UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

EXTRAÇÃO E CONCENTRAÇÃO DE COMPOSTOS BIOATIVOS DE CAMU-CAMU E UVAIA POR PROCESSOS LIMPOS (ULTRASSOM ASSISTIDO E OSMOSE REVERSA)

LETÍCIA MISTURINI RODRIGUES

Maringá
2021

LETÍCIA MISTURINI RODRIGUES

EXTRAÇÃO E CONCENTRAÇÃO DE COMPOSTOS BIOATIVOS DE CAMU-CAMU E UVAIA POR PROCESSOS LIMPOS (ULTRASSOM ASSISTIDO E OSMOSE REVERSA)

Tese apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos

Profa. Dr. Adriano Costa de Camargo

Prof. Dr. André Álvares Monge Neto

Prof. Dra. Maysa Ariane Formigoni Fasolin

Dra. Ana Paula Stafussa

Profa. Dra. Grasiele Scaramal Madrona

Orientadora

Maringá
2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

R696e

Rodrigues, Letícia Misturini

Extração e concentração de compostos bioativos de camu-camu e uvaia por processos limpos (ultrassom assistido e osmose reversa) / Letícia Misturini Rodrigues. -- Maringá, PR, 2021.

54 f.: il., figs., tabs.

Orientadora: Profa. Dra. Grasiele Scaramal Madrona.

Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Ciências Agronômicas, Programa de Pós-Graduação em Ciência de Alimentos, 2021.

1. Myrtaceae. 2. Alimentos funcionais. 3. Atividade antioxidante. 4. Compostos fenólicos. I. Madrona, Grasiele Scaramal, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Ciências Agronômicas. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDD 23.ed. 641.302

Márcia Regina Paiva de Brito - CRB-9/1267

Orientadora

Grasiele Scaramal Madrona

BIOGRAFIA

LETÍCIA MISTURINI RODRIGUES, nasceu em vinte e nove de setembro de mil novecentos e noventa e um, NA CIDADE DE Planaltina do Paraná-PR. Possui graduação em ENGENHARIA DE ALIMENTOS pela Universidade Tecnológica Federal do Paraná - UTFPR e mestrado em Ciência de Alimentos pela UEM. Tem experiência nas áreas acadêmica e industrial atuando principalmente nos seguintes temas: controle de qualidade, pesquisa e desenvolvimento de alimentos, gestão e inovação, extração de compostos bioativos de frutas exóticas, identificação de compostos por métodos cromatográficos e melhoramento de processos industriais.

Dedico

Dedico esta tese de doutorado a minha família, sem eles não teria chegado até aqui.
Á equipe Innov. food product group por estes anos de trabalho e amizade.
Obrigada.

AGRADECIMENTOS

Agradeço primeiramente, a Deus por ter me proporcionado esse tempo de estudo e por permitir a conclusão do meu doutorado. Por ter colocado pessoas que foram essenciais durante a minha trajetoria, que nao mediram esforços para me ajudar, realizando parcerias e compartilhando de suas amizades e conhecimento para meu desenvolvimento pessoal e profissional. Agradeço ainda, por me mostrar cada dia mais que praticar a honestidade é o que alicerça meus passos e que sem esforço, dedicação e fé nada seria possível.

Aos meus pais José Augusto e Iliete, e irmão José Augusto Rodrigues Júnior, por me apoiarem a todo o tempo, por serem meus pilares durante os tempos difíceis e por sempre acreditarem em mim, me incentivando e comemorando junto por todas conquistas.

A toda equipe Innov food product group, em especial ao Me. Edilson Bruno Romanini, que me ajudou durante o trabalho e se tornou um grande parceiro amigo. Serei eternamente grata.

Aos Professores Doutores Adley Forti Rubira (DQI-UEM), Marcos Roberto Mauricio (DQI- UEM), Eduardo Jorge Pilau (DQI-UEM), Silvio Cláudio da Costa (NERPRON-UEM), que intermediaram parceiros e disponibilizaram alunos e recursos para colaborar de maneira essencial a este trabalho.

Aos pesquisadores, Me. Evandro Silva por todo auxílio durante o trabalho no UHPLC-Q-TOF-MS/MS, Me. Antônio Sergio Dacome (NERPRON-UEM), ao técnico do COMCAP Dr. Angelo de Oliveira Caleare que prestou todo suporte durante e utilização do HPLC-DAD/UV-vis, e aos pesquisadores do GMPC (Grupo de Materiais Poliméricos e Compósitos), Me. Nayane Queiroz, Me. Andressa R. Simão e Me. Otavio A. da Silva. Muito Obrigado.

A Profª. Dr. Grasiele Scaramal Madrona, que me acompanhou desde o mestrado e nunca mediu esforços para me orientar durante esses anos. Sempre com muita paciência e amor pelo que faz conosco. Obrigada por todo conhecimento dividido, pelo carinho e compreensão que teve comigo durante essa trajetoria. Deus não poderia ter me colocado em melhores mãos.

Finalmente, agradeço ao Programa de Pós-graduação em Ciência de Alimentos, à Universidade Estadual de Maringá, à Capes e CNPq pelo financiamento da pesquisa.

APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de três artigos científicos:

1. Leticia Misturini Rodrigues; Edilson Bruno Romanini; Evandro Silva; Eduardo Jorge Pilau; Silvio Cláudio da Costa; Grasiele Scaramal Madrona. Uvaia pulp: obtaining a functional product by ultrasound assisted extraction and reverse osmosis. Artigo enviado para revista Journal of Food Measurement and Characterization. Qualis Capes: B1.

2. Leticia Misturini Rodrigues; Edilson Bruno Romanini; Evandro Silva; Eduardo Jorge Pilau; Silvio Cláudio da Costa; Grasiele Scaramal Madrona. *Camu-camu* bioactive compounds extraction by ecofriendly sequential processes (ultrasound assisted extraction and reverse osmosis). Ultrasonics Sonochemistry, v. 64, p. 1-8, 2020. Qualis Capes: A1. Artigo publicado.

3. Leticia Misturini Rodrigues; Edilson Bruno Romanini; Evandro Silva; Eduardo Jorge Pilau; Silvio Cláudio da Costa; Grasiele Scaramal Madrona. Uvaia (*eugenia pyriformis cambess*) residue as a source of antioxidants: an approach to ecofriendly extraction. LWT, v. 138, p. 110785, 2021. Qualis Capes: A1. Artigo publicado.

GENERAL ABSTRACT

INTRODUCTION

Camu-camu (*Myrciaria dubia*) is a reddish-colored fruit found typically in the Amazon region. Uvaia (*Eugenia pyriformis Cambess*), also known as Uvalha, Uvaia do mato, is yellow to orange in color. Both fruits belong to the Myrtaceae family and have acidic flavors. These fruits have aroused the interest of industries (pharmaceutical, nutraceutical, food), due to the presence of vitamin C and other substances beneficial to health. Factors like these contribute to the development of extraction, identification and quantification of these compounds. Currently, the use of clean technologies that improve the extracts yield, reduce the extraction time and are low cost has been sought. Associated with clean solvents such as water, it is possible to avoid toxicity and reduce the environmental impact, in addition to being considered potentially green and ecofriendly. In this context, among several extraction techniques, it can be highlight ultrasound-assisted extraction (UAE) for facilitating mass transfer and reducing the size of fragments through the cavitation effect, in addition, it has greater efficiency and reduced energy when compared to conventional techniques. The reverse osmosis (RO) membrane concentration is a simple operation process, economically viable, and stands out for performing the separation / concentration of the compounds at room temperature, allowing the thermolabile compounds to be processed without modification or functional properties losses. There are few reports in the literature that evaluate the association of green technologies (ultrasound and reverse osmosis) for the extraction of bioactive compounds from camu-camu and uvaia, and there are few studies that point out alternatives for the reuse of uvaia residues.

AIMS

The objective of the study was to evaluate the best conditions for aqueous extraction of bioactive compounds from the camu-camu and Uvaia fruit (pulp and residue) using clean technologies, extraction assisted by ultrasound followed by reverse osmosis concentration, as well as characterizing the process steps and all obtained products.

MATERIAL AND METHODS

First, an experimental project was used to obtain the aqueous extract of the fruits (1: 4 w / v), camu-camu and uvaia (pulp and residue), by means of ultrasound-assisted extraction (UAE) to evaluate different variables, being they, time (X1 = 2.5, 5 and 10 min), amplitude (X2 = 20, 30 and 40%) and temperature (X3 = 40, 50 and 60 °C). After obtaining the best extraction conditions, the samples were concentrated by reverse osmosis (R25a, 500 Da, polyamide and and 5 bar area 3 ft^2), and the flow rate was monitored. The process flow and the volumetric concentration factor were calculated. At the end of the sequential process, the products obtained (control sample, sample obtained after extraction and concentrated sample), were characterized according to the contents of phenolic compounds, antioxidant activity (DPPH, FRAP, ABTS), total flavonoids, anthocyanins (for camu-camu) and carotenoids (uvaia), quantification of compounds (vitamin C, myricetin, cyanidin-3-glucoside, p-cumaric acid, rutin, gallic acid, quercetin, chlorogenic acid and ellagic acid) using HPLC-DAD UV / vis, and identification of compounds using UHPLC-MS / MS. All analyzes were subject to analysis of variance and Tukey test ($p < 0.05$) using the Sisvar 5.6 statistical program, and the standard curves for the antioxidant tests were plotted using the GraphPad Prism 5 program.

