor (model CSA 78, Schulz S/A, Brazil), a recirculator (model F08400796, Polyscience, USA) and a CO_2 flowmeter (model M 5SLPM, Alicat Scientific, USA). The extraction cell used had internal diameter of 0.032 m and height of 0.126 m, constituting a total internal volume of 9.96×10^{-5} m^3. The operating conditions were: temperature of 343.15 K, pressure of 49 MPa, and density of 900 kg/m^3, according to Pires et al. [23].

2.3 Preparation of the starting emulsions

The starting emulsions were prepared based on the literature studies [19,24,25]. All formulations were prepared with water, murici oil, emulsifier, acetone and thickening agents. The percentages of emulsifier (1 %), water (84 %), thickening agent (0.25 %) and acetone (14.75 %) were fixed. Twenty-four formulations were made with different emulsifiers (tween

20, tween 80, stargel and csgel), thickening agents (purity gum and xanthan gum) and oil concentrations (6, 9 and 12 mg/mL). The formulations of the starting emulsions are shown in Table 1. Firstly, the aqueous phase was prepared, where the gums were solubilized in water and left to stand for 43200 s. The oily phase was prepared by homogenizing the oil, acetone and emulsifier, which were subjected to an ultrasound bath (P=340 W/ F=50/60 Hz) for 600 s. The oil phase was dripped, under constant agitation, on the aqueous phase. The mixture was homogenized for 180 s and submitted to an ultrasound bath for 360 s.

2.4 SFEE process

For SFEE, the starting emulsion formulation that showed the greatest stability and the smallest particle diameter was used. SFEE was performed in a bubble column configuration using a reaction unit containing stainless steel autoclave (model 2777 1000, Top Industrie, France) with an internal volume of 9.50×10^{-5} m^3. The equipment scheme is presented in the study by Bezerra et al. [26]. The output of this system was modified by coupling a micrometric valve (V4) (model 30VRMM4812, Autoclave Engineers, USA), a liquid acetone collection bottle and a flow meter (model SLA5861, Brooks Instrument's, France), as shown in Fig. 1. Approximately 3.00×10^{-5} m^3 of the starting emulsions were added to the autoclave, occupying 0.048 m of the internal height. The V1 valve was opened and supercritical CO_2 (Sc) was added to the autoclave. Then, valve V2 was opened and the flow of 8.33×10^{-6} m^3/s was controlled by the micrometric valve V4. The CO_2 dragged the acetone out of the system, where after depressurization, the liquid acetone was recovered in a collecting flask and the gaseous CO_2 went to the flow meter. After the time of operation, the V1 valve was closed, the autoclave was slowly depressurized, the V3 valve was opened

and the murici oil nanoemulsion was recovered. The temperature and pressure used were 313.15 K and 8.00 MPa, respectively, since they are the most moderate phase equilibrium conditions of the CO_2+acetone binary system [27]. The SFEE process was performed using a static period and a dynamic period, as shown in Table 1. The use of a static period was proposed in this work in order to achieve the thermodynamic balance between the emulsion and CO_2, increasing the contact time between the mixture to solubilize acetone in supercritical CO_2. The dynamic periods used were based on the works of literature [25,28,29]. All determinations were made in triplicate.

2.5 Characterization of the emulsions

2.5.1 Determination of phase behavior

The determination of the phase behavior of the initial emulsions involved the visual inspection of the samples. About 3.00×10^{-5} m^3 of the emulsions were added to glass tubes and were properly sealed to prevent evaporation of the acetone. The tubes were stored, in the absence of light, at room temperature for 24 hours. The formulations were classified according to aspect (liquid, semi-liquid or gelled), turbidity (opaque, translucent or transparent) and stability (phase separation, creaming or stable). All determinations were made in triplicate.

2.5.2 Stability assessment

For stability assessment, the starting emulsions were added to sealed glass tubes and protected from light. The stability determination was carried out in 3 consecutive steps: 1) CI T_{romm}: after 24 hours stored at room temperature (298.15 K); 2) CI $T_{cooling}$: after 24 hours

stored under refrigeration (278.15 K); and 3) CI Post-centr.: after centrifugation (523.60 rad. s^{-1}/1800 s/ 298.15 K) (model 5804 R, Eppendorf AG, Germany). The volumes of the aqueous phase were measured in all conditions. The emulsion stability was evaluated by calculating the creaming index [30], defined by the ratio between the height of the aqueous phase and the initial height. The creaming index was determined in triplicate and the results were expressed in %.

2.5.3 Lutein content

The lutein contents were determined for SFEE emulsions, according to the methodology proposed by Rodriguez-Amaya & Kimura [31]. The extraction solvent used was acetone. The partition was performed with ethyl ether and petroleum ether. The reading extract was reconstituted in ethanol. The readings were performed on a spectrophotometer (model UV-M90, Bel Engineering, Italy). The total lutein contents were calculated through absorbance measurements carried out at 445 nm, and with the absorption coefficient of lutein in ethanol (2550). Quantifications were performed in triplicate and the results expressed in μg/g.

2.5.4 Retention percentage of lutein

The percentage of lutein retention was determined for SFEE emulsions by the ratio between the concentration of lutein in the suspension (Section 2.5.3) and the theoretical concentration of lutein in the suspension, assuming that at SFEE there was a 5 % loss of lutein during the process [21]. The determinations were made in triplicate and the results were expressed in %.

2.5.5 Particle size, Polydispersity and Zeta potential

The average particle sizes, Pdi and the ZP of starting emulsions and SFEE emulsions were measured by photon correlation spectroscopy using a Zetasizer Nano Series (Malvern Instrument, Royston, United Kingdom). A dilution of the samples with Milli-q water (2:100) was necessary to achieve the appropriate optical density. All measurements were made at 298 K. The measurements were made in triplicate and the results were expressed in nm.

2.5.6 Residual solvent of SFEE emulsions

The SFEE emulsions used to determine the residual levels of solvent (acetone) were those obtained in the static periods of 0, 1800 and 3600 s, and in the dynamic period of 18000 s (assays 3, 6 and 9). Residual levels of acetone in SFEE emulsions were determined by NMR spectrometry (model Ascend NMR Magnets TM 400 MHz, Bruker, Germany), coupled with Avance III HD console (400 MHz for 1H). The high-field 1H nuclear magnetic resonance (NMR) spectra were generated, read and analyzed using the TopSpin 3.6.3 software. (© 2021 Bruker, Germany). The samples were solubilized in deuterium oxide (D_2O). The high-field 1H nuclear magnetic resonance (NMR) spectra were generated, read and analyzed using the TopSpin 3.6.3 software. (©2021 Bruker, Germany). The areas of the spectra were standardized in the ranges of 0.50 to 7.00 ppm and the percentages of acetone were determined using their chemical displacement (δ=2.05). The percentages of acetone were converted into mass, according to the amount initially added to the emulsions. The results were expressed as a percentage (%) and ppm (part per million).

2.5.7 Optical microscopy

SFEE emulsions were analyzed using an optical microscope (model BIO2, Bel Enginnering, Italy), with a 100× magnifying glass. Aliquots of the suspensions were placed on glass slides and covered with coverslips, operating under oil immersion.

2.6 Statistical analysis

The means and standard deviations were calculated for all analyses. The results of the analyses were submitted to Tukey test, at a significance level of 5 % ($p<0.05$). Excel 2000 SR-1 (Microsoft, Troy, USA), Statistica Kernel Release 7.1 (StartSoft Inc., Tulsa, USA) and TopSpin 3.6.3. (©2021 Bruker, Germany) programs were used as tools.

3 Results and Discussion

3.1 Selection of the starting emulsions

3.1.1 Determination of phase behavior and stability assessment of the starting emulsions

The phase behavior and stability of starting emulsions containing murici oil (*B. crassifolia*) are shown in Table 1. In emulsions with purity gum 1773 (1-12), all those prepared with tween 20 (1-3) and tween 80 (4-6) were liquid, translucent and presented phase separation (CI between 1.88 % to 3.65 %). The phase separation observed occurred due to the coalescence caused by the melting of dispersed particles, forming a totally separate layer of oil on the surface of the suspensions [32,33]. The emulsions prepared with stargel (7-9) and csgel (10-12) were semi-liquid, opaque and presented creaming (CI between 2.19 % to 3.62 %). The presence of creaming in the emulsions occurred due to the movement of dispersed particles to the surface of the suspension, however, without melting [34]. The

translucent and opaque turbidity of the 1773 purity gum emulsions were characteristic of the emulsifiers used. Such turbidity characteristics indicated that emulsions with tween 20 and tween 80 had smaller particle sizes than those obtained with stargel and csgel, since translucent emulsions are generally characterized by having smaller particle sizes [35,36]. In addition, oil concentrations and types of emulsifier did not influence the stability of starting emulsions containing purity gum 1773 (CI>0). This allows us to say that the use of purity gum 1773 as a thickening agent was not suitable for the formulation of emulsions based on murici oil.

All formulations containing xanthan gum (13-24) presented a gelled aspect, which is characteristic of the gum. The formulations with tween 20 (13-15) were translucent and those with tween 80 (16-18) were opaque, however both showed creaming (CI T_{room} between 0.34 % and 1.41 %). All emulsions formed with stargel (19-21) and csgel (22-24) were opaque and stable (CI T_{room}=0) after 24 hours at rest in ambient conditions. However, after 24 hours of refrigeration, csgel formulations containing 9 mg/mL (23) and 12 mg/mL of murici oil (24) showed creaming (CI $T_{cooling}$ of 3.76 % and 3.16 %, respectively). This demonstrates that csgel emulsions were influenced by the increase in oil concentration. Therefore, all stargel formulations (19-21) and the 6 mg/mL csgel formulation (22) were subjected to centrifugation, where after centrifugation they also did not show creaming (CI Post-centr.=0). This allows us to say that xanthan gum provided an increase in the stability of the emulsions, and that the type of emulsifier and the oil concentration had an influence on the stability of the emulsions, where both the stargel and csgel proved to be suitable for use as emulsifiers for murici oil emulsion formulations. A similar behavior was also observed in the study by

Prieto & Calvo [25], when comparing the use of glycerol and xanthan gum as thickening agents in the SFEE process with fish oil.

3.1.2 Particle size, Polydispersity and Zeta potential of the starting emulsions

The particle data were obtained only for the starting emulsions that did not show phase separation and creaming after the third stage of the stability assessment (CI Post-centr.=0). Thus, the analyzed emulsions were those prepared with xanthan gum and with stargel (19-21) and csgel (22). The particle sizes, Pdi and ZP of murici oil (*B. crassifolia*) starting emulsions are shown in Table 1. It was possible to observe that the particle sizes varied from 726.01 nm (csgel-6 mg/mL of oil) at 1202.30 nm (stargel-12 mg/mL oil). The high particle sizes can be attributed to the use of xanthan gum, since the increase in viscosity increases the particle diameters [37]. For formulations with stargel, it was demonstrated that the increase in oil concentration increased the diameter of the particles. This behavior was also observed by Reis et al. [19]. This possibly occurred due to the flocculation, characterized by the union of dispersed particles, generated by the reduction of the emulsifier/oil ratio [38,39]. The Pdi's ranged from 0.68 (csgel-6 mg/mL of oil) to 0.92 (stargel-9 mg/mL of oil), which indicates that the starting emulsions had heterogeneous drop sizes [40]. Despite the polydispersity of the particles, the results of ZP indicate that all formulations were stable, since they presented high values (in modulus) (-43.37 to -54.90 mV), which indicates that the particles tend to repel and reduce the possibility of phase separation [41,42].