RESULTS AND DISCUSSION

It was verified that the Ultrasound Assisted Extraction (UAE) process, applied to obtain extracts rich in bioactive compounds of camu-camu and uvaia, generated satisfactory results, when followed by Reverse Osmosis membrane (OR) in order to concentration of the compounds. In the case of camu-camu, the best region for compounds extracting was 5 min, 60 °C and 30% amplitude according to the experimental design with the response to total phenolics compounds (TPC) and vitamin C. During the sample concentration, the permeate flow was measured as a function of the operating time for the reverse osmosis process at intervals of 3 minutes to 48 minutes, and varied from 15.0 L / (h.m^2) at the beginning of the process to 1.8 L / (h.m^2), when the solids content reached 4.1 ° Brix. The reduction in permeate flow occurs because the membrane used is very dense and almost all soluble solids are retained as the process time increases. Fouling of the membrane impairs its useful life and performance, with low fouling being best. In the present study, the level of membrane fouling was relatively low (19%), and the concentration factor was 4.1 times that the initial volume (feed). The final concentrated sample (CC) of camu-camu showed the following results of phenolic compounds (25.798 mg GAE / g fw) and total anthocyanins (66.169 mg of cyanidin-3-glucoside / 100 g total, being 3.2 and 6.5 times higher respectively than initial sample (CS). For the antioxidant analyzes the best result was obtained by the FRAP method (528.667 mmol TE / g) for final sample (CC). A total of twenty bioactive compounds were identified by UHPLC-Q – TOF-MS/MS, such as Cyanidin-3-O-glucoside, ellagic acid and Alnusiin for the first time detected in camu-camu. The quantification of some compounds was performed using HPLC-DAD / UV vis, and vitamin C was highlighted with 7.0 times higher in the final concentrated sample (52.01 mg /g) in relation to CS, followed by gallic acid (97.298 mg / 100 g), rutin (9.783 mg / 100 g) and Cyanidin-3-glucoside (2.783 mg / 100 g) The same processes mentioned above were applied to pulp and residues (peel and seed) of uvaia. Best region of extraction for both was similar: 40 ° C temperature, 40% amplitude, with a difference only for the extraction time, residue (2.5 min) and for the pulp (10 min). For the residue concentration, the fouling found was slightly higher (39%) than for the pulp (31%), probably due to the presence of more solids in the sample. The concentration factor was 7.02 times higher than feed for residue sample, and 4.4 times for pulp of uvaia. The samples final concentration, of residue (CF) and pulp (CP), increased by 6.2 times (332.225 mg GAE / 100 g) and 3.7 times (189.542 mg GAE / 100 g), respectively, for total phenolic content, in relation to the initial extract (RS). For flavonoids an increase of 7.8 (1300.179 mg QE / 100 g) and 6.5 (207.870 mg QE / 100 g) with respect to the initial sample. For both concentrated final samples (CF and CP) the best results for antioxidant were obtained by the FRAP method (136.761 and 39.396 mmol TE / g). Fifteen and fourteen compounds were identified in the residue and pulp samples by UHPLC-Q –TOF-MS / MS, respectively. Quantification by HPLC-DAD / UV vis, allowed to highlight compounds such as: vitamin C (4.420 and 93.367 mg / 100g), galic acid (8.119 and 3.181 mg / 100 g), rutin (2.243 and 1.034 mg / 100g) and ellagic acid (9.407 and 1.876 mg / 100 g), for the final samples (CF and CP). The combination of the two techniques (UAE and OR) applied to camu-camu and uvaia (residue and pulp), showed great potential for recovery of bioactive compounds and can be applied in food, nutraceutical and cosmetic matrices, bringing their benefits to consumers.

CONCLUSIONS

It can be concluded that a combination of aqueous extraction assisted by ultrasound followed by the reverse osmosis concentration, were efficient for the bioactive compounds extraction, with emphasis on being clean and viable techniques. At the end of the processes, products with higher levels of phenolic compounds, total flavonoids,

antioxidant activity were obtained, when compared to each other (control sample, after extraction and concentrated sample), both for camu-camu and for the different parts analyzed for uvaia. The uvaia residue (seed and peel) used as raw material, was highly promising for the bioactive compounds recovery. The final commercial fruit concentrate can be considered functional and viable for use by the food, nutraceutical and cosmetic industries.

Keywords: Myrtaceae, functional foods, antioxidant activity, phenolic compounds, HPLC-DAD/UV-vis**,** UHPLC-QTOF-MS/MS.

RESUMO GERAL

INTRODUÇÃO

Camu-camu (*Myrciaria dubia*) é uma fruta de cor avermelhada encontrada tipicamente na região amazônica. Uvaia (*Eugenia pyriformis* Cambess), também conhecida como Uvalha, Uvaia do mato, apresenta coloração de amarelo a laranja. Ambas frutas, pertencem à família Myrtaceae e apresentam sabores ácidos. Esses frutos tem despertado o interesse das indústrias (farmacêuticas, nutracêuticas, alimentícias), devido a presença de vitamina C e outras substancias benéficas a saúde. Fatores como esses, contribuem para o desenvolvimento de extração, identificação e quantificação desses compostos. Atualmente, tem se buscado o uso de tecnologias limpas que melhorem o rendimento de extratos, diminuam o tempo de extração e sejam de baixo custo. Associado a solventes limpos como a água, pode-se evitar a toxicidade e reduzir o impacto ambiental, além de ser considerado potencialmente verde. Nesse contexto, dentre várias tenicas de extração, a extração assistida por ultrassom (EAU) tem se destacado por facilitar a transferencia de massa e redução do tamanho dos fragmentos por meio do efeito de cavitação, além disso, apresenta maior eficiência e diminuição de energia em comparação às técnicas convencionais. A concentração por membrana de osmose reversa é um processo de operação simples, economicamente viável, e destaca-se por realizar a separação/concentração dos compostos em temperatura ambiente, permitindo que os compostos termolabeis sejam processados sem modificação ou perda de propriedades funcionais. Existem poucos relatos na literatura que avaliam a associação de tenologias ecologicamente viáveis (ultrassom e osmose reversa) para a extração de compostos bioativos de camu-camu e uvaia, e são poucos os estudos que apontam alternativas para a reutilização dos resíduos de uvaia.

OBJETIVOS

O objetivo deste estudo foi, avaliar as melhores condicões de extração aquosa de compostos bioativos do fruto camu-camu e da Uvaia (polpa e resíduo) por meio de tecnologias limpas, extração assistida por ultrassom seguida de concentração por osmose reversa, bem como caracterizar as etapas do processo e todos os produtos obtidos.

MATERIAL E METODOS

Primeiramente, um projeto experimental foi utilizado para obtenção do extrato aquoso dos frutos (1:4 v/v), camu-camu e uvaia (polpa e resíduo), por meio da extração assistida por ultrassom (EAU) para avaliar diferentes variáveis, sendo elas, tempo (X1= 2,5, 5 e 10 min), amplitude (X2= 20, 30 e 40%) e temperatura (X3= 40, 50 e 60 °C). Após a obtenção das melhores condições de extração , as amostras foram concentradas por osmose reversa (R25a, 500 Da, poliamida e área de 5 bar 3 pés^2), e a vazão do processo foi monitorada. Foram calculados o fluxo do processo, bem como o fator de concentração volumétrica. Ao final do processo sequencial, os

produtos obtidos (amostra controle - CS, amostra obtida após extração - CE e amostra concentrada - CC), foram caracterizados quanto aos teores de compostos fenólicos, atividade antioxidante (DPPH, FRAP, ABTS), flavonoides totais, antocianinas (para camu-camu) e carotenoides (uvaia), quantificação de compostos (vitamina C, miricetina, cianidina-3-glucoside, ácido p-cumárico, rutina, ácido gálico, quercetina, ácido clorogênico e ácido elágico) por meio do HPLC-DAD UV/vis, e identificação de compostos através de UHPLC-MS/MS. Todas as análises foram submetidas à análise de variância e teste de Tukey ($p < 0,05$) no programa estatístico Sisvar 5.6, e as curvas padrões para os testes de antioxidantes foram plotadas no programa GraphPad Prism 5.

RESULTADOS EDISCUSSÃO

Foi verificado que o processo de Extração Assistida por Ultrassom (EAU), aplicado para obtenção de extratos ricos em compostos bioativos de camu-camu e uvaia, gerou resultados satisfatórios, acoplados com a concentração dos compostos apartir da utilização de membrana de Osmose Reversa (OR). No caso do camu-camu, a melhor região para extração dos compostos, foi de 5 min, 60 °C and 30% amplitude de acordo com o delineamento experimental tendo como resposta para fenolicos totais (FT) e vitamina C. Durante a concentração da amostra, o fluxo do permeado foi medido em função do tempo de operação para o processo de OR em intervalos de 3 minutos até 48 minutos, e variou de 15,0 L / ($h.m^2$) no início do processo a 1,8 L / ($h.m^2$), quando o teor de sólidos atingiu 4,1 ° Brix. A redução no fluxo de permeado aconteceu possivelmente pois a membrana usada é muito densa e quase todos os sólidos solúveis são retidos à medida que o tempo do processo aumenta. A incrustação da membrana prejudica a vida util e o desempenho da mesma, sendo melhor uma baixa incrutação. No presente estudo, o nível de incrustação da membrana foi relativamente baixo (19%), e o fator de concentração foi de 4,1 vezes o da alimentação. A amostra final concentrada (CC) de camu-camu apresentou os seguintes resultados de compostos fenólicos (25,798 mg GAE/g fw) e antocianinas totais (66,169 mg de cianidina-3-glucosídeo / 100 g totais, sendo respectivamente 3,2 e 6,5 vezes maior que amostra inicial (CS). Para as análises antioxidantes o melhor resultado foi obtido pelo método de FRAP (528,667 mmol TE/g) para amostra final (CC). Um total de vinte compostos bioativos foram identificados por UHPLC-Q–TOF-MS/MS, como Cianidina-3-O-glucosídeo, Ácido Elágico e Alnusiina pela primeira vez detectado em camu-camu. A quantificação de alguns compostos foi realizada por meio do HPLC-DAD/UV vis, e a vitamina C apresentou destaque com um valor 7,0 vezes maior na amostra final concentrada (52,01mg/g) em realção ao CS, seguido do ácido gálico (97,298 mg/100 g), rutina (9,783 mg/100 g) e Cianidina-3-glucosideo (2,783 mg/100 g). Os mesmos processos citados acima, foram aplicados para polpa e resíduos (casca e semente) de uvaia. A região ótima de extração para ambos foi semelhante: 40 °C de temperatura, 40% de amplitude, tendo diferença apenas para o tempo de extração, resíduo (2,5 min) e para a polpa (10 min). Para a concentração do extrato do resíduo, o *fouling* (incrustação) encontrado foi um pouco acima (39%) do que para a polpa (31%), devido provavelmente a presença de mais sólidos na amostra. O fator de concentração foi 7,02 vezes maior do que