Therefore, according to the results of phase behavior, stability, particle size, Pdi and ZP, the starting emulsion selected for the SFEE process was formulation 22, containing xanthan gum, csgel and a concentration oil of 6 mg/mL.

3.3 SFEE process

3.3.1 Lutein content and percentage of retention of SFEE emulsions

The lutein contents and retention percentages in the SFEE emulsions of murici oil (*B. crassifolia*) are shown in Table 2. The highest level of lutein (214.71 µg/g) was obtained with a static period of 3600 s and a dynamic period of 18000 s. It was possible to observe that in all conditions the levels of lutein decreased with the use of a static period of 1800 s, but showed an increase when a static period of 3600 s was used. This reduction may have occurred due to turbulence observed in the SFEE when 1800 s static period was used, which may have caused oil losses during the process caused by the release of the oil to the aqueous phase and its subsequent dissolution in supercritical CO_2 [22]. Such loss may also have occurred due to the coextraction of murici oil together with acetone, since both are miscible in supercritical CO_2. The levels of lutein increased with the increase of the dynamic period. This suggests that the concentration of lutein occurred with the periodic removal of acetone from the emulsions. These results are in accordance with the retention values, since the murici oil SFEE emulsions exhibited high percentages of lutein retention (96.53 %). This result was superior to those obtained by Trucillo et al. [43] when applying the Supercritical assisted Liposome formation process (SuperLip) in the encapsulation of olive pomace extract (58 %), by Santos et al. [16] when using Rapid Expansion of Supercritical Solution (RESS) in the encapsulation of anthocyanin extract from jabuticaba skins (79.78 %), and by Tirado et al. [44] when using SFEE in Astaxanthin encapsulation in ethyl cellulose carriers (84 %). This demonstrates that under conditions of greater static and dynamic period, it was possible to obtain emulsions with a lutein content similar to that added in the formulation (224.46

µg/g). It is important to point out that until the brief moment, there are no studies in the literature that evaluate the influence of the static period in the SFEE in the configuration of the bubble column type. Therefore, it is extremely important to collect this information.

3.3.2 Particle size, Polydispersity and Zeta potential of SFEE emulsions

The particle sizes, Pdi and ZP of the SFEE emulsions of murici oil (*B. crassifolia*) are shown in Table 2. It was observed that the particle sizes varied from 364.03 nm (static period=3600 s/ dynamic period=18000 s) at 656.07 nm (static period=3600 s/ dynamic period=3600 s). These values were higher than those found in other studies that addressed the use of SFEE. However, it is important to note that most studies use different equipment, where the particle size characteristics are influenced. This was confirmed in the study by Prieto et al. [29], on the performance comparison of different supercritical fluid extraction equipments for the production of vitamin E in polycaprolactone by SFEE, where it was possible to observe that the equipment similar to that used in the present work (bubble column) presented larger particle diameters compared to the emulsions obtained with other equipment (bubble column with a gas redistributor, spray column and packed column). It can be considered that oil-in-water (O/W) nanoemulsions were formed, since they had sizes between 100 nm and 1000 nm [33]. In general, particle sizes decreased after SFEE, with a reduction range of approximately 9.65 % to 48.87 %. This behavior was also observed by Mendonça et al. [18] when using the SFEE process. Such reduction is generally attributed to the particle narrowing effect, caused by the removal of the solvent from the emulsion droplets [45]. It was possible to observe that the increase in the static period reduced the particle sizes when 1800 s was used and increased when 3600 s were used, except in test 9. The increase

in the dynamic period also reduced the particle sizes in all SFEE conditions. The reduction in the size of the droplets is usually associated with a decrease in the concentration of acetone in the suspensions with an increase in the dynamic periods of extraction. However, the reduction observed in the 1800 s static period was possibly due to the reduction in acetone caused by the turbulence observed in this condition, which caused the CO_2 flow to increase in certain extraction periods, intensifying the mass transfer inside the vessel high pressure [46]. The Pdi ranged from 0.48 (static period=3600 s/ dynamic period=18000 s) to 0.79 (static period=3600 s/ dynamic period=10800 s), showing similar to the values of the starting emulsion. Pdi within this range of values were also obtained from SFEE to nanoencapsulate vitamin E in polycaprolactone (0.54) [28]. The ZP values ranged from -35.37 mV (static period=0 s/ dynamic period=3600 s) to -51.73 mV (static period=3600 s/ dynamic period=3600 s). These results demonstrate that the emulsions remained stable after SFEE, since high values of ZP (positive or negative) indicate that the emulsions are electrically stabilized [47]. This suggests that the Pdi values did not interfere with the stability of the emulsions. Therefore, these data prove that it was possible to obtain stable SFEE nanoemulsions from murici oil. In addition, the negative signs of the ZP suggest that nanocapsules were formed from murici oils covered by xanthan gum, since the xanthan gum molecules have a negative charge [48].

3.3.3 Residual solvent of SFEE emulsions

The High-field 1H nuclear magnetic resonance (NMR) spectra of SFEE emulsions of murici oil (*B. crassifolia*) of assays 3, 6 and 9 are shown in Fig. 2. The percentages of acetone in the SFEE emulsions were 2.14 %, 1.79 % and 1.61 %, which corresponded to the

concentrations of 17009 ppm, 14185 ppm and 12795 ppm for tests 3, 6 and 9, respectively. Acetone reduction rates ranged from 85.48 % to 89.08 %. Although acetone is a Class 3 solvent, considered less toxic and less risky to human health, the results were above the established concentration limit (5000 ppm) [49]. This may have occurred due to the increase in the viscosity of the aqueous phase, by the addition of xanthan gum, which decreased the rate of extraction of acetone [25]. This behavior was also observed by Aguiar et al. [21] in the encapsulation of pepper oleoresin by SFEE. Therefore, for the application of these nanoemulsions in the formulation of pharmaceutical or food products, an exhaustive washing of the particles must be carried out with subsequent drying of them, in order to remove the amount of residual solvent. It was possible to observe that the acetone content decreased with the increase of the static extraction time. This was possibly due to the increased solvation power of acetone with supercritical CO_2, caused by the longer contact time between the solvent and the anti-solvent.

3.3.4 Optical microscopy of SFEE emulsions

The images obtained by optical microscopy of the SFEE emulsions of murici oil (*B. crassifolia*) are shown in Fig. 3. All formulations presented spherical and non-aggregated particles. The SFEE emulsions showed different spacing and particle size behaviors, where the droplets of emulsions of smaller particle size (6 and 9) showed to be closer to each other, while the others of larger size were more distant. This behavior was possibly due to the greater evaporation of acetone in these conditions, which caused the concentration of droplets in the aqueous phase [24]. It was also possible to observe the presence of capsules in some images, where the core containing oil appears clearly concentrated in the central region of

the particles, being surrounded by a thin layer of the encapsulating agent, proving the occurrence of encapsulation, a phenomenon also suggested by the signs of ZP. The same was observed by Cruz et al. [50] when using SFEE in the encapsulation of yacon (*Smallanthus sonchifolius*) leaf extract. The size variability explains the Pdi values, however, despite being close, no instability phenomenon was observed as flocculation and coalescence, which explains the high values of ZP.

4 Conclusions

The stability and particle diameters were influenced by the concentrations of murici oil, types of emulsifier and thickening agent in the initial formulations, where the best starting emulsion was obtained with csgel, xanthan gum and 6 mg/mL murici oil. In general, the increases in the static and dynamic periods of SFEE increased the levels of lutein, the retention of lutein and the stability of the emulsions, and decreased the diameters of particles. The increase in the static period was also responsible for the reduction of the residual solvent concentration, however, the dynamic period was not enough to reduce the application levels. Thus, it is necessary to improve the internal mass transfer, where future tests are suggested under constant agitation in equipment with this type of configuration. The images of the nanoemulsions showed small spherical and non-aggregated particles with different spacing and particle size behaviors, where it was possible to observe the presence of capsules, proving the occurrence of encapsulation. Therefore, it was possible to produce murici oil particles with good concentration/retention of lutein and high stability, using supercritical CO_2 in all stages of obtaining nanoemulsions (SFE and SFEE). This confirms that supercritical

technology can be used in the production chain of several natural products applied in the food and pharmaceutical industries.

Acknowledgements

Flávia Cristina Seabra Pires thanks the laboratories LABEX/UFPA, LABTECS/UFPA, Laboratory P&D Pharmaceutical and Cosmetic/UFPA, Laboratory of Pharmaceutical Nanotechnology/UFPA and LACOS/UFPA for the scientific and structural contributions.

Funding sources

This work was supported by CAPES - PROPESP/UFPA [process number 1662244/2016].

References

[1] F.C.S. Pires, A.P. de S. e. Silva, M. de los A.R. Salazar, W.A. da Costa, H.S.C. da Costa, A.S. Lopes, H. Rogez, R.N. de Carvalho Junior, Determination of process parameters and bioactive properties of the murici pulp (Byrsonima crassifolia) extracts obtained by supercritical extraction, J. Supercrit. Fluids. 146 (2019) 128–135. https://doi.org/10.1016/j.supflu.2019.01.014.

[2] C.M. Rezende, S.R.G. Fraga, Chemical and aroma determination of the pulp and seeds of murici (Byrsonima crassifolia L.), J. Braz. Chem. Soc. 14 (2003) 425–428. https://doi.org/10.1590/S0103-50532003000300014.

[3] L.R.B. Mariutti, E. Rodrigues, A.Z. Mercadante, Carotenoids from Byrsonima crassifolia: Identification, quantification and in vitro scavenging capacity against peroxyl radicals, J. Food Compos. Anal. 31 (2013) 155–160.

https://doi.org/10.1016/j.jfca.2013.05.005.

[4] I.D. Nwachukwu, C.C. Udenigwe, R.E. Aluko, Lutein and zeaxanthin: Production technology, bioavailability, mechanisms of action, visual function, and health claim status, Trends Food Sci. Technol. 49 (2016) 74–84. https://doi.org/10.1016/j.tifs.2015.12.005.

[5] J. Widomska, Why has Nature Chosen Lutein and Zeaxanthin to Protect the Retina?, J. Clin. Exp. Ophthalmol. 05 (2014) 1–22. https://doi.org/10.4172/2155-9570.1000326.

[6] R. Ravikrishnan, S. Rusia, G. Ilamurugan, U. Salunkhe, J. Deshpande, J. Shankaranarayanan, M.L. Shankaranarayana, M.G. Soni, Safety assessment of lutein and zeaxanthin (Lutemax^{TM} 2020): Subchronic toxicity and mutagenicity studies, Food Chem. Toxicol. 49 (2011) 2841–2848. https://doi.org/10.1016/J.FCT.2011.08.011.