alimentação para amostra de resíduo, e 4,0 vezes para a polpa de uvaia. A concentração final das amostras, de resíduo (CF) e polpa (CP), apresentou acréscimo de 6,2 vezes (332,225 mg GAE/100 g) e 3,7 vezes (189,542 mg GAE/100 g) respectivamente para teores de fenólicos totais, com relação ao extrato inicial (RS). Para flavonoides o aumento foi de 7,8 (1300,179 mg QE/100 g) e 6,5 (207,870 mg QE/100 g) com relação a amostra inicial. Para ambas amostras finais concentradas (CF e CP) os melhores resultados para antioxidante foi obtido pelo método de FRAP (136,761 e 39,396 mmol TE /g, respectivamente). Quinze e quatorze compostos foram identificados nas amostras de resíduo e polpa, respectivamente por UHPLC-Q–TOF-MS/MS. A quantificação por HPLC-DAD/UV vis, permitiu destacar compostos como: vitamina C (4,420 e 93,367 mg/100 g), ácido gálico (8,119 e 3,181 mg/100 g), rutina (2,243 e 1,034 mg/100 g) e ácido elágico (9,407 e 1,876 mg/100 g), para as amostras finais (CF e CP). Assim, a combinação das duas técnicas (EAU e OR) aplicadas em camu-camu e uvaia (resíduo e polpa), apresentaram grande potencial para recuperação de compostos bioativos podendo utiliza-los como ingredientes em matrizes de alimentos, nutracêuticos e cosméticos, proporcionando benefícios aos consumidores.

CONCLUSÕES

Pode-se concluir que a combinação da extração aquosa assistida por ultrassom e seguida da concentração por osmose reversa, foram eficientes para extração dos compostos bioativos, tendo destaque por serem tecnologias limpas e viavéis. Ao final dos processos, foi obtido produtos com níveis mais altos de compostos fenólicos, flavonóides totais, atividade antioxidante, quando as amostras foram comparadas entre si (amostra controle, após extração e amostra concentrada), tanto para o camu-camu como para as diferentes partes da uvaia analisadas. O resíduo da uvaia (semente e casca) utilizado como matéria prima, foi altamente promissor para recuperação dos compostos bioativos. O concentrado final obtido das diferentes frutas, pode ser considerado funcional e viável para uso pelas industrias de alimentos, nutraceuticos e cosméticos.

Palavras-chaves: Myrtaceae, alimentos funcionais, atividade antioxidante, compostos fenólicos, HPLC-DAD/UV-vis**,** UHPLC-QTOF-MS/MS.

ARTICLE 01

Uvaia pulp: obtaining a functional product by ultrasound assisted extraction and reverse osmosis

Leticia Misturini Rodrigues [a]
Edilson Bruno Romanini [a]
Evandro Silva [b]
Eduardo Jorge Pilau [b]
Silvio Cláudio da Costa [c]
Grasiele Scaramal Madrona [d]

[a] Postgraduate Program in Food Science, State University of Maringa, Av. Colombo 5790, CEP 87020-900 Maringa, PR, Brazil.

[b] Department of Chemistry, State University of Maringa, Av. Colombo 5790, CEP 87020-900Maringá, PR, Brazil.

[c] Department of Biochemistry, State University of Maringa, Av. Colombo 5790, CEP 87020-900 Maringá, PR, Brazil.

[d] Department of Food Engineering, State University of Maringa, Av. Colombo 5790, ZIP Code 87020-900 Maringa, PR, Brazil

Corresponding Author: Letícia Misturini Rodrigues

Email address: leticia_misturini@hotmail.com

*e-mail: leticia_misturini@hotmail.com

ORCID ID https://orcid.org/0000-0002-5359-6915

Abstract

Aqueous ultrasound assisted extraction, followed by reverse osmosis were used to concentrate the bioactive compounds of Uvaia pulp. An experimental design with different extraction times (2.5, 5, 10 minutes), amplitude (20, 30 and 40%) and temperature (40, 50 and 60 ° C) was used, response variables being phenolic and carotenoid compounds. The ideal extraction conditions were obtained at a temperature of 40 °C, 10 min and 40 % amplitude. After extraction the product was concentrated by reverse osmosis, obtaining relatively low fouling (31%). The product obtained showed an increase in bioactive compounds when extracted by ultrasound and concentrated by reverse osmosis. Fourteen compounds were identified by UHPLC-MS/MS. The concentrate was 3.7 times higher in phenolic content (189.542 mg GAE / 100g); and the concentration of total flavonoids was increased by 6.5 times (207.870 mg / 100g). In total the concentrate was 7.0 times higher in antioxidant activity. Finally, the concentrated Uvaia pulp showed potential to be used as a functional product, in the development of new food matrices, or in nutraceuticals and cosmetics.

Keywords: aqueous extract, bioactive compounds, E*ugenia pyriformis* C., UHPLC-MS/MS.

Introduction

Uvaia (*Eugenia pyriformis Cambess*) belongs to the Myrtaceae family and is considered an exotic fruit, also known as uvalha, uvalha-do-mato and uvalheira. The name comes from the Tupi native language and means sour fruit. Uvaia is spherical, yellow and has a juicy and bitter pulp. It is used for the manufacture of jelly, juices and ice cream. Studies indicate that this fruit has high antioxidant and anti-inflammatory power when compared to other species, containing high levels of phytochemicals, including phenolic compounds [1,2].

In order to determine commercial uses for this fruit, it is essential to know its functional compounds and chemical composition. In this aspect, research on Brazilian native fruits has increased due to evidence that healthy foods, have positive effects on human health, reducing inflammatory processes through bioactive compounds, such as carotenoids, phenolic compounds and vitamins [3].

However, there are major limitations to the consumption of fresh fruit, such as high perishability and rapid changes in appearance due to water loss, resulting in a short shelf life. In addition, the Uvaia fruit has a strong acid flavor. These limitations can be minimized through processing and applied technologies that can maintain their bioactive compounds or minimize the loss of such substances [4].

Through research it is possible to identify biological properties as notes De Paulo Farias et al. [5] who reported on the need for further research on Brazilian native fruits to establish more species as commercial crops, emphasizing that the volume of information on fruits it is still very poor.

To improve extraction efficiency of bioactive compounds, ultrasound assisted extraction technique is suggested as an alternative, which reduces processing time, solvent

consumption and improves solid-liquid extraction. Therefore, ultrasound assisted extraction is considered a clean technology. The efficacy of ultrasound assisted extraction can be attributed to bubble cavitation, which facilitates the opening of biological matrices and thus the release of compounds [5].

In addition to UAE, other membrane separation processes, when compared to traditional technologies, are viable clean possibilities, since they have low operating cost, reasonable conditions of pressure and temperature, easy control, and high selective separations. In addition, the process does not require an extraction agent or chemical additives, preventing contamination of products and protecting the biological activity of the compounds of interest.

Pressure-driven membrane processes, such as micro (MF), ultra (UF) and nanofiltration (NC), and also reverse osmosis (RO) are well-established technologies in the food industry for the treatment of numerous products and by-products [6]. Reverse osmosis was highlighted in recent years for its ability to concentrate non-thermal fruit juices [7]. Some works have already carried out tropical fruit's juice extraction followed by membrane concentration, [8,9] however the present work is the first to use a sequential process (UAE + RO) for Uvaia extraction.

The main objective was to investigate the use of clean technologies, ultrasound assisted extraction (UAE) and reverse osmosis, for aqueous extraction of bioactive compounds from Uvaia pulp.

Materials and methods

Chemicals reagents

6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2

diphenyl-1-picrylhydrazyl (DPPH), Calcium gallate, Folin- Ciocalteu reagent, TPTZ (2,4,6 -tripyridyls-triazine), ascorbic acid, cyanidin 3-glucoside, ellagic acid, gallic acid, quercetin, rutin, p-coumaric acid, myricetin, chlorogenic acid, and cyanidin chloride were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Milli-Q ultrapure water was used in the required analyzes.

Pulp sample and Chemical analyses

Uvaia pulp was purchased in Paraibuna - SP, Brazil and kept frozen (-18ºC) until analysis. Pulp composition was analyzed in triplicate by: total soluble solids (^{0}brix) using an HI96801 digital refractometer (Hanna Instruments); pH, moisture, ashes, protein (micro Kjeldahl) lipids and carbohydrates by difference (100 – (moisture+protein+lipids)) [10]; and the color parameters were determined by a portable colorimeter Minolta® CR400.

Ultrasound assisted extraction (UAE) by experimental design

Uvaia pulp samples were diluted in water to a concentration of 50 g of solids in 200 mL of solvent (1: 4) [8,11].

The ultrasonic probe system used, provided a power of 750 W and a frequency of 20 kHz (collective Parmer 750-Watt Ultrasonic Processors). By means of the automatic amplitude compensation provided by the equipment, adjustment to the desired level was carried out on a scale of 0-40% (0-20 kHz), allowing the adjustment of ultrasonic vibrations through the probe tip (titanium, 13 mm diameter), maintaining the desired extraction amplitude. The probe was inserted directly into the sample and the approximate volume of each test was 250 mL. Based on the experimental design, the following

parameters were studied: ultrasound extraction time (X1 = 2.5, 5 and 10 min), amplitude (X2 = 20, 30 and 40%) and temperature (X3 = 40, 50 and 60 ° C) added by three central points, and response variables were the concentration of phenolic compounds and carotenoids, being evaluated by response surface (statistical program 7.0).

All extractions were kept pulsating (5 s pause and 5 s pulse) according to preliminary tests and following literature [8,12].

After extraction, the entire extracts were vacuum filtered and stored in a freezer (-18 °C), for further analysis.

Reverse Osmosis (RO) Membrane Concentration

The extract obtained under optimized conditions of extraction was cooled to 25 °C and subjected to filtration (0.45 µm) to remove possible dirt or larger particles, preventing damage to the membrane. Then the extract was concentrated on a R25A, 500 Da, polyamide membrane, pressure of 5 bar and filtration area of 3 $feet^2$. The process took place at room temperature (25 °C), and the flow rate was monitored until stable.

The flow was calculated using the weight of the collected concentrate and measured at different time intervals, together with the flow data of distilled water in the membrane in its clean and dirty state as well. Thus, the percentage of fouling (% F) was calculated according to equation 1, using the data collected during the concentration process. The feed and permeate volume were used to calculate the volumetric concentration factor (VCF) [13].

$$\%F = \frac{(Ji - Jf)}{Ji} X100 \quad (1)$$

Following that, the initial extracts (PS), the extract obtained under ideal conditions

150 in the ultrasonic extraction (PE) and the concentrate obtained in the membrane process
151 (CP) were analyzed.
152
153 **Bioactive compounds**
154 **Total phenolic compounds (TPC)**
155 Total phenolic compounds were determined by colorimetric analysis using the
156 Folin-Ciocalteu reagent, as described by Singleton et al. [16] & Pierpoint [17]. The
157 obtained measurement was compared with a gallic acid calibration curve ($R^2 = 0.99$).
158 Results were expressed in milligrams of gallic acid equivalent (mg GAE) per 100 g of
159 fresh pulp (f.p).
160
161 **Total carotenoids**
162 Total carotenoid was evaluated according to Lichtenthaler [16], where 2 mL of
163 extract was homogenized with 18 mL of 80% acetone. The mixture was then filtered on
164 filter paper, in the absence of light, and read sequentially on a spectrophotometer at
165 absorbance of 647, 663 and 470 nm. The concentration was determined according to
166 equation 2:
167 $C_{(x+c)}\,(\mu g/mL) = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198$ (2)
168 Where $C_a\,(\mu g/mL) = 12.25\ A_{663} - 2.79\ A_{647}$; $C_b\,(\mu g/mL) = 21.50\ A_{646} - 5.10\ A_{663}$.
169 The concentration of pigments was expressed in µg per 100g of f.p.
170
171 **Vitamin C by high performance liquid chromatography (HPLC)**
172 Vitamin C was quantified by using the peak area obtained in HPLC according to
173 Rodrigues et al. [8] results are expressed in mg per g^{-1} f.p applying the standard

curve (Y=4.84e^006X-4.02e^005, R^2=0.998106).

Total flavonoids (TF)

Total flavonoids were determined in 510 nm absorbance according to Alothman et al.[17]. The results are expressed in mg of quercetin equivalent (QE).100g $^{-1}$ f.p.

Antioxidant activity (DPPH, FRAP and ABTS)

The reduction of the stable radical DPPH (2,2 diphenyl-1 picrylhydrazine) was measured by spectrophotometric assay [18].

The antioxidant activity on the ABTS (2,2'- azinobis (3 ethylbenzothiazoline-6-sulfonic acid) method was performed using a colorimetric assay [19].

For the FRAP (Ferric Reducing Antioxidant Power) method the extracts were mixed with distilled water and FRAP reagent, then kept at 37 °C (during 30 min) in a water bath, after that a spectrophotometer reading was performed (595 nm) [20].

Results of all antioxidant analysis were presented in mmol of Trolox equivalent (TE).g^{-1} product.

Bioactive compounds by UHPLC-MS/MS and HPLC-DAD/UV vis

Bioactive compounds from uvaia extracts were analyzed by a UHPLC-MS/MS (Ultra-high performance liquid chromatography tandem mass spectrometry) system (Shimadzu, Nexera X2, 194 Japan). Chromatographic separation was performed using an Acquity UPLC BEH C18 column (Waters, USA, 1.7 μm, 2.1 × 100 mm), the parameters were: elution with a gradient mixture solvents: A (water with 0.1% formic acid, v:v) and B (acetonitrile with 0.1% formic acid, v-v), 5% B (0–1 min), 70% B (1–

198 5 min), 98% B (5-15 min) and maintained at 5% B (15–20 min) at 40 °C. The flow rate
199 was at 0.250 mL min^{-1} [21].

200 The compounds were analyzed by a Q-TOF (Impact II, Bruker Daltonics
201 Corporation, Germany) equipped with an electrospray ionization source (ESI), the
202 parameters settings were: the capillary voltage was operated in positive ionization mode
203 set at 4500 V, with a potential plate end of −500 V, dry gas flow 8 L min−1 at 180 °C,
204 nebulization gas pressure 4 bar. The acquisition data were monitored as mass range from
205 m/z 50 to 1300 with an acquisition rate of 5 Hz. The 5 most intense ions were selected for
206 automatic tandem mass spectrometry (AutoMS/MS) [24].

207 Compounds were quantified by high performance liquid chromatography (HPLC-
208 DAD UV/vis) as described in [8].

209

210 **Statistical Analysis**

211 The data were presented as mean ± standard deviation and analysis of variance
212 (ANOVA) and Tukey's test for the minimum significant difference ($p < 0.05$) between the
213 means using the Sisvar 5.6 statistical program [23]. Calibration curves for antioxidant
214 analyzes were performed using the GraphPrism 5 program [24].

215

216 **Results and discussion**

217 **Initial pulp characterization**

218 Uvaia pulp had an average total soluble solids (TSS) content of 4.40 ± 0.173 °brix,
219 pH 4.27, humidity of approximately 95.0 ± 0.00% and 0.150 ± 0.017% of ash. The pulp
220 color analysis showed a luminosity (L) of 31.9 ± 0.035, a tendency to red (a *) of 1.32 ±
221 0.017 and yellow (b *) to 17.41 ± 0.021. The lipid content was 0.5 ± 0.015 (g per 100 g)

222 and protein 0.770 ± 0.006 (g per 100 g) on a wet basis.

223 The whole Uvaia has already been characterized previously, with TSS values of
224 7.75 and pH 3.06, corroborating with our study where only pulp was evaluated [25]. Other
225 authors reported values for physical-chemical evaluation of two different varieties (Dura
226 and Pêra) during the 4-day post-harvest storage period showing values for pH 2.95 to 3.2
227 and stated that fruits with low values for pH are suitable for the processing of sweets,
228 however they prevent the fresh fruit consumption [26].

229 A study characterized three species of the Myrtaceae family (yellow guava,
230 guabiroba and Uvaia), determining the antioxidant potential and evaluating the chemical
231 composition of the fruits. The authors used uvaia pulp and peel, obtaining values of 0.52
232 for lipids, 15.82 g per 100g dry matter for protein, 94.50 for moisture and 0.23 g per 100
233 g fresh matter for ashes, [27] values similar to those found in the present study.

234

235 **Experimental planning - ultrasound assisted extraction**

236 The experimental results of TP and carotenoids (Table 1) of the Uvaia pulp extract
237 ranged from 50.600 to 54.900 mg of EAG / 100g of f.p., and 139.134 to 354.250 µg total
238 carotenoids / 100g f.p. respectively.

239 **Table 1.** Results of total phenolic content and carotenoids at different extraction
240 conditions according to a central composite design.

241

Run	Extraction conditions			Response	
	Time (min)	Amplitude (%)	Temperature (°C)	TPC (mg/100g)	Carotenoids (µg /100g)
1	2.5	20	40	51.600	145.711
2	10	20	40	53.700	183.187

3	2.5	40	40	53.300	241.255
4	10	40	40	53.200	335.495
5	2.5	20	60	54.900	190.795
6	10	20	60	53.600	274.371
7	2.5	40	60	51.500	242.113
8	10	40	60	53.800	354.250
9	5	30	50	50.800	139.134
10	5	30	50	51.400	144.492
11	5	30	50	50.600	298.608

TPC total phenolic compounds.

Figure 1 (A-F) shows the response surface according to the effect of the sonication time, temperature and amplitude variables. For the extraction of TP (Fig 1. AB), time (X1) was significant ($p < 0.05$), and amplitude (X2) and temperature (X3) interaction had a negative influence according to the equation ($Y = 2.28\ X12 - 0.65X2X3$). For the extraction of carotenoids (Fig. 1 D-F) the two variables of extraction time (X1) and amplitude (X2) were significant ($p < 0.05$), and amplitude is slightly more important than time, analyzing the equation obtained ($Y = 40.96\ X1 + 47.44\ X2$). According to the results, the combination of variables presented the following points as the ideal extraction region: 10 minutes, 40% amplitude at 40 °C, a condition that was used in other stages of this work.