[7] F.C.S. Pires, A.P. de S. e. Silva, I.Q. Silva, J.C. de Oliveira, E.M.O. Gama, W.A. Costa, R.N. de Carvalho Junior, Application of supercritical technology in the production of dietary supplement based on plant extracts, in: Inamuddin, R. Boddula, M.I. Ahamed, A.M. Asiri (Eds.), Green Sustain. Process Chem. Environ. Eng. Sci., Solventes, Elsevier, 2021: pp. 161–183. https://doi.org/10.1016/B978-0-12-821885-3.00006-2.

[8] V. Kitryte, A. Laurinavičiene, M. Syrpas, A. Pukalskas, P.R. Venskutonis, Modeling and optimization of supercritical carbon dioxide extraction for isolation of valuable lipophilic constituents from elderberry (Sambucus nigra L.) pomace, J. CO2 Util. 35 (2020) 225–235. https://doi.org/10.1016/j.jcou.2019.09.020.

[9] M.P. Silva, V.M.B. Cunha, S.H.B. Sousa, E.G.O. Menezes, P.D.N. Bezerra, J.T. De Farias Neto, G.N.R. Filho, M.E. Araújo, R.N. De Carvalho, Supercritical CO2 extraction of lyophilized Açaí (Euterpe oleracea Mart.) pulp oil from three municipalities in the state of Pará, Brazil, J. CO2 Util. 31 (2019) 226–234. https://doi.org/10.1016/j.jcou.2019.03.019.

[10] R.H.H. Pinto, E.G.O. Menezes, L.C. Freitas, E.H. de A. Andrade, R.M. Ribeiro-Costa, J.O.C. Silva Júnior, R.N. Carvalho Junior, Supercritical CO2 extraction of uxi (Endopleura uchi) oil: Global yield isotherms, fatty acid profile, functional quality and thermal stability, J. Supercrit. Fluids. 165 (2020) 1–8. https://doi.org/10.1016/j.supflu.2020.104932.

[11] R.T. Buratto, M.I. Chinchilla, M.J. Cocero, Á. Martín, Formulation of açaí (E. oleracea Mart.) Pulp and seeds extracts by co-precipitation in Supercritical Antisolvent (SAS) technology, J. Supercrit. Fluids. 169 (2020) 1–8. https://doi.org/10.1016/j.supflu.2020.105090.

[12] D.F. Tirado, A. Latini, L. Calvo, The encapsulation of hydroxytyrosol-rich olive oil in Eudraguard® protect via supercritical fluid extraction of emulsions, J. Food Eng. 290 (2021) 1–9. https://doi.org/10.1016/j.jfoodeng.2020.110215.

[13] N. Ganañ, M.G. Bordón, P.D. Ribotta, A. González, Study of chia oil microencapsulation in soy protein microparticles using supercritical CO2-assisted impregnation, J. CO2 Util. 40 (2020) 1–10. https://doi.org/10.1016/j.jcou.2020.101221.

[14] V.M.B. Cunha, M.P. da Silva, W.A. da Costa, M.S. de Oliveira, F.W.F. Bezerra, A.C. de Melo, R.H.H. Pinto, N.T. Machado, M.E. Araujo, R.N. de Carvalho Junior,

Carbon Dioxide Use in High-Pressure Extraction Processes, in: Carbon Dioxide Chem. Capture Oil Recover., IntechOpen, 2018: pp. 211–240. https://doi.org/10.5772/intechopen.71151.

[15] G. Brunner, Supercritical fluids: Technology and application to food processing, J. Food Eng. 67 (2005) 21–33. https://doi.org/10.1016/j.jfoodeng.2004.05.060.

[16] D.T. Santos, J.Q. Albarelli, M.M. Beppu, M.A.A. Meireles, Stabilization of anthocyanin extract from jabuticaba skins by encapsulation using supercritical CO2 as solvent, Food Res. Int. 50 (2013) 617–624. https://doi.org/10.1016/j.foodres.2011.04.019.

[17] D. Hu, C. Lin, L. Liu, S. Li, Y. Zhao, Preparation, characterization, and in vitro release investigation of lutein/zein nanoparticles via solution enhanced dispersion by supercritical fluids, J. Food Eng. 109 (2012) 545–552. https://doi.org/10.1016/J.JFOODENG.2011.10.025.

[18] F.M.R. Mendonça, A.E. Polloni, A. Junges, R.S. da Silva, A.F. Rubira, G.R. Borges, C. Dariva, E. Franceschi, Encapsulation of neem (Azadirachta indica) seed oil in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by SFEE technique, J. Supercrit. Fluids. 152 (2019) 1–9. https://doi.org/10.1016/j.supflu.2019.104556.

[19] P.M.C.L. Reis, N. Mezzomo, G.P.S. Aguiar, E.M.T.L. Senna, H. Hense, S.R.S. Ferreira, Ultrasound-assisted emulsion of laurel leaves essential oil (Laurus nobilis L.) encapsulated by SFEE, J. Supercrit. Fluids. 147 (2019) 284–292. https://doi.org/10.1016/J.SUPFLU.2018.11.018.

[20] P. Chattopadhyay, B.Y. Shekunov, J.S. Seitzinger, R.W. Huff, Particles from supercritical fluid extraction of emulsion, US6998051, 2006.

[21] A.C. Aguiar, L.P.S. Silva, C.A. Rezende, G.F. Barbero, J. Martínez, Encapsulation of pepper oleoresin by supercritical fluid extraction of emulsions, J. Supercrit. Fluids. 112 (2016) 37–43. https://doi.org/10.1016/j.supflu.2016.02.009.

[22] J.L.P. Reátegui, F.P. Fernandes, P. dos Santos, C.A. Rezende, A. Sartoratto, C.L. Queiroga, J. Martínez, Production of copaiba (Copaifera officinalis) oleoresin particles by supercritical fluid extraction of emulsions, J. Supercrit. Fluids. 140 (2018) 364–371. https://doi.org/10.1016/j.supflu.2018.07.021.

[23] F.C.S. Pires, J.C. de Oliveira, E.G.O. Menezes, A.P. de S. e Silva, M.C.R. Ferreira, L.M.M. Siqueira, A.O. Almada-Vilhena, H. Rogez, J.C. Pieczarka, C.Y. Nagamachi, R.N. de Carvalho Junior, Bioactive compounds and evaluation of antioxidant, cytotoxic and cytoprotective effects of murici pulp extracts (Byrsonima crassifolia) obtained by supercritical extraction in HepG2 cells treated with H2O2, Foods. 10 (2021) 1–22. https://doi.org/10.3390/foods10040737.

[24] J.L.P. Reátegui, F.M. Barrales, C.A. Rezende, C.L. Queiroga, J. Martínez, Production of Copaiba oleoresin particles from emulsions stabilized with modified starches, Ind. Crops Prod. 108 (2017) 128–139. https://doi.org/10.1016/j.indcrop.2017.06.027.

[25] C. Prieto, L. Calvo, The encapsulation of low viscosity omega-3 rich fish oil in polycaprolactone by supercritical fluid extraction of emulsions, J. Supercrit. Fluids. 128 (2017) 227–234. https://doi.org/10.1016/j.supflu.2017.06.003.

[26] F.W.F. Bezerra, W.A. da Costa, M.S. de Oliveira, E.H. de Aguiar Andrade, R.N. de Carvalho, Transesterification of palm pressed-fibers (Elaeis guineensis Jacq.) oil by supercritical fluid carbon dioxide with entrainer ethanol, J. Supercrit. Fluids. 136

(2018) 136–143. https://doi.org/10.1016/j.supflu.2018.02.020.

[27] C.Y. Day, C.J. Chang, C.Y. Chen, Phase equilibrium of ethanol + CO2 and acetone + CO2 at elevated pressures, J. Chem. Eng. Data. 41 (1996) 839–343. https://doi.org/10.1021/je960049d.

[28] C. Prieto, L. Calvo, Supercritical fluid extraction of emulsions to nanoencapsulate vitamin E in polycaprolactone, J. Supercrit. Fluids. 119 (2017) 274–282. https://doi.org/10.1016/j.supflu.2016.10.004.

[29] C. Prieto, C.M.M. Duarte, L. Calvo, Performance comparison of different supercritical fluid extraction equipments for the production of vitamin E in polycaprolactone nanocapsules by supercritical fluid extraction of emulsionsc, J. Supercrit. Fluids. 122 (2017) 70–78. https://doi.org/10.1016/j.supflu.2016.11.015.

[30] A. Ye, H. Singh, Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: Influence of polysaccharides, Food Hydrocoll. 20 (2006) 269–276. https://doi.org/10.1016/j.foodhyd.2005.02.023.

[31] D. Rodriguez-Amaya, M. Kimura, HarvestPlus Handbook for Carotenoid Analysis, 1st ed., Breeding Crops for Better Nutrition, Washington, 2004.

[32] S. Tamilvanan, Oil-in-water lipid emulsions: implications for parenteral and ocular delivering systems, Prog. Lipid Res. 43 (2004) 489–533. https://doi.org/10.1016/J.PLIPRES.2004.09.001.

[33] S.M. Jafari, E. Assadpoor, Y. He, B. Bhandari, Re-coalescence of emulsion droplets during high-energy emulsification, Food Hydrocoll. 22 (2008) 1191–1202. https://doi.org/10.1016/j.foodhyd.2007.09.006.

[34] E. Dickinson, Hydrocolloids as emulsifiers and emulsion stabilizers, Food

Hydrocoll. 23 (2009) 1473–1482. https://doi.org/10.1016/J.FOODHYD.2008.08.005.

[35] J. Surh, G.T. Vladisavljevicä, S. Mun, D.J. Mcclements, Preparation and characterization of water/oil and water/oil/water emulsions containing biopolymer-gelled water droplets, J. Agric. Food Chem. 55 (2007) 175–184. https://doi.org/10.1021/jf061637q.

[36] D. Guzey, D.J. McClements, Formation, stability and properties of multilayer emulsions for application in the food industry, Adv. Colloid Interface Sci. 128–130 (2006) 227–248. https://doi.org/10.1016/J.CIS.2006.11.021.

[37] M. Li, O. Rouaud, D. Poncelet, Microencapsulation by solvent evaporation: State of the art for process engineering approaches, Int. J. Pharm. 363 (2008) 26–39. https://doi.org/10.1016/j.ijpharm.2008.07.018.

[38] F. Leal-Calderon, M. Cansell, The design of emulsions and their fate in the body following enteral and parenteral routes, Soft Matter. 8 (2012) 10213–10225. https://doi.org/10.1039/c2sm26215k.

[39] D.J. McClements, Food emulsions: Principles, Practices, and Techniques, 2nd ed., CRC Series, Florida, 2005. https://doi.org/doi:10.1201/b18868-13.

[40] S.S. Galooyak, B. Dabir, Three-factor response surface optimization of nano-emulsion formation using a microfluidizer, J. Food Sci. Technol. 52 (2015) 2558–2571. https://doi.org/10.1007/s13197-014-1363-1.

[41] A. González-Saucedo, L.L. Barrera-Necha, R.I. Ventura-Aguilar, Z.N. Correa-Pacheco, S. Bautista-Baños, M. Hernández-López, Extension of the postharvest quality of bell pepper by applying nanostructured coatings of chitosan with

Byrsonima crassifolia extract (L.) Kunth, Postharvest Biol. Technol. 149 (2019) 74–82. https://doi.org/10.1016/j.postharvbio.2018.11.019.

[42] P.-H. Li, W.-C. Lu, Effects of storage conditions on the physical stability of d-limonene nanoemulsion, Food Hydrocoll. 53 (2016) 218–224. https://doi.org/10.1016/J.FOODHYD.2015.01.031.

[43] P. Trucillo, R. Campardelli, B. Aliakbarian, P. Perego, E. Reverchon, Supercritical assisted process for the encapsulation of olive pomace extract into liposomes, J. Supercrit. Fluids. 135 (2018) 152–159. https://doi.org/10.1016/j.supflu.2018.01.018.

[44] D.F. Tirado, I. Palazzo, M. Scognamiglio, L. Calvo, G. Della Porta, E. Reverchon, Astaxanthin encapsulation in ethyl cellulose carriers by continuous supercritical emulsions extraction: A study on particle size, encapsulation efficiency, release profile and antioxidant activity, J. Supercrit. Fluids. 150 (2019) 128–136. https://doi.org/10.1016/j.supflu.2019.04.017.

[45] S.K. Luther, A. Braeuer, High-pressure microfluidics for the investigation into multi-phase systems using the supercritical fluid extraction of emulsions (SFEE), J. Supercrit. Fluids. 65 (2012) 78–86. https://doi.org/10.1016/j.supflu.2012.02.029.

[46] R. Guha, M. Vinjamur, M. Mukhopadhyay, Demonstration of mechanisms for coprecipitation and encapsulation by supercritical antisolvent process, Ind. Eng. Chem. Res. 50 (2011) 1079–1088. https://doi.org/10.1021/ie101449a.

[47] C.E. Mora-Huertas, H. Fessi, A. Elaissari, Polymer-based nanocapsules for drug delivery, Int. J. Pharm. 385 (2010) 113–142. https://doi.org/10.1016/J.IJPHARM.2009.10.018.

[48] P. erik Jansson, L. Kenne, B. Lindberg, Structure of the extracellular polysaccharide

from xanthomonas campestris, Carbohydr. Res. 45 (1975) 275–282. https://doi.org/10.1016/S0008-6215(00)85885-1.

[49] European Medicines Agency, Impurities: Guideline for Residual Solvents Q3C (R6), 2019.

[50] P.N. Cruz, P.M.C. Lima Reis, S.R.S. Ferreira, M.L. Masson, M.L. Corazza, Encapsulation of yacon (Smallanthus sonchifolius) leaf extract by supercritical fluid extraction of emulsions, J. Supercrit. Fluids. 160 (2020) 1–11. https://doi.org/10.1016/j.supflu.2020.104815.

Table 1. Formulations, phase behavior, creaming index, particle size, Pdi and Zeta potential of starting emulsions containing murici oil (*B. crassifolia*).

Formulation	Aqueous phase	Oily phase	Aspect	Turbidity	Stability	CI T_{room} (%) *	CI $T_{cooling}$ (%) *	CI Post-centr. (%) *	Particle size (nm)	Pdi	ZP (mV)
1	Purity gum 1773 + water	Oil (6 mg/mL) + acetone + Tween 20	Liquid	Translucent	Phase separation	2.20	-	-	-	-	-
2	Purity gum 1773 + water	Oil (9 mg/mL) + acetone + Tween 20	Liquid	Translucent	Phase separation	2.50	-	-	-	-	-
3	Purity gum 1773	Oil (12 mg/mL)	Liquid	Translucent	Phase separation	1.88	-	-	-	-	-

	+ water	+ acetone + Tween 20									
4	Purity gum 1773 + water	Oil (6 mg/mL) + acetone + Tween 80	Liquid	Translucent	Phase separation	2.80	-	-	-	-	-
5	Purity gum 1773 + water	Oil (9 mg/mL) + acetone + Tween 80	Liquid	Translucent	Phase separation	2.77	-	-	-	-	-
6	Purity gum 1773 + water	Oil (12 mg/mL) + acetone + Tween 80	Liquid	Translucent	Phase separation	3.65	-	-	-	-	-

7	Purity gum 1773 + water	Oil (6 mg/mL) + acetone + Stargel	Semi-liquid	Opaque	Creaming	2.19	-	-	-	-	-
8	Purity gum 1773 + water	Oil (9 mg/mL) + acetone + Stargel	Semi-liquid	Opaque	Creaming	3.62	-	-	-	-	-
9	Purity gum 1773 + water	Oil (12 mg/mL) + acetone + Stargel	Semi-liquid	Opaque	Creaming	3.14	-	-	-	-	-
10	Purity gum 1773 + water	Oil (6 mg/mL) + acetone + Csgel	Semi-liquid	Opaque	Creaming	2.73	-	-	-	-	-
11	Purity gum 1773 + water	Oil (9 mg/mL) + acetone	Semi-liquid	Opaque	Creaming	3.14	-	-	-	-	-

		+ Csgel									
12	Purity gum 1773 + water	Oil (12 mg/mL) + acetone + Csgel	Semi-liquid	Opaque	Creaming	2.83	-	-	-	-	-
13	Xanthan gum + water	Oil (6 mg/mL) + acetone + Tween 20	Gelled	Translucent	Creaming	1.11	-	-	-	-	-
14	Xanthan gum + water	Oil (9 mg/mL) + acetone + Tween 20	Gelled	Translucent	Creaming	0.34	-	-	-	-	-
15	Xanthan gum + water	Oil (12 mg/mL) + acetone + Tween 20	Gelled	Translucent	Creaming	0.49	-	-	-	-	-
16	Xanthan gum	Oil (6 mg/mL)	Gelled	Opaque	Creaming	1.41	-	-	-	-	-

	+ water	+ acetone + Tween 80									
17	Xanthan gum + water	Oil (9 mg/mL) + acetone + Tween 80	Gelled	Opaque	Creaming	1.12	-	-	-	-	-
18	Xanthan gum + water	Oil (12 mg/mL) + acetone + Tween 80	Gelled	Opaque	Creaming	0.47	-	-	-	-	-
19	Xanthan gum + water	Oil (6 mg/mL) + acetone + Stargel	Gelled	Opaque	Stable	0.00	0.00	0.00	1074.00 ± 31.11 [a]	0.84 ± 0.11 [a]	-54.90 ± 0.14 [a]
20	Xanthan gum + water	Oil (9 mg/mL) + acetone + Stargel	Gelled	Opaque	Stable	0.00	0.00	0.00	1084.00 ± 83.32 [a]	0.92 ± 0.07 [a]	-47.80 ± 0.99 [b]

21	Xanthan gum + water	Oil (12 mg/mL) + acetone + Stargel	Gelled	Opaque	Stable	0.00	0.00	0.00	1203.30 ± 93.98 [a]	0.81 ± 0.07 [a]	-43.37 ± 2.20 [c]
22	Xanthan gum + water	Oil (6 mg/mL) + acetone + Csgel	Gelled	Opaque	Stable	0.00	0.00	0.00	726.10 ± 34.19 [b]	0.68 ± 0.05 [a]	-49.77 ± 1.16 [b]
23	Xanthan gum + water	Oil (9 mg/mL) + acetone + Csgel	Gelled	Opaque	Stable	0.00	3.76	-	-	-	-
24	Xanthan gum + water	Oil (12 mg/mL) + acetone + Csgel	Gelled	Opaque	Stable	0.00	3.16	-	-	-	-

* Different letters in the same column showed a difference in significance level of 5 % ($p<0.05$). CI T_{room}: creaming index obtained after 24 hours of rest at room temperature; CI $T_{cooling}$: creaming index obtained after 24 hours of rest under refrigeration; CI Post-centr.: creaming index obtained after centrifugation.

Table 2. Conditions of static period and dynamic period of SFEE of murici oil (*B. crassifolia*) and characterization of emulsions.

Assay	Static period (s)	Dynamic period (s)	Lutein content (µg/g)	Retention of lutein (%)	Particle size (nm)	Pdi	ZP (mV)
1	0	3 600	131.15 ± 0.54 ^{h}	58.96 ± 0.24 ^{h}	614.53 ± 40.27 ^{ab}	0.66 ± 0.06 ^{ab}	-35.37 ± 0.25 ^{a}
2	0	10 800	137.49 ± 0.58 ^{f}	61.81 ± 0.26 ^{f}	525.63 ± 27.95 ^{bc}	0.65 ± 0.11 ^{ab}	-49.00 ± 0.26 ^{cde}
3	0	18 000	145.27 ± 0.87 ^{e}	65.31 ± 0.39 ^{e}	494.03 ± 20.15 ^{c}	0.48 ± 0.07 ^{b}	-40.07 ± 1.82 ^{ab}
4	1 800	3 600	101.56 ± 0.63 ^{i}	45.66 ± 0.28 ^{i}	496.47 ± 28.16 ^{c}	0.53 ± 0.06 ^{ab}	-44.53 ± 1.14 ^{bcd}
5	1 800	10 800	132.96 ± 0.48 ^{g}	59.77 ± 0.22 ^{g}	478.50 ± 23.08 ^{c}	0.71 ± 0.20 ^{ab}	-45.87 ± 2.80 ^{bcde}
6	1 800	18 000	159.94 ± 0.74 ^{c}	71.90 ± 0.33 ^{c}	382.30 ± 30.47 ^{d}	0.64 ± 0.08 ^{ab}	-50.87 ± 0.55 ^{de}
7	3 600	3 600	154.99 ± 0.49 ^{d}	69.68 ± 0.22 ^{d}	656.07 ± 23.44 ^{a}	0.62 ± 0.07 ^{ab}	-51.73 ± 1.76 ^{e}
8	3 600	10 800	188.39 ± 0.67 ^{b}	84.69 ± 0.30 ^{b}	642.10 ± 19.23 ^{a}	0.79 ± 0.17 ^{a}	-46.63 ± 2.12 ^{cde}
9	3 600	18 000	214.71 ± 0.34 ^{a}	96.53 ± 0.15 ^{a}	364.03 ± 54.59 ^{d}	0.58 ± 0.01 ^{ab}	-43.50 ± 5.12 ^{bc}

* Different letters in the same column showed a difference in significance level of 5 % ($p<0.05$).