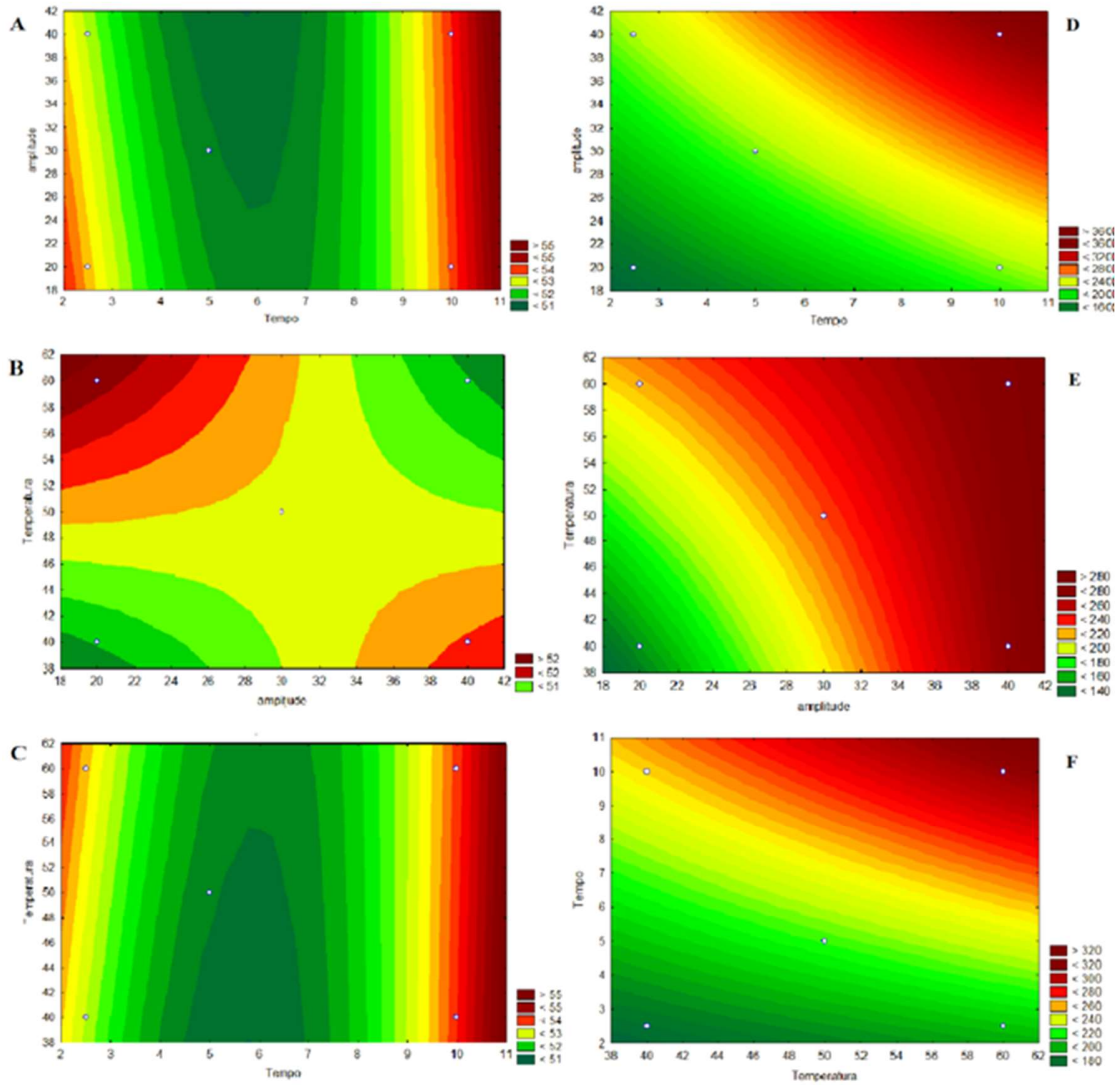

Figure 1. Response surface between three variables (time, amplitude, and temperature) in the extraction of Total Phenolic compounds (TP mg GAE/ 100g ff, A - C) and carotenoids (µg /100g f.w, D - F.). (A, B, C) TP = 2.28 X12 - 0.65X2X3 (D, E, F) Carotenoids = 40.96 X1+ 47.44 X2.

In the present study, the extraction of carotenoids was facilitated by ultrasound in combination with time. Cavitation, which occurs during the ultrasound process, is so called because it produces several mechanical effects, such as rupture of the cell wall and particle collisions, which allow the solvent to penetrate the sample more easily. The ultrasonic power can weaken the cell wall and thus increase the contact of the compounds

264 and the solvent, allowing a decrease in the sonication time [28].

265 Another work used the same methodology to determine carotenoids in Uvaia pulp
266 and reported lower values (91.0 µg total carotenoids / g) for its sample compared to the
267 values obtained through UAE (139.134 to 354.250 µg total carotenoids) / 100g f.p). The
268 authors also remark the importance of carotenoids in health and in focusing the interest of
269 food industries for its natural dye properties [29].

270 A recent study also used ultrasound assisted extraction (UAE) to extract total
271 carotenoids from peach peel, using soybean oil as the extraction solvent. The optimized
272 extraction parameters were 48 °C, and 28 minutes of extraction to obtain the highest
273 content of carotenoids (151.50 mg / 100 g) [30].

274 In the study reported by Aware et al. [31], different extraction techniques and
275 different solvents were evaluated to obtain maximum extraction of phytocomponents
276 (total phenolics, flavonoids and antioxidant activity) from Mucuna macrocarpa beans.
277 Water and ultrasound were found to be the most effective solvent and technique. The
278 response surface methodology was also used to investigate the ideal process conditions
279 (time and ultrasonic power) for maximum compound extraction. (Chakraborty, Uppaluri,
280 & Das, 2020) also stated in their research that the pulsed ultrasound process represents a
281 promising opportunity in relation to conventional extraction methods for production of
282 aqueous extracts from various sources for future food, medicinal and functional
283 applications.

284

285 **Pulp concentration process**

286 The permeate flow was measured as a function of the operating time for the reverse
287 osmosis process at intervals of 5 min up to 70 min, and varied from 14.0 L / (h.m^2), at the

beginning of the process, to 3.0 L / (h.m^2), when the solids content reached 3.2 °Brix for PE. During the process, a reduction in the permeate flow is noted, which may be justified by the type of membrane used (pore size) and probably the deposition or adsorption of high molecular weight of the sample that lead to partial blocking of the membrane pores [32,33]. However, the use of RO has the advantages of obtaining high quality concentrated products because of low operating temperature, easy operation and lower energy consumption, and results mainly in the retention of nutritional compounds [32].

Fouling is the accumulation of unwanted deposits inside the pores or on the membrane surface, which causes a reduction in the flow of permeate. In addition, it is one of the main factors that reduces membrane useful life, as it reduces the productivity and quality of the permeate, while simultaneously increasing operation cost due to higher energy demand, and additional pretreatments [34].

The membrane fouling level was relatively low (31%), and the concentration factor was 4.4 times that of the feed. Another study used the same membrane, to concentrate bioactive compounds from the Camu-camu fruit, and according to results, the reverse osmosis membrane presented low fouling (19%) and was a suitable process, with a permeate flow of 15 to 1.8 L / (h.m^2) for 48 min showing excellent results for a concentrated sample [8].

Researchers used RO to increase the concentration of bioactive compounds in apples, blueberries and cranberry juice, to prepare a functional drink with antioxidant properties. Through the technique used, they obtained greater constituents of polyphenols such as catechin, chlorogenic acid, anthocyanins and greater antioxidant capacity [9]. And prior to the work cited, the same author used ultrasound assisted extraction to prepare an aqueous extract with ginger, rich in bioactive compounds [35].

312

313 **Bioactive compounds**

314 Table 2 shows the analysis of TPC, antioxidants (DPPH, ABTS and FRAP) and
315 total flavonoids from samples of PS, PE and CP.

316 Regarding the TPC levels, we noticed an increase of 3.7 times (189.542 ± 2.933mg
317 GAE / 100g) of the concentrate (CP), in relation to the initial extract (PS) which was
318 51.154 ± 0.391, and also an increase of 1.2 times (62.560 ± 0.391) of the optimal extract
319 (PE) in relation to the initial (PS), with a significant difference in relation to the other two
320 samples (Table 2).

321

322 **Table 2.** Bioactive compounds from different Uvaia pulp samples (p.f)

	PS	PE	CP
TPC (mg GAE/100g)	$51.154^{c} \pm 0.391$	$62.560^{b} \pm 0.391$	$189.542^{a} \pm 2.933$
TF (mg QE/100 g)	$31.544^{c} \pm 1.601$	$71.932^{b} \pm 3.203$	$207.870^{a} \pm 14.413$
ABTS (mmol TE/g)	$4.555^{c} \pm 0.0749$	$5.588^{b} \pm 0.075$	$21.926^{a} \pm 0.337$
DPPH (mmol TE/g)	$3.698^{b} \pm 0.000$	$3.699^{b} \pm 0.000$	$21.006^{a} \pm 0.709$
FRAP (mmol TE /g)	$3.684^{b} \pm 0.309$	$3.267^{b} \pm 0.037$	$39.396^{a} \pm 1.081$
Vitamin C (mg/100g)	$49.574^{c} \pm 0.880$	$54.781^{b} \pm 0.023$	$93.367^{a} \pm 0.540$
Myricetin (mg/100g)	$0.216^{a} \pm 0.000$	$0.228^{a} \pm 0.000$	$0.279^{a} \pm 0.002$
Chlorogenic acid (mg/100g)	$0.132^{b} \pm 0.000$	$0.138^{b} \pm 0.000$	$0.246^{a} \pm 0.000$
Ellagic acid (mg/100g)	$0.807^{c} \pm 0.000$	$0.898^{b} \pm 0.000$	$1.876^{a} \pm 0.001$
p-coumaric acid (mg/100g)	$0.061^{a} \pm 0.000$	$0.067^{a} \pm 0.000$	$0.199^{b} \pm 0.001$
Quercetin (mg/100g)	$0.000^{b} \pm 0.000$	$0.005^{b} \pm 0.000$	$0.1410^{a} \pm 0.000$
Rutin (mg/100g)	$0.342^{c} \pm 0.000$	$0.383^{b} \pm 0.000$	$1.034^{a} \pm 0.000$
Gallic acid (mg/100g)	$0.834^{c} \pm 0.000$	$1.033^{b} \pm 0.001$	$3.181^{a} \pm 0.000$