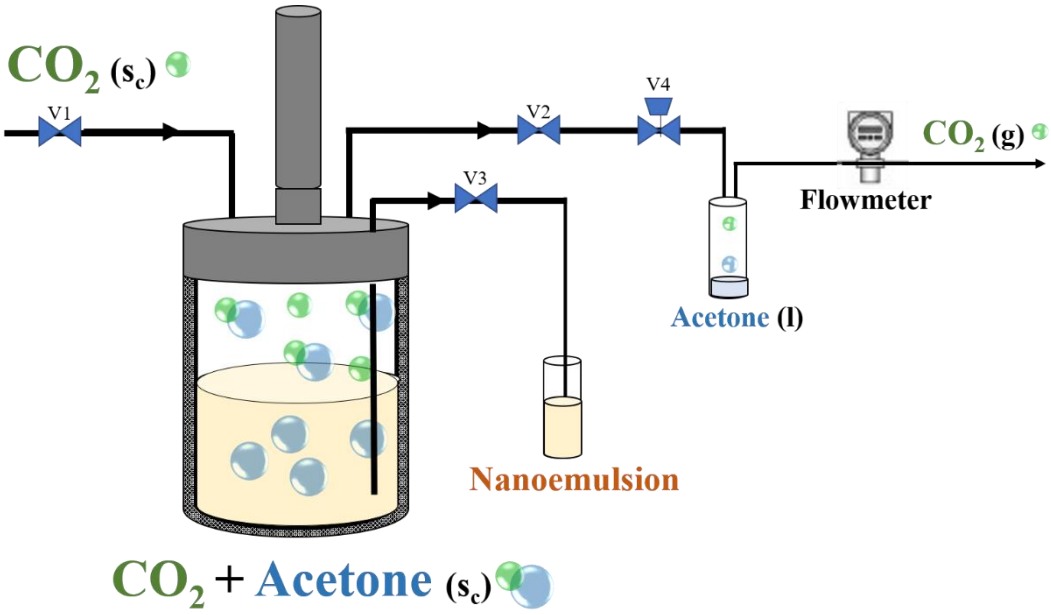


Fig. 1. Schematic representation of SFEE of murici oil (*B. crassifolia*). V1-V3: Flow control valves, V4: Micrometric flow control valve.

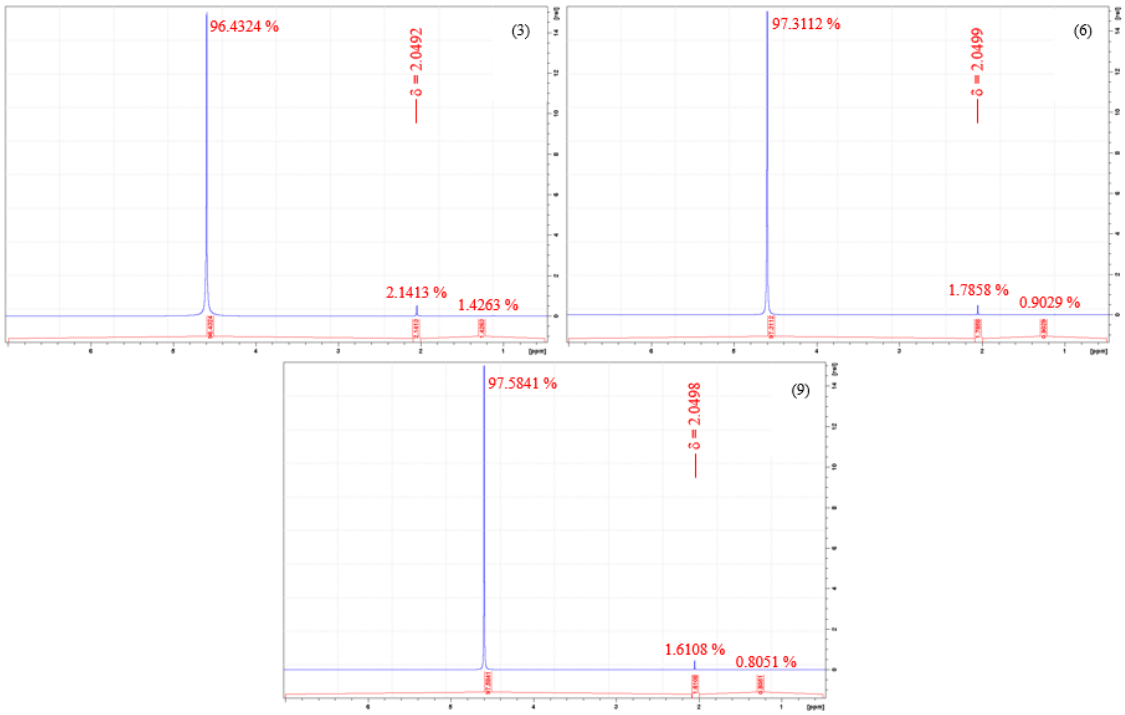


Fig 2. High-field 1H nuclear magnetic resonance (NMR) spectra of SFEE emulsions of murici oil (*B. crassifolia*) of assays 1, 3 and 9.

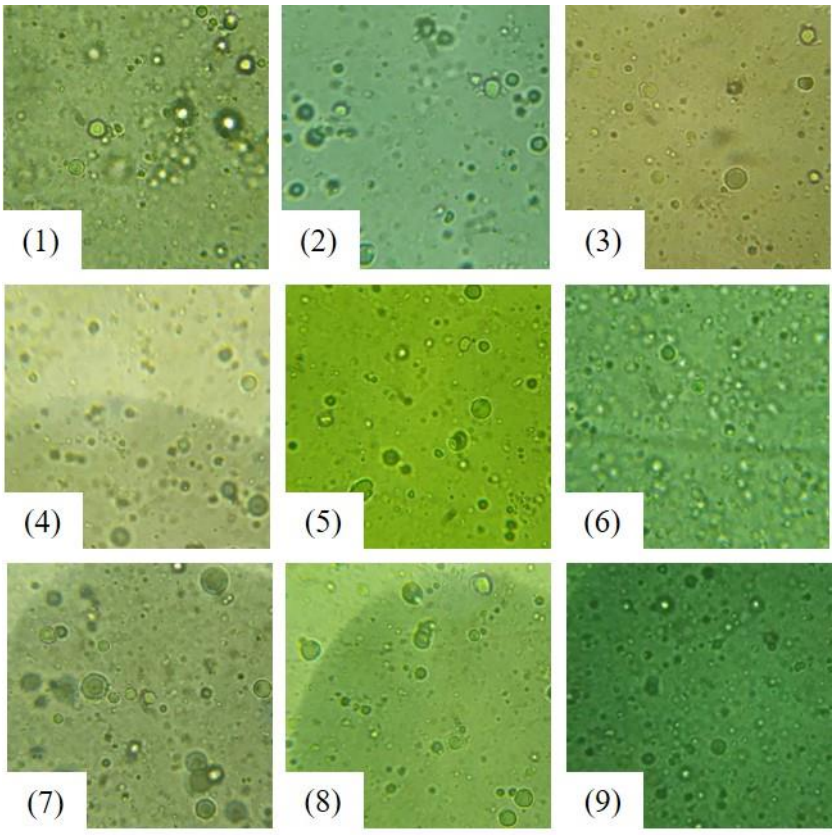


Fig 3. Optical microscopy of the SFEE emulsions of murici oil (*B. crassifolia*).

* The numbers shown in the images correspond to the assays listed in table 2.

CAPÍTULO V

Produção de partículas secas e de suplemento alimentar à base de óleo de murici

Flávia Cristina Seabra Pires, Leticia Maria Martins Siqueira, Ana Paula de Souza e Silva, Maria Caroline Rodrigues Ferreira, Raul Nunes de Carvalho Junior

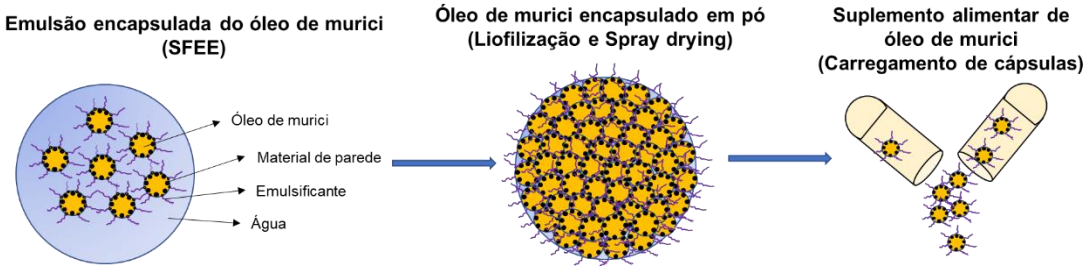
Submetido no Instituto Nacional da Propriedade Industrial (INPI), 2021:
PIRES, F. C. S.; SIQUEIRA, L. M. M.; SILVA, A. P. S.; FERREIRA, M. C. R.; CARVALHO JUNIOR, R. N. Produção de partículas secas e de suplemento alimentar à base de óleo de murici.

RESUMO

PRODUÇÃO DE PARTÍCULAS SECAS E DE SUPLEMENTO ALIMENTAR À BASE DE ÓLEO DE MURICI

O óleo de murici apresenta como carotenoide majoritário a luteína, que é um composto bastante comercializado na forma de suplementos alimentares, com apelo sobre a prevenção de doenças oculares. Para garantir a estabilidade da luteína, foi necessário realizar o encapsulamento do óleo, na forma de emulsão, seguido da secagem para garantir a proteção do óleo às condições de armazenamento do produto e aumentar a vida útil. Os pós obtidos por liofilização e spray drying apresentaram características desejáveis para a aplicação do óleo encapsulado em suplementos alimentares obtidos pelo carregamento destes pós em cápsulas vegetais. Diante disto, a presente invenção refere-se ao processo de produção de partículas secas e de suplemento alimentar à base de óleo de murici, onde os produtos apresentam-se ricos em luteína, sendo promissores ao uso na complementação da alimentação humana.

DESENHOS


Emulsão encapsulada do óleo de murici (SFEE)
Óleo de murici
Material de parede
Emulsificante
Água
Óleo de murici encapsulado em pó (Liofilização e Spray drying)
Suplemento alimentar de óleo de murici (Carregamento de cápsulas)

TÍTULO

[001] Produção de partículas secas e de suplemento alimentar à base de óleo de murici.

CAMPO DE UTILIZAÇÃO

[002] O aumento da consciência dos consumidores que almejam melhoras na qualidade de vida e optam por hábitos mais saudáveis fez com que houvessem mudanças de perspectiva no desenvolvimento de novos produtos na indústria alimentícia e farmacêutica.

[003] Há uma tendência mundial que aponta para a necessidade de que os alimentos não sejam mais somente vistos como uma fonte sensorial, mas também como fonte de bem-estar e de saúde para os indivíduos. Esta mudança de perspectiva tem exigido mudanças de paradigma no desenvolvimento de novos produtos, onde aumentou-se o interesse no encapsulamento de compostos bioativos de fontes naturais para o desenvolvimento de suplementos alimentares, visto que ingestão continuada de compostos bioativos vegetais está associada à prevenção/tratamento de doenças.

[004] Tendo em vista este panorama, os métodos de encapsulamento de compostos bioativos vegetais que utilizam a tecnologia supercrítica, como a extração de emulsões por fluido supercrítico (SFEE), têm se destacado devido ao apelo ambiental global pelo uso de tecnologias limpas, associado às restrições de aplicação de produtos obtidos com solventes tóxicos nas indústrias alimentícias e farmacêuticas.