323 The data are expressed as means ± standard deviations. Means followed by the same letter in the lines do not differ significantly from
324 each other by the Tukey test at 5% significance. PS = control sample, PE = ultrasound optimal extraction, CP = concentrated sample,
325 TPC= total phenolic compounds, TF=total flavonoids, GAE= gallic acid equivalent, QE= quercetin equivalent, TE = Trolox equivalent.
326

A study evaluated three different solvents (aqueous, ethanolic and hydroethanol) for the extraction of bioactive compounds from Uvaia pulp, and reported values for the aqueous extraction of TPC (34.70 ± 0.99 mg (GAE) 100 g^{-1} pulp) and total flavonoids (0.04 ± 0.00 mg (QE) 100 g^{-1} pulp) below those found in the present study. The same was noted for antioxidant activity by the analyzes of DPPH (249.45 ± 62.15 mg (TEAC) 100 g^{-1} pulp), ABTS (27.00 ± 0.57 mg (TEAC) 100 g^{-1} pulp) and FRAP (64.74 ± 1.28 mg (TEAC) 100 g^{-1} pulp) [36].

For the analysis of antioxidant activity by DPPH, all samples were different from each other ($p < 0.05$), revealing that the techniques used (UAE and RO) were efficient for the extraction and concentration of compounds. In the ABTS and FRAP method, the samples showed a significant difference between them only for the concentrated sample (CP). The greatest increase was noted for FRAP analysis where the concentrate (CP) was 10.7 times greater (39.396 ± 1.081) than the control sample (PS) 3.684 ± 0.309.

For total flavonoids, all three samples showed a significant difference between them ($p < 0.05$), and RO favored the increase of this class by 6.5 times (207.870 ± 14.413 mg QE / 100 g) than the control sample (31.544 ± 1.601 mg QE / 100 g). Total flavonoids are extremely important according to literature, as they have beneficial effects on human health and are characterized as important antioxidants due to their high potential redox, acting as reducing agents and singlet oxygen quenchers [37,38].

In a work previously reported by da Silva et al. [26] the authors analyzed bioactive compounds and antioxidant capacity from six different Uvaia species (‘Common’, ‘Rugosa’, ‘Doce de Patos de Minas’, ‘Pêra’, ‘Rugosa Doce’ and ‘Dura’). Values of total flavonoids ranging from 22.97 ± 3.15 to 38.58 ± 1.48 mg 100 g^{-1} f.w., were obtained. DPPH antioxidant activity ranged from 9.94 ± 0.78 to 29.71 ± 0.62 mmol trolox 100 g^{-1}

351 d.w. and the vitamin C content of 1.37 ± 0.03 to 64.82 ± 14.98 mg 100 g^{-1} f.w. All values
352 reported are below that found in our paper (Table 2) for the PE and CP samples,
353 confirming once again the efficiency of the techniques used in the process.

354 Vitamin C was increased 1.9 times (93.367 ± 0.540 mg per 100g) in the
355 concentrate (CP) in relation to initial sample (PS) (49.574 ± 0.880 mg per 100g), showing
356 a significant difference between the three samples. The Uvaia species 'Doce de Patos de
357 Minas', weighs approximately 11.4 g, and may be an alternative source of natural vitamin
358 C, taking into account human daily needs, the fruit can supply around 72.04% (for men)
359 and 86.45% (for women) [3].

360 Pereira et al. [27] determined the antioxidant potential of three species of the
361 Myrtaceae family, including Uvaia, which presented a value of 0.7 ± 0.37 mg equivalent
362 chlorogenic acid per 100 g dry matter for vitamin C, and explained that there are many
363 factors that influence the amount of vitamins in fruits, such as the stage of ripening at
364 harvest, species, genetic variation, storage and processing conditions.

365 The bioactive compounds quantified by HPLC / UV DAD vis (Table 2), among
366 which those that stood out were gallic acid, ellagic acid, vitamin C and rutin and the three
367 samples showed significant difference ($p < 0.05$) between them (PS, PE and CP).
368 Chlorogenic acid and quercetin were significantly different for the CP sample only.
369 Myricetin and p-coumaric acid did not vary between samples. The CP when compared
370 with PS showed 3.8, 3.2 and 3.0 times higher levels of gallic acid, p-coumaric acid and
371 rutin respectively. Quercetin was not noticed in the PS, and subtly appeared in the PE
372 sample, but it is 28 times higher in the CP sample, indicating that the concentration process
373 was feasible for this target compound.

374 A study used Uvaia purée to evaluate the extraction of phenolic compounds, and

375 performed the quantification of individual phenolic compounds by HPLC-DAD / UV,
376 obtaining the following values for extraction with distilled water: Gallic acid (255.8 ±
377 6.91); Chlorogenic acid (33.4 ± 1.54); p-coumaric acid (2.9 ± 0.12); Rutin (0.83 ± 0.01);
378 Myricetin (23.0 ± 1.25); Quercetin (114.2 ± 3.52) all values were expressed in mg / Kg of
379 fresh weight [11].
380
381 **Bioactive compounds identification by UHPLC-MS/MS**
382 The analysis of UHPLC-MS/MS allowed the detection and identification of
383 bioactive compounds of Uvaia pulp. So, 14 compounds were identified (in CF sample),
384 12 compounds in the PE sample, 11 compounds in the PS sample. The identification of
385 these compounds is carried out through the interpreted mass spectra (MS) and
386 fragmentation spectra (MS/MS) by comparing with literature spectra and public databases
387 such as GNPS, [39] KEGG [40] and PubChem [41]. The compounds were manually
388 confirmed and mass errors ranged from 0.0 to 4.6 ppm (Table 3). These compounds
389 belong to the phenolic acid and flavonoid classes.
390 Gallic acid compounds $[M+H]^+$ *m/z* 171.0288; ascorbic acid $[M+H]^+$ *m/z*
391 177.0394; p-Coumaric acid $[M+H]^+$ *m/z* 165.0540; chlorogenic acid $[M+H]^+$ *m/z*
392 355.1028 (Table 3), belong to the class of phenolic acids, which are antioxidant
393 compounds that reduce some chronic diseases related to oxidative stress through anti-
394 inflammatory, antibacterial, antiproliferative, and anticarcinogenic activities [42].
395 The class of flavonoids is predominant in all samples, being: myricetin $[M+H]^+$
396 *m/z* 319.0449; quercetin $[M+H]^+$ *m/z* 303.0485; rutin $[M+H]^+$ *m/z* 611.1584;
397 dihydrotricetin $[M+H]^+$ *m/z* 305.0647; isoquercitin $[M+H]^+$ *m/z* 465.1029; quercitrin
398 $[M+H]^+$ *m/z* 449.1076; kaempferol 3-O-alpha-L-rhamnoside $[M+H]^+$ *m/z* 433.1121;

399 quercetin-3-O-pentoside $[M+H]^+$ *m/z* 435.0916 the last three compounds are glycoside
400 flavonoids. Tribuloside $[M+H]^+$ *m/z* 595.1438 derived from a kaempferol, also belongs to
401 the class of flavonoids, as well as Bellidifolin-8-O-glucoside (swertianolin) which is a
402 potential compound for the development of therapeutic drugs [43].

403 There are reports on this group of compounds working against heart disease,
404 coronary artery disease, and even in reducing the incidence of type 2 diabetes. In addition,
405 they show important antioxidant effects, anti-obesity action, anticancer activity, reducing
406 oxidative stress and inflammation [44]. These are some benefits mentioned, such as
407 disease reduction, which are associated with the consumption of foods containing
408 flavonoids [42,45].

409 **Table 3.** Bioactive compounds of different Uvaia samples

410

Compound identify	RT (min)	Molecular Formula	$[M+H]^+$ Measured	$[M+H]^+$ Accuracy	Error (ppm)	Sample PS	PE	CP
Gallic acid	1.97	$C_7H_6O_5$	171.0288	171.0288	0.0	✓	✓	✓
Ascorbic Acid	1.19	$C_6H_8O_6$	177.0394	177.0394	0.2	✓	✓	✓
p-Coumaric acid	1.23	$C_9H_8O_3$	165.0540	165.0546	-3.6	✓	✓	✓
Myricetin	3.97	$C_{15}H_{10}O_8$	319.0449	319.0448	0.3	✓	✓	✓
Chlorogenic acid	3.95	$C_{16}H_{18}O_9$	355.1028	355.1024	1.2	✓	✓	✓
Quercetin	4.00	$C_{15}H_{11}O_7$	303.0485	303.0499	-4.6	nd	nd	✓
Dihydrotricetin	3.86	$C_{15}H_{12}O_7$	305.0647	305.0655	-2.6	✓	✓	✓
Kaempferol 3-O-alpha-L-rhamnoside	4.49	$C_{21}H_{20}O_{10}$	433.1121	433.1129	-1.8	nd	nd	✓
Quercetin-3-O-pentoside	4.17	$C_{20}H_{18}O_{11}$	435.0916	435.0922	-1.4	✓	✓	✓
Bellidifolin-8-O-glucoside	3.83	$C_{20}H_{20}O_{11}$	437.1064	437.1078	-3.2	✓	✓	✓
Quercitrin	4.22	$C_{21}H_{20}O_{11}$	449.1076	449.1078	-0.4	✓	✓	✓
Isoquercitin	4.00	$C_{21}H_{20}O_{12}$	465.1029	465.1027	0.4	✓	✓	✓
Tribuloside	5.09	$C_{30}H_{26}O_{13}$	595.1438	595.1446	-1.4	nd	✓	✓
Rutin	3.87	$C_{27}H_{30}O_{16}$	611.1584	611.1606	-3.6	✓	✓	✓

411 *nd= not detect; RT- retention time; PS - control sample; PE - ultrasound optimal extraction; CP - concentrated sample.