[005] As emulsões encapsuladas por SFEE apresentam os compostos bioativos protegidos aos fatores de armazenamento como temperatura, umidade, e luz, o que permite que não ocorra a degradação destes compostos até a ingestão. Entretanto, para a aplicação dessas emulsões em suplementos alimentares, se faz necessário o emprego de técnicas de desidratação, como a liofilização e o spray drying, que são processos que possibilitam a obtenção de pós encapsulados com características desejáveis de uso.

[006] Nas últimas duas décadas, o mercado de suplementos cresceu substancialmente, tendo destaque os produtos que são constituídos por óleos e por luteína, composto este associado ao tratamento e prevenção de doenças oculares. Tendo em vista isto, se torna de extrema importância a aplicação do óleo de murici na produção de suplemento alimentar, visto que ele é rico em luteína.

ESTADO DA TÉCNICA

[007] Murici é uma fruta que produz óleo com propriedades antioxidantes, anti-hiperlidêmicas, antitrombogênicas e anti-aterogênicas, rico em luteína (Pires et al., 2019; Rezende & Fraga, 2003), que é um composto amplamente associado ao tratamento e prevenção de doenças maculares degenerativas relacionadas ao envelhecimento (Nwachukwu, Udenigwe, & Aluko, 2016).

[008] O processo de extração de emulsões por fluido supercrítico (SFEE) representa um método de encapsulamento que combina processos convencionais de preparação de emulsão com processo antissolvente supercrítico para a produção de partículas (US6998051, 2006).

[009] As técnicas de desidratação como a liofilização e spray drying permitem a expansão do uso de materiais encapsulados em suplementos alimentares na forma de cápsulas. A liofilização é um processo de desidratação realizado em baixas temperaturas, que remove a água através da sublimação. Já a secagem por spray dryer é uma técnica que utiliza elevadas temperaturas associadas ao uso de uma alimentação realizada por aspersão, onde o ar seco em contracorrente remove rapidamente a água das partículas, sem o aquecimento demasiado dos pós (Santo et al. 2013).

[010] As cápsulas são formas farmacêuticas sólidas nas quais as substâncias ativas (pós), adicionadas ou não de adjuvantes, encontram-se contidas em um invólucro mais ou menos flexível (Loyd; Popovich; Ansel, 2013). Em cápsulas rígidas, os invólucros apresentam duas partes desmontáveis, o corpo e a tampa. Na manipulação, as duas partes são separadas, onde o corpo é preenchido com o pó e, então, a tampa é recolocada (Thompson; Davidow, 2013). Estes produtos oferecem uma forma de dosagem individualizada e representam uma forma farmacêutica empregada para a administração oral de compostos com elevada potência biológica (Thompson; Davidow, 2013).

[011] O processo de carregamento de cápsulas rígidas envolve as seguintes etapas: pesagem dos componentes da formulação, moagem ou tamisação dos pós (opcional), mistura dos pós, enchimento das cápsulas, fechamento e selagem das cápsulas, limpeza e polimento externo, acondicionamento e rotulagem (Ferreira, 2011). Todas essas etapas de processamento são influenciadas pelas características de cada componente adicionado na formulação. O ideal é que os pós presentes na formulação apresentem propriedades semelhantes. As principais características dos componentes que determinam a qualidade das cápsulas são: tamanho e forma, densidade, umidade, entre outros (Le Hir, 1997).

INVENÇÃO

[012] A presente invenção refere-se a produção de partículas secas e de suplemento alimentar à base de óleo de murici. A produção de partículas secas à base do óleo de murici proporciona a obtenção de pós encapsulados, ou seja, permite a proteção dos compostos bioativos do óleo, em especial da luteína. Além disso, permite a aplicação do uso do óleo de murici no carregamento de cápsulas duras para a produção de suplemento alimentar à base de óleo de murici rico em luteína. A comercialização das partículas secas à base do óleo na forma de suplementos possibilita o controle da dosagem adequada para os consumidores e torna o produto mais atrativo e competitivo no mercado de suplementação.

DESCRIÇÃO DETALHADA DA INVENÇÃO

[013] As emulsões foram preparadas com óleo de murici, emulsificante csgel, água, goma xantana e acetona. Primeiramente foi preparada a fase aquosa, onde a goma xantana foi solubilizada em água. A fase oleosa foi preparada homogeneizando o óleo, a acetona e o emulsificante. A fase oleosa foi gotejada na fase aquosa, onde a mistura foi homogeneizada e submetida a banho de ultrassom por 360 s.

[014] Na SFEE foi realizada em uma unidade de reação supercrítica (modelo 2777 1000, Top Industrie, França), utilizando o CO_2 supercrítico como antissolvente. As emulsões foram adicionadas na autoclave. O processo foi realizado nas faixas de pressão de 80-110 bar, temperaturas de 40-60 ºC, vazões de saída de 0,5-2,0 NL/min, períodos estáticos de 0-1 hora e períodos dinâmicos de 5-10 horas.

[015] A emulsão SFEE foi seca por liofilização e por spray drying.

[016] Para a liofilização, a emulsão SFEE foi adicionada em bandejas de aço inoxidável e foi congelada (-15 ºC). A amostra foi liofilizada em liofilizador de bancada (modelo L101, Liobras, São Carlos, Brasil) a -48 ºC durante 2 dias.

[017] Para a secagem em spray dryer, foi utilizado um mini secador de spray (modelo B-290, BUCHI Labortechnik AG, Flawil, Suíça). Na secagem da emulsão SFEE, foram utilizadas temperaturas de entrada na faixa de 170-220 ºC, alimentações de 30-50 % e pressões de 0,04-0,06 bar. A emulsão em pó foi coletada em um recipiente na base do ciclone.

[018] O carregamento das cápsulas foi realizado com as emulsões secas (liofilizadas e spray dryer). Foram utilizados cápsulas vegetais. As cápsulas vegetais foram carregadas

com as emulsões secas utilizando encapsuladora semi-automática (modelo 120 cáp., Mawin, Santo André, Brasil).

REIVINDICAÇÕES

1. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar as emulsões preparadas com óleo de murici, emulsificante csgel, água, goma xantana e acetona.
2. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar o processo de extração de emulsões por fluido supercrítico (SFEE) nas pressões de 80-110 bar, temperaturas de 40-60 ºC, vazões de saída de 0,5-2,0 NL/min, períodos estáticos de 0-1 hora e períodos dinâmico de 5-10 horas.
3. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar a emulsão por fluido supercrítico (SFEE) seca por liofilização na T= -48 ºC durante 2 dias.
4. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar a emulsão por fluido supercrítico (SFEE) seca por spray drying, com temperaturas de entrada na faixa de 170-220 ºC, alimentações de 30-50 % e pressões de 0,04-0,06 bar.
5. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar o carregamento das cápsulas com as emulsões secas (liofilizadas e spray dryer).
6. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar o uso de cápsulas vegetais para produzir o suplemento a base de óleo de murici.
7. Produção de partículas secas e de suplemento alimentar à base de óleo de murici, **caracterizado por** reivindicar as cápsulas vegetais serem carregadas com as emulsões secas utilizando encapsuladora semi-automática (modelo 120 cáp., Mawin, Santo André, Brasil).

MATERIAL SUPLEMENTAR

1 MATERIAIS E MÉTODOS

1.1 EXTRAÇÃO DE EMULSÕES POR FLUIDO SUPERCRÍTICO (SFEE)

As emulsões foram preparadas com óleo de murici (6mg/mL), emulsificante csgel (1 %), água (84 %), goma xantana (0,25 %) e acetona (14,75 %). Primeiramente foi preparada a fase aquosa, onde a goma xantana foi solubilizada em água e deixada em repouso por 43200 s para garantir sua completa saturação. A fase oleosa foi preparada homogeneizando o óleo, a acetona e o emulsificante, os quais foram submetidos a um banho de ultrassom (Potência = 340 W/ Frequência = 50/60 Hz) por 600 s para garantir a homogeneidade completa. Para a formação da emulsão, a fase aquosa foi agitada com o auxílio de um agitador magnético a 313,15 K por 300 s. Em seguida, a fase oleosa foi gotejada, sob agitação constante, na fase aquosa. A mistura foi homogeneizada por 180 s e submetida a banho de ultrassom por 360 s. Na SFEE foi realizada em uma unidade de reação contendo autoclave de aço inoxidável (modelo 2777 1000, Top Industrie, França) com um volume interno de $9{,}50 \times 10^{-5}$ m^3 (Ø 0,128 × 0,152 m), colar de aquecimento (modelo 2777 1021, Top Industrie, França), agitador magnético (modelo 616 0100, Top Industrie, França), termopar (modelo TKA 15 × 20 HMP, Erciat, França), cabeça de segurança (modelo 728 0350, Top Industrie, França), disco de ruptura (modelo 728 0400, Sitec, Suíça), transdutor de pressão (modelo PA-23SY, Keller, Suíça), painel de controle (modelo TS1070, Monitouch Technoshot, Holanda), bomba de líquido, compressor de ar (modelo CSA, Schulz S/A, Brasil), válvula micrométrica (modelo 30VRMM4812, Autoclave Engineers, EUA), medidor de fluxo (modelo SLA5861, Brooks Instrument's, França) e um cilindro e recirculador de CO_2 (modelo F08400796, Polyscience, EUA). As emulsões foram adicionadas na autoclave, que foi fechada com o auxílio de um torquímetro. O processo foi realizado na pressão de 80 bar, temperatura de 40 ºC, vazão de saída de 0,5 NL/min, período estático de 1 hora e período dinâmico de 5 horas.

1.2 SECAGEM DAS EMULSÕES SFEE

1.2.1 Liofilização

A emulsão SFEE fora adicionada em bandejas de aço inoxidável e foi congelada (-15 ºC). A amostra foi liofilizada em liofilizador de bancada (modelo L101, Liobras, São Carlos,

Brasil) a -48 ºC durante 2 dias. A emulsão em pó foi coletada posteriormente moída em moinho (modelo A11, Ika, Campinas, Brasil) e foi armazenada em frascos de vidro hermético, sob proteção de luz, sob congelamento (-10 ºC).

1.2.2 Spray Drying

Um mini secador de spray (modelo B-290, BUCHI Labortechnik AG, Flawil, Suíça) equipado com um painel de controle, elemento de aquecimento elétrico, bomba peristáltica, dois bicos de fluido, câmara de secagem e ciclone feito de classe transparente espessa foi usado na secagem da emulsão SFEE. Para o processo de secagem, o ar ambiente foi aquecido com um elemento de aquecimento elétrico e soprado em fluxo paralelo ao líquido atomizado dentro da câmara de secagem a 35 m^3/h. A câmara de secagem foi alimentada por uma bomba peristáltica de dois bicos (diâmetro interno do bocal de alimentação de 0,7 mm) acionada por ar comprimido (8 bar) e controlada por um medidor de vazão. Na secagem da emulsão SFEE foi utilizado temperatura de entrada de 170 ºC, temperatura de saída de 92 ºC, alimentação de 30 % e pressão de 0,04 bar. A emulsão em pó foi coletada em um recipiente na base do ciclone, colocada em frascos de vidro hermético, sob proteção de luz, e armazenada sob congelamento (-10 ºC).