412

413

Conclusions

Ultrasound assisted extraction combined with reverse osmosis, appears to be a good alternative to improve the availability of phenolic compounds, through sonication and membrane concentration, of the bioactive substances from Uvaia pulp extract. In addition, water used as a solvent is an inexpensive and non-toxic source, proving to be ideal for extraction of bioactive compounds.

The final concentrate obtained was rich in bioactive compounds, presenting higher values than the initial sample. Total phenolics were 3.7 times greater; flavonoids 6.5 times greater and superior antioxidant activity in 4.8, 5.7 and 10.7 times for ABTS, DPPH, and FRAP respectively, highlighting gallic acid (3.8), p-coumaric acid (3.2), rutin (3.0) and vitamin C (1.9) times higher than the initial extract, concluding in the identification of 14 compounds by UHPLC-Q-TOF-MS / MS.

In summary, the study carried out was highly promising because it obtained a final concentrate of Uvaia pulp rich in phenolic compounds and with high antioxidant activity, indicated for functional foods, medicinal and cosmetic products. In addition, this concentrate has potential application as a natural dye.

Acknowledgements

The authors acknowledge the financial support from CAPES scholarship, and CNPQ (10936/16). As well as the support by the Central Research Support Complex (COMCAP) of the State University of Maringa (UEM).

Author Contributions

Letícia M. Rodrigues and Edilson B. Romanini, was responsible for the investigation, data curation, formal analysis, Software (extracting, spectral and HPLC-DAD UV/vis); Evandro Silva and Eduardo J. Pilau for the investigation, data curation, formal analysis (UPLC-QTOF-MS/MS data); Grasiele S. Madrona for the conceptualization, project administration, Software, resources

441 and writing, review, and editing; Silvio C. da Costa for Visualization and review; Letícia M.
442 Rodrigues also were responsible for writing the original draft, reviewing and editing.
443
444 **References**
445 1. J. M. M. Lopes, N. N. Lage, J. F. C. Guerra, M. Silva, L. F. Bonomo, A. H. S. Paulino, A. L.
446 R. S. Regis, M. L. Pedrosa, and M. E. Silva, Food Res. Int. **105**, 563 (2018).
447 2. M. R. Ramirez, C. E. Schnorr, L. B. Feistauer, M. Apel, A. T. Henriques, J. C. F. Moreira,
448 and J. Â. S. Zuanazzi, J. Food Biochem. **36**, 405 (2012).
449 3. A. P. G. da Silva, P. C. Spricigo, E. Purgatto, S. M. de Alencar, S. F. Sartori, and A. P.
450 Jacomino, Food Chem. **294**, 547 (2019).
451 4. I. G. Branco, T. T. Kikuchi, E. J. S. Argandoña, I. C. F. Moraes, and C. W. I. Haminiuk, Int.
452 J. Food Sci. Technol. **51**, 1703 (2016).
453 5. M. Hadidi, F. B. Khaksar, J. Pagan, and A. Ibarz, Food Res. Int. **130**, 108907 (2020).
454 6. C. Conidi, A. Cassano, F. Caiazzo, and E. Drioli, J. Food Eng. **195**, 1 (2017).
455 7. P. O. Bagci, M. Akbas, H. A. Gulec, and U. Bagci, Innov. Food Sci. Emerg. Technol. **52**,
456 213 (2019).
457 8. L. M. Rodrigues, E. B. Romanini, E. Silva, E. J. Pilau, S. C. da Costa, and G. S. Madrona,
458 Ultrason. Sonochem. **64**, 105017 (2020).
459 9. K. D. P. P. Gunathilake, L. J. Yu, and H. P. V. Rupasinghe, Food Chem. **148**, 335 (2014).
460 10. AOAC, *Official Method of Analysis*, 18th ed. (Washington, 2005).
461 11. C. W. I. Haminiuk, M. S. V. Plata-Oviedo, G. de Mattos, S. T. Carpes, and I. G. Branco, J.
462 Food Sci. Technol. **51**, 2862 (2014).
463 12. Z. Pan, W. Qu, H. Ma, G. G. Atungulu, and T. H. McHugh, Ultrason. Sonochem. **19**, 365
464 (2012).
465 13. F. C. Cianci, L. F. M. Silva, L. M. C. Cabral, and V. M. Matta, Ciência e Tecnol. Aliment.
466 **25**, 579 (2005).
467 14. Singleton V L & Rossi J A Jr, Am. J. Enol. Vitic. **16(3)**, 144 (1965).

15. W. S. Pierpoint, Methods Mol. Biol. **244**, 65 (2004).

16. H. K. Lichtenthaler, Methods Enzymol. **148**, 350 (1987).

17. M. Alothman, R. Bhat, and A. A. Karim, Food Chem. **115**, 785 (2009).

18. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, and D. Hawkins Byrne, J. Food Compos. Anal. **19**, 669 (2006).

19. N. Nenadis, L. F. Wang, M. Tsimidou, and H. Y. Zhang, J. Agric. Food Chem. **52**, 4669 (2004).

20. R. Pulido, L. Bravo, and F. Saura-Calixto, J. Agric. Food Chem. **48**, 3396 (2000).

21. T. Luiza, C. Oldoni, N. Merlin, M. Karling, S. Teresinha, S. Matias, D. Alencar, R. Gustavo, F. Morales, E. Aparecido, and E. Jorge, Food Res. Int. **125**, 108647 (2019).

22. E. Silva, J. P. da Graça, C. Porto, R. Martin do Prado, C. B. Hoffmann-Campo, M. C. Meyer, E. de Oliveira Nunes, and E. J. Pilau, Sci. Rep. (2020).

23. H. J. Motulsky, in *GraphPad Softw. Inc* (San Diego, CA, 2017).

24. D. F. Ferreira, Rev. Bras. Biometria **37**, 529 (2019).

25. A. P. G. da Silva, T. de O. Tokairini, S. M. de Alencar, and A. P. Jacomino, Rev. Bras. Frutic. **40**, (2018).

26. A. P. G. Da Silva, I. C. Espindola, A. C. A. Miguel, P. C. Spricigo, S. Sartori, and A. P. Jacomino, Acta Hortic. **1194**, 959 (2018).

27. M. C. Pereira, R. S. Steffens, A. Jablonski, P. F. Hertz, A. De O. Rios, M. Vizzotto, and S. H. Flôres, J. Agric. Food Chem. **60**, 3061 (2012).

28. B. He, L. L. Zhang, X. Y. Yue, J. Liang, J. Jiang, X. L. Gao, and P. X. Yue, Food Chem. **204**, 70 (2016).

29. R. ZILLO, P. SILVA, S. ZANATTA, and M. SPOTO, Bioenergia Em Rev. Diálogos (ISSN 2236-9171) **4**, (2015).

30. L. E. Ordoñez-santos, J. Martínez-girón, and D. X. Rodríguez-rodríguez, **86**, 98 (2019).

31. C. B. Aware, R. R. Patil, G. D. Vyavahare, S. T. Gurme, and J. P. Jadhav, J. Am. Coll. Nutr. **38**, 364 (2019).

32. K. D. P. P. Gunathilake, *Emerging Technologies Available for the Enhancement of Bioactives Concentration in Functional Beverages* (Elsevier Inc., 2020).

33. E. Yilmaz and P. O. Bagci, Food Bioprocess Technol. **12**, 1273 (2019).

34. S. Jiang, Y. Li, and B. P. Ladewig, Sci. Total Environ. **595**, 567 (2017).

35. K. D. P. P. Gunathilake, H. P. V. Rupasinghe, and N. L. Pitts, Food Res. Int. **52**, 535 (2013).

36. W. Gustavo Sganzerla, P. Carolina Beling, J. Peruzzo Ferrareze, R. Akitoshi Komatsu, M. Ramos Nunes, and A. P. de Lima Veeck, Commun. Plant Sci. 1 (2018).