1.3 CARACTERIZAÇÃO DAS EMULSÕES SECAS

1.3.1 Umidade

Os teores de umidade foram obtidos de acordo com AOAC (2006), em estufa de circulação de ar a 105 ºC. As determinações foram feitas em triplicata e os resultados foram expressos em %.

1.3.2 Atividade de água

A atividade de água (a_w) foi determinada a 25 ºC em um medidor de atividade de água (modelo Aqualab 3TE, Decagon Devices Inc., EUA). A determinação foi realizada em triplicata.

1.3.3 Tamanho Médio, Polidispersividade e Potencial Zeta

Os tamanhos médios de partícula, índices de polidispersidade (Pdi) e os potenciais Zeta (ZP) das emulsões SFEE secas foram medidos por espectroscopia de correlação de fótons usando um Zetasizer Nano Series (Malvern Instrument, Royston, Reino Unido). As emulsões obtidas por liofilização e por spray drying foram reconstituídas em água a 0,2 mg/mL. Uma diluição das amostras com água foi necessária para atingir a densidade óptica apropriada (1:99). Todas as medições foram feitas a 298 K. As medições foram feitas em triplicata e os resultados foram expressos em nm.

1.3.4 Teores de Luteína

Os teores de luteína foram determinados de acordo com a metodologia proposta por Rodriguez-Amaya & Kimura (2004). Na extração foi utilizada a acetona. Na partição foramm utilizados o éter etílico e o éter de petróleo. Para as leituras, o extrato foi reconstituído em etanol. As leituras foram realizadas em espectrofotômetro (modelo UV-M90, Bel Engineering, Italy). Os teores de luteína total foram calculados a partir das absorvâncias obtidas a 445 nm e com o coeficiente de absortividade da luteína em etanol (2550). As quantificações foram realizadas em triplicate e os resultados foram expressos em μg/g.

1.4 CARREGAMENTO DAS CÁPSULAS

O carregamento das cápsulas foi realizado com as emulsões secas (liofilizadas e spray dryer) sem o uso de materiais de enchimento. Foram utilizados cápsulas vegetais de HPMC (Hypromellose-Hydroxypropyl Methylcellulose) nº 0 (500 mg). As cápsulas vegetais HPMC foram carregadas com as emulsões secas utilizando encapsuladora semi-automática (modelo 120 cáp., Mawin, Santo André, Brasil). As cápsulas carregadas foram colocadas em frascos de vidro herméticos, sob proteção de luz, e armazenadas sob congelamento (-10 ºC).

1.5 CARACTERIZAÇÃO DAS CÁPSULAS CARREGADAS

1.5.1 Peso médio das cápsulas manipuladas ($P_{Médio}$)

Foi calculado pela média aritmética do peso das cápsulas manipuladas (FARMACOPÉIA BRASILEIRA, 2012).

1.5.2 Desvio padrão relativo (DPR)

O DPR foi calculado pela equação 1 e será expresso em % (FARMACOPÉIA BRASILEIRA, 2012).

$$DPR\ (\%) = (\text{Desvio padrão do } P_{Médio}/\ P_{Médio}) \times 100 \quad (1)$$

1.5.3 Variação do conteúdo teórico (%)

Para determinar a variação do conteúdo teórico nas cápsulas, foi necessário determinar o peso médio das cápsulas vazias e o peso teórico das cápsulas. O peso médio das cápsulas vazias foi obtido pesando-se, individualmente, 20 cápsulas vazias e calculando-se a média aritmética. O peso teórico das cápsulas foi obtido com a soma de peso médio das cápsulas vazias e os pesos teóricos das substâncias adjuvantes e fármacos que compõem a fórmula. A variação teórica de conteúdo das cápsulas foi estimada determinando a Quantidade teórica mínima de pó ($Q_{teor.mín.}$) e a Quantidade teórica máxima de pó ($Q_{teor.máx.}$), de acordo com os extremos de pesos obtidos na pesagem das cápsulas (equações 2 e 3) (FARMACOPÉIA BRASILEIRA, 2012).

$$Q_{teor.mín.} = (P_{cápsula\ mais\ leve}/\ P_{teórico}) \times 100 \quad (2)$$

$$Q_{teor.máx.} = (P_{cápsula\ mais\ pesada}/\ P_{teórico}) \times 100 \quad (3)$$

1.5.4 Tempo de desintegração

Sete cápsulas foram colocadas em um tubo agitador com água durante 900 s. Em seguida, o material foi passado em uma peneira de 10 mesh. A cápsula foi considerada totalmente desintegrada quando nenhum material ficou retido na peneira (PUJARA; PARMAR, 2013).

1.6 ANÁLISE ESTATÍSTICA

As médias e desvios-padrão foram calculados para todas as análises. Os resultados das análises foram submetidos ao teste de Tukey, ao nível de significância de 5 % ($p<0,05$). O programas Excel 2000 SR-1 (Microsoft, Troy, EUA) e Statistica Kernel Release 7.1

(StartSoft Inc., Tulsa, EUA) foram usados como ferramentas.

2 RESULTADOS E DISCUSSÃO

2.1 UMIDADE E Aw

Os teores de umidade e de Aw das emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentadas na Tabela 1. Os resultados de umidade foram de 1,78 e 2,02 %, enquanto que os valores de Aw foram de 0,21 e 0,37 para as emulsões obtidas por spray drying e por liofilização, respectivamente, onde somente a Aw apresentou diferença significativa ($p \geq 0,05$ %). Estes valores demonstram que os teores foram relativamente baixos, onde segundo a legislação vigente, os alimentos comercializados sob forma seca, como em muitos dos suplementos alimentares (comprimidos, cápsulas de gel duro e pós), o teor de umidade dos ingredientes deve estar no nível mais baixo possível, consistente com o processo de fabricação do produto (ANVISA, 2018a). Desse modo, foi possível observar que os resultados estão de acordo com seus processos de fabricação, visto que os valores de umidade e de Aw foram similares aos resultados observados por outros estudos sobre a obtenção de emulsões em pó por liofilização e por spray drying (FERNANDES; BORGES; BOTREL, 2013; REÁTEGUI et al., 2017; SANTANA et al., 2014). Além disso, foi observado que faixa de Aw obtida nos pós pode ser considerada segura em relação ao crescimento microbiano, visto que fungos se desenvolvem a partir de Aw de 0,6, bactérias a partir de 0,7 e toxinas são produzidas a partir de 0,8 (BARBOSA-CÁNOVAS et al., 2003; LABUZA et al., 1972). Portanto, ambas emulsões secas mostram-se dentro dos padrões de processamento e estabilidade no que diz respeito à estes resultados.

2.2 TAMANHO MÉDIO, POLIDISPERSIVIDADE E POTENCIAL ZETA

Os resultados de diâmetro médio, polidispersividade e potencial Zeta das emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentados na Tabela 1. Os resultados de tamanho de partícula foram de 656,90 e 707,20 nm, os valores de Pdi foram de 0,70 e 0,50, e os valores de ZP foram de -34,30 e 33,53 para as emulsões obtidas por spray drying e por liofilização, respectivamente, onde somente a ZP não apresentou diferença significativa ($p \geq 0,05$ %). Os valores de tamanhos

de partícula foram bastante inferiores aos obtidos por Reátegui et al. (2018) ao estudar a produção de partículas de oleorresina de copaíba (*Copaifera officinalis*) por SFEE, onde as emulsões em pó obtidas por liofilização apresentaram tamanhos de 238,72 a 277,75 µm, enquanto que as obtidas por spray drying foram na faixa de 10,52 a 14,68 µm, embora os tamanhos as emulsões SFEE antes das secagens tenham sido bastante inferiores (261,7 nm) aos obtidos no presente estudo (364,03 nm). Isto demonstra que as condições de secagem não alteraram de forma drástrica a estabilidade das emulsões, onde tal estabilidade tambem pode ser observada pelos valores de ZP, visto que foram elevados (distantes de zero). Os valores de Pdi foram elevados possívelmente devido ao comportamento bimodal das emulsões, visto que apresentaram duas populações de partículas, uma em aproximadamente 450 nm (87.3 %) e outra em 72 nm (12.7 %) (GONZÁLEZ-SAUCEDO et al., 2019; LI; LU, 2016). Foi possível observar que as partículas obtidas por liofilização apresentaram menor tamanho médio de partícula e maiores Pdi e ZP em comparação às obtidas por spray drying. Embora tenha ocorrido tal diferença, ambas emulsões secas apresentaram tamanhos na faixa nanométrica e foram consideradas estáveis, o que demonstrou adequação para serem utilizadas em suplementos alimentares, visto que partículas reduzidas possuem mecanismos de liberação controlada de compostos bioativos, onde tal mecanismo de liberação é ativado pelo intumescimento do polímero, onde as cadeias poliméricas são quebradas pela clivagem de suas ligações intermoleculares, de modo que durante essa degradação ocorre a liberação do agente ativo (MCCLEMENTS, 2014; SALVIA-TRUJILLO; MCCLEMENTS, 2016).

Tabela 1. Umidade, atividade de água, tamanhos médios, polidispersividade, potencial Zeta e teor de luteína das emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying.

Determinação	Emulsão liofilizada	Emulsão spray dryer
Umidade (%)	2,02 ± 0,64 [a]	1,78 ± 0,06 [a]
Aw	0,37 ± 0,02 [a]	0,21 ± 0,04 [b]
Tamanho médio (nm)	656,90 ± 10,48 [b]	707,20 ± 1,10 [a]
Pdi	0,70 ± 0,04 [a]	0,50 ± 0,09 [b]
ZP (mV)	34,30 ± 1,30 [a]	33,53 ± 0,42 [a]
Luteína (µg/g)	10599,15 ± 0,72 [b]	11993,65 ± 0,81 [a]

2.2 TEORES DE LUTEÍNA

Os resultados dos teores de luteína das emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentados na Tabela 1. Os resultados de luteína foram de 10599,15 μg/g (10.60 mg/g) para a emulsão obtida por liofilização e 11993,65 μg/g (11.99 mg/g) para a obtida por spray drying. Foi possível observar que ocorreu a perda de luteína em ambos os processos, onde na liofilização houve perda de 14 % e no spray drying de 32 %. De acordo com o mercado de suplementos à base de luteína, estes valores são satisfatórios, visto que produtos comerciais são encontrados no mercado em concentrações a partir de 10 mg/g, onde é possível obter tal concentração carregando estes pós em cápsulas vegetais de nº 0 (500 mg) com ingestão diária de 2 cápsulas ou em cápsulas vegetais de nº 00 (1000 mg) com ingestão diária de 1 cápsula. Embora o mercado estabeleça limites de concentração, segundo a legislação vigente, os suplementos de luteína são classificados como produtos sem limites de concentração devido à ausência de valores de IDR (Ingestão Diária Recomendada). Entretanto, a proposta inicial de limite máximo é de 20 mg para adultos, onde o produto deve apresentar a seguinte advertência: "Este produto não deve ser consumido por gestantes, lactantes e crianças" (ANVISA, 2017). A justificativa para não ser consumido por estes grupos populacionais se deve à ausência de informações sobre a segurança de uso da luteína em suplementos alimentares para obter um nível seguro de consumo por crianças, grávidas e lactantes. Assim, a autorização de uso de luteína em suplementos alimentares indicados para esses grupos populacionais necessita de comprovação de segurança e eficácia (ANVISA, 2018b). Portanto, ambos os pós mostram-se adequados para serem utilizados em suplementos alimentares de murici, ricos em luteína, voltados aos consumidores adultos que desejam prevenir ou tratar o envelhecimento da retina dos olhos e melhorar a saúde ocular.

2.3 PESO MÉDIO DAS CÁPSULAS MANIPULADAS ($P_{MÉDIO}$)

Os $P_{Médio}$ das cápsulas carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentados na Tabela 1. Os resultados foram de 480,13 ± 7,21 mg para as cápsulas carregadas com o pó obtido por liofilização e de 492,05 ± 11,14 mg para as cápsulas carregadas com o pó obtido por spray drying. Os limites de variação de ambas cápsulas estão dentro do tolerados pela legislação,

que determina que o $P_{Médio}$ deve ser de ± 7,5 % para cápsulas com peso de 300 mg ou mais (FARMACOPÉIA BRASILEIRA, 2012).

Tabela 2. Peso médio, desvio padrão relativo, quantidade teórica mínima e quantidade teórica máxima das cápsulas vegetais carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying.

Determinação	**Cápsulas (Pó liofilização)**	**Cápsulas (Pó spray drying)**
Peso médio das cápsulas (mg)	480,13 ± 7,21 [a]	492,05 ± 11,14 [a]
Desvio Padrão relativo (%)	1,50	2,26
Quantidade teórica mínima (%)	97,25	94,49
Quantidade teórica máxima (%)	103,28	102,39
Tempo de desintegração (min)	10 ± 0,10 [b]	12 ± 0.16 [a]

2.4 DESVIO PADRÃO RELATIVO (DPR)

Os DPR das cápsulas carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying foram de 1,50 % e 2,26 %, respectivamente, como mostrado na Tabela 2. Estes valores estão dentro do limite de variação tolerado para o DPR que é de 4 % (FARMACOPÉIA BRASILEIRA, 2012).

2.5 VARIAÇÃO DO CONTEÚDO TEÓRICO (%)

As variações dos conteúdos teóricos, representadas pelas quantidades teóricas mínima e máxima, das cápsulas carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentadas na Tabela 2. As cápsulas carregadas com o pó obtido por liofilização apresentaram quantidades teóricas mínima e máxima de 97,25 % e 103,28 %, respectivamente. Já as cápsulas carregadas com o pó obtido por spray drying apresentaram quantidades teóricas mínima e máxima de 94,49 % e 102,39 %, respectivamente. Apesar de distintos, tais percetuais se encontram dentro do intervalo estipulado pela farmacopéia brasileira, que varia de 90 a 110 % (FARMACOPÉIA BRASILEIRA, 2012).

2.6 TEMPO DE DESINTEGRAÇÃO

Os tempos de desintegração das cápsulas carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) obtidas por liofilização e por spray drying estão apresentadas na Tabela 2. Os resultados foram de 10 minutos para as cápsulas carregadas com o pó obtido por liofilização e de 12 minutos para as cápsulas carregadas com o pó obtido por spray drying. Estes resultados foram considerados adequados, visto que o limite máximo é de 45 minutos (ANVISA, 2010).

3. CONCLUSÃO

Foi possível obter emulsões SFEE em pó do óleo de murici (*B. crassifolia*) por liofilização e por spray drying com umidades, atividades de água, tamanhos de partícula, estabilidades e teores de luteína adequados para serem utilizados em suplementos alimentares carregados em cápsulas vegetais. As cápsulas vegetais carregadas com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) por liofilização e por spray drying apresentaram-se dentro dos padrões de carregamento para comercialização. Portanto, foi possível obter suplementos alimentares de óleo de murici, ricos em luteína.

REFERÊNCIAS

ANVISA. Farmacopeia Brasileira Volume 1. **Farmacopeia Brasileira, 5ª edição**, v. 1, 2010.

ANVISA. Suplementos alimentares: Documento de base para discussão regulatória. **Diário da União**, p. 1–94, 2017.

ANVISA. Guia para determinação de prazos de validade de alimentos. **Diário da União**, p. 1–76, 2018a.

ANVISA. Justificativas para os limites mínimos e máximos de nutrientes, substâncias bioativas e enzimas da proposta regulatória de suplementos alimentares. **Diário da União**, p. 1–36, 2018b.

AOAC. Official methods of analysis of the Association of Official Analytical Chemists (method 920.39): Official methods of analysis Proximate Analysis and Calculations Crude Fat (CF). In: **Official methods of analysis of the Association of Official Analytical Chemists**. 17. ed. Gaithersburg: Association of Analytical Communities, 2006.

BARBOSA-CÁNOVAS, G. V. et al. GENERAL CONSIDERATIONS FOR

PRESERVATION OF FRUITS AND VEGETABLES. In: **TECHNICAL MANUAL FAO Agricultural Services Bulletin 149: Handling and Preservation of Fruits and Vegetables by Combined Methods for Rural Areas**. Rome: FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2003. p. 1–13.

FARMACOPÉIA BRASILEIRA. **Formulário Nacional Da Farmacopeia Brasileira**, 2012. (Nota técnica).

FERNANDES, R. V. DE B.; BORGES, S. V.; BOTREL, D. A. Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. **Food Science and Technology**, v. 33, n. SUPPL.1, 2013.

GONZÁLEZ-SAUCEDO, A. et al. Extension of the postharvest quality of bell pepper by applying nanostructured coatings of chitosan with Byrsonima crassifolia extract (L.) Kunth. **Postharvest Biology and Technology**, v. 149, p. 74–82, 2019.

LABUZA, T. P. et al. STABILITY OF INTERMEDIATE MOISTURE FOODS. 1. Lipid Oxidation. **Journal of Food Science**, v. 37, n. 1, 1972.

LI, P.-H.; LU, W.-C. Effects of storage conditions on the physical stability of d-limonene nanoemulsion. **Food Hydrocolloids**, v. 53, p. 218–224, 2016.

MCCLEMENTS, D. **Nanoparticle- and Microparticle-based Delivery Systems**. Boca Raton: CRC Press, 2014.

PUJARA, N. D.; PARMAR, R. B. Formulation and Evaluation of Hard Gelatin Capsule of Losartan Potassium. **Inventi Journals**, v. 2013, n. 2, p. 21–29, 2013.

REÁTEGUI, J. L. P. et al. Production of Copaiba oleoresin particles from emulsions stabilized with modified starches. **Industrial Crops and Products**, v. 108, p. 128–139, 2017.

REÁTEGUI, J. L. P. et al. Production of copaiba (Copaifera officinalis) oleoresin particles by supercritical fluid extraction of emulsions. **The Journal of Supercritical Fluids**, v. 140, p. 364–371, 2018.

RODRIGUEZ-AMAYA, D.; KIMURA, M. **HarvestPlus Handbook for Carotenoid Analysis**. 1. ed. Washington: Breeding Crops for Better Nutrition, 2004.

SALVIA-TRUJILLO, L.; MCCLEMENTS, D. J. Improvement of β-Carotene Bioaccessibility from Dietary Supplements Using Excipient Nanoemulsions. **Journal of Agricultural and Food Chemistry**, v. 64, p. 4639–4647, 2016.

SANTANA, A. A. et al. Microencapsulation of pequi pulp by spray drying: Use of modified

starches as encapsulating agent. **Engenharia Agricola**, v. 34, n. 5, 2014.

CONCLUSÃO GERAL

De acordo com o que foi apresentado no Capítulo I, o uso de tecnologia supercrítica na cadeia produtiva de suplementos alimentares demonstra a preocupação com o meio ambiente e com a qualidade dos bioativos obtidos, aumentando assim sua aplicabilidade e a sustentabilidade das indústrias alimentícia e farmacêutica.

O estudo da extração supercrítica da polpa de murici (*B. crassifolia*), apresentado nos Capítulos II e III, possibilitou a obtenção de óleos não-tóxicos com elevado teor de luteína, constituídos de triglicerídeos de cadeia longa ricos em ácidos graxos insaturados como os ômega 3 e 9, e com atividades antioxidante, antihipercolesterolemica, antiaterosclerogênica, antitrombogênica e citoprotetora, onde a melhor condição de extração foi obtida a 343,15 K/ 49 MPa/ 900 kg/m^3. Também possibilitou a obtenção de extratos etanólicos não-tóxicos a partir da polpa desengordurada, um subproduto nas indústrias alimentícias, contendo luteína, compostos fenólicos, flavonóides e atividade antioxidante, onde a melhor condição de extração foi obtida a 343,15 K/ 22 MPa/ 775 kg/m^3. Estes resultados mostraram o notável valor nutricional/nutracêutico dos extratos da polpa de murici, o que os qualificou como seguro para o desenvolvimento de produtos alimentícios e farmacêuticos, incluindo suplementos alimentares à base de luteína.

Devido ao efeito citoprotetor, o óleo de murici foi utilizado para a produção de partículas por meio do processo SFEE, como apresentado no Capítulo IV, onde foi possível produzir nanopartículas de óleo de murici com boa concentração/retenção de luteína, tamanho reduzido de partículas e com alta estabilidade, onde a melhor formulação das emulsões de partida foi obtida com o espessante goma xantana, com o emulsificante csgel e em uma concentração de 6 mg/mL de óleo de murici, e a melhor condição do processo SFEE foi a de 8 MPa, 313,15 K, período estático de 3600 s e período dinâmico de 18000 s. Portanto, o uso do CO_2 supercrítico em todas as etapas de obtenção das nanoemulsões (SFE e SFEE) do óleo de murici demonstrou que a tecnologia supercrítica pode ser utilizada na cadeia produtiva de diversos produtos naturais aplicados nas indústrias alimentícia e farmacêutica.

Foi possível obter emulsões SFEE em pó do óleo de murici por liofilização e por spray drying, como apresentado no Capítulo V, com umidades, atividades de água, tamanhos de partícula, estabilidades e teores de luteína adequados para serem utilizados em suplementos alimentares carregados em cápsulas vegetais. As cápsulas vegetais carregadas

com as emulsões SFEE em pó do óleo de murici (*B. crassifolia*) por liofilização e por spray drying apresentaram-se dentro dos padrões de carregamento para comercialização. Portanto, foi possível obter suplementos alimentares de óleo de murici, ricos em luteína.

Portanto, a presente tese evidenciou que é possível utilizar a tecnologia supercrítica na cadeia produtiva de suplementos alimentares a base de murici, para facilitar o acesso e a estabilidade dos compostos aos quais são atribuídos os potenciais terapêuticos do murici. Este trabalho verticalizou a importância de pesquisas sobre o efeito funcional do murici e sobre o uso da tecnologia supercrítica, o que contribuiu para o desenvolvimento da bieconomia da região e de novos produtos pela ciência, tecnologia e engenharia de alimentos, com um grande retorno à sociedade.