37. F. Ren, Y. Nian, and C. A. Perussello, Food Res. Int. 108953 (2019).

38. S. Yan, M. Xie, Y. Wang, Q. Xiao, N. Ding, and Y. Li, Tetrahedron **76**, 130950 (2020).

39. M. Wang, J. J. Carver, V. V. Phelan, L. M. Sanchez, N. Garg, Y. Peng, D. D. Nguyen, J. Watrous, C. A. Kapono, T. Luzzatto-Knaan, C. Porto, A. Bouslimani, A. V. Melnik, M. J. Meehan, W. T. Liu, M. Crüsemann, P. D. Boudreau, E. Esquenazi, M. Sandoval-Calderón, R. D. Kersten, L. A. Pace, R. A. Quinn, K. R. Duncan, C. C. Hsu, D. J. Floros, R. G. Gavilan, K. Kleigrewe, T. Northen, R. J. Dutton, D. Parrot, E. E. Carlson, B. Aigle, C. F. Michelsen, L. Jelsbak, C. Sohlenkamp, P. Pevzner, A. Edlund, J. McLean, J. Piel, B. T. Murphy, L. Gerwick, C. C. Liaw, Y. L. Yang, H. U. Humpf, M. Maansson, R. A. Keyzers, A. C. Sims, A. R. Johnson, A. M. Sidebottom, B. E. Sedio, A. Klitgaard, C. B. Larson, C. A. P. Boya, D. Torres-Mendoza, D. J. Gonzalez, D. B. Silva, L. M. Marques, D. P. Demarque, E. Pociute, E. C. O'Neill, E. Briand, E. J. N. Helfrich, E. A. Granatosky, E. Glukhov, F. Ryffel, H. Houson, H. Mohimani, J. J. Kharbush, Y. Zeng, J. A. Vorholt, K. L. Kurita, P. Charusanti, K. L. McPhail, K. F. Nielsen, L. Vuong, M. Elfeki, M. F. Traxler, N. Engene, N. Koyama, O. B. Vining, R. Baric, R. R. Silva, S. J. Mascuch, S. Tomasi, S. Jenkins, V. Macherla, T. Hoffman, V. Agarwal, P. G. Williams, J. Dai, R. Neupane, J. Gurr, A. M. C. Rodríguez, A. Lamsa, C. Zhang, K. Dorrestein, B. M. Duggan, J. Almaliti, P. M. Allard, P. Phapale, L. F. Nothias, T. Alexandrov, M. Litaudon, J. L. Wolfender, J. E. Kyle, T. O. Metz, T. Peryea, D. T. Nguyen, D. VanLeer, P. Shinn, A. Jadhav, R. Müller, K. M. Waters, W. Shi, X. Liu, L. Zhang, R. Knight, P. R. Jensen,

B. Palsson, K. Pogliano, R. G. Linington, M. Gutiérrez, N. P. Lopes, W. H. Gerwick, B. S. Moore, P. C. Dorrestein, and N. Bandeira, Nat. Biotechnol. **34**, 828 (2016).

40. H. Ogata, S. Goto, K. Sato, W. Fujibuchi, H. Bono, and M. Kanehisa, Nucleic Acids Res. **27**, 29 (1999).

41. S. Kim, P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, and S. H. Bryant, Nucleic Acids Res. **44**, D1202 (2016).

42. L. A. de la Rosa, J. O. Moreno-Escamilla, J. Rodrigo-García, and E. Alvarez-Parrilla, *Phenolic Compounds* (Elsevier Inc., 2019).

43. C. Tian, T. Zhang, L. Wang, Q. Shan, and L. Jiang, J. Ethnopharmacol. **154**, 259 (2014).

44. C. R. Ballard and M. R. Maróstica, *Health Benefits of Flavonoids* (Elsevier Inc., 2019).

45. U. Farooq, A. Shafi, K. Akram, and Z. Hayat, 1 (2020).

Ultrasonics - Sonochemistry 64 (2020) 105017

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

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

Camu-camu bioactive compounds extraction by ecofriendly sequential processes (ultrasound assisted extraction and reverse osmosis)

Leticia Misturini Rodrigues[a,*], Edilson Bruno Romanini[a], Evandro Silva[b], Eduardo Jorge Pilau[b], Silvio Cláudio da Costa[c], Grasiele Scaramal Madrona[d]

[a] Postgraduate Program in Food Science, State University of Maringa, Av. Colombo 5790, CEP 87020-900 Maringa, PR, Brazil
[b] Department of Chemistry, State University of Maringa, Av. Colombo 5790, CEP 87020-900Maringá, PR, Brazil
[c] Department of Biochemistry, State University of Maringa, Av. Colombo 5790, CEP 87020-900 Maringá, PR, Brazil
[d] Department of Food Engineering, State University of Maringa, Av. Colombo 5790, ZIP Code 87020-900 Maringa, PR, Brazil

ARTICLE INFO

Keywords:
Vitamin C
Functional foods
Myrciaria dubia
HPLC-DAD/UV-vis
UPLC-QTOF-MS/MS

ABSTRACT

Technical feasibility of an ecofriendly sequential process (ultrasound assisted extraction and reverse osmosis, or UAE and RO) was evaluated in order to obtain a functional Camu-camu (Myrciaria dubia) product with high vitamin C content. Water was used in the assisted extraction by probe ultrasound (UAE) in an experimental design to evaluate different times, amplitudes and temperatures. The best region for total phenolic (TP) and vitamin C (VC) extraction was 5 min, 60 °C and 30% amplitude. Following extraction, the sample was concentrated by reverse osmosis (R25a, 500 Da, polyamide, and 5 bar area 3 ft2), obtaining a relatively low fouling of 19%. At the end of the sequential process (by HPLC-DAD/UV vis), was obtained a concentrated camu-camu (CC) with high Vitamin C (52.01 ± 0.889 mg/g) and cyanidin-3-glucoside, being respectively 7.0 and 4.5 times higher; also the concentration of phenolic compounds was increased by 3.2 times (25.798 mg GAE/g), and anthocyanins in 6.5 times (66.169 mg of cyanidin-3-glucoside/100 g) as well as high antioxidant activity by all three methods evaluated (increased 3.0, 4.6 and 2.38 times for ABTS, DPPH, FRAP, respectively) by comparing the CC with the initial extract (CS). Twenty compounds were identified by UHPLC-QTOF-MS/MS, highlighting quercetin, gallic acid, p- coumaric, ellagic acid and cyanidin-3-glucoside, and at the first time alnusiin was detected in camu-camu. Therefore, the combination of ultrasound assisted extraction and reverse osmosis can be a promising profitable alternative in order to apply bioactive compounds in food, nutraceuticals and cosmetic matrices, bringing their benefits to consumers.

1. Introduction

Camu-camu (*Myrciaria dubia*) is a reddish-colored fruit belonging to the Myrtaceae family found typically in the Amazon region [1]. This fruit is an important source of antioxidants because it has high levels of vitamin C (ascorbic acid) and high amounts of phenolic compounds, classifying it as a functional food [2].

This type of fruit is popular due to its high nutritional content and also stands out for the presence of bioactive compounds, such as the phenolic group that includes flavonoids, carotenoids and anthocyanins, that have disease prevention effects and provide health benefits by acting as natural antioxidants [3,4].

In addition to the use of Camu-camu as natural food coloring, this plant has also aroused interest in the pharmaceutical industry for the production of cosmetic and nutraceuticals mainly due to the presence of ascorbic acid and other beneficial substances. These factors contribute to the development of extraction, identification and quantification studies of these compounds [5].

Increasing interest has emerged in the use of clean technologies that are able to provide high extract yield simultaneously preventing any solvent-associated toxicity [6]. The water was used in this study as extraction solvent, according to the literature is considered potentially green, as it reduces the environmental impact, is not toxic to health, being considered safe, low cost, short extraction time and high

Abbreviations: FW, fresh weigh; CS, control sample; OE, ultrasound optimal extraction; CC, concentrated sample; UAE, ultrasound assisted extraction; RO, reverse osmosis; TP, total phenolics; VC, vitamin C; GAE, gallic acid equivalent; TE, trolox equivalent; EQ, quercetin equivalent; DPPH, 2,2-diphenyl-1 picrylhydrazine; FRAP, Ferric Reducing Antioxidant Power; ABTS, 2,2′ azinobis (3 ethylbenzothiazoline-6-sulfonic acid); HPLC-DAD UV/vis, high efficiency liquid chromatography; UPLC-QTOF-MS/MS, Ultra Performance Liquid Chromatography and Mass Spectrometry

* Corresponding author.
E-mail address: gsmadrona@uem.br (L.M. Rodrigues).

https://doi.org/10.1016/j.ultsonch.2020.105017
Received 12 December 2019; Received in revised form 22 January 2020; Accepted 8 February 2020
Available online 10 February 2020
1350-4177/

efficiency [7,8]. Ultrasound assisted extraction (UAE) is an efficient, simple and economical technique. Using this technique can improve solid-liquid extractions, reducing extraction time and increasing yield. The use of ultrasound can be seen as a green technique because it takes less solvent, shorter extraction time and simpler operation [9,10].

To Arent et al [11], membrane separation is an alternative to minimize nutritional and sensory loss in fruit processing. The advantages detected by this process are energy saving, simplicity of operation, no special chemical requirements, economically viable for concentration and purification, and separations are highly selective. In addition, separation can be performed at mild or room temperature, allowing thermolabile compounds to be processed without modification or loss of functional and sensory properties. This makes membrane separation a viable alternative to conventional methods used in liquid food processing [12–14].

The reverse osmosis (RO) process has been in evidence in recent years for being an efficient non-thermal process to concentrate fruit juices. This is relatively new, and it works by the difference in water vapor pressure occurring through the membrane, transferring from the higher to the lower pressure side. In addition, concentrated osmosis solutions can be used for feed without drastically decreasing permeability, thus maintaining flow stability at high concentration levels [15].

Other technologies have been studied for this fruit, a study evaluated camu-camu as a new source of Vitamin C, as it is widely used in the food, pharmaceutical and cosmetics industries. The authors evaluated three different extraction techniques: pressurized liquid extraction (PLE), acid extraction, and maceration, being the pressurized liquid extraction (PLE), (a green technology), a viable alternative for obtaining extracts rich in vitamin C [5].

This study presented as innovation factor the Vitamin C concentration by ultrasound assisted extraction with water, in order to obtain a concentrated camu-camu product (with a sequential process UAE and RO). The literature reported only the separated process, as an example, the thermosonication (in different conditions) was used to evaluate physicochemical changes in camu–camu nectars [16]. RO was evaluated together with the osmotic evaporation process (OE), in order to concentrate the camu – camu juice, the authors concluded that such integrated processes were effective being an alternative to the concentration of thermosensitive juices [17].

Thus, the objective of this study was: 1) to evaluate the best conditions for aqueous extraction of phenolic compounds and vitamin C from Camu-camu fruit through UAE; 2) concentrate the extract obtained in the optimal region by RO; 3) characterize all products obtained.

2. Materials and methods

2.1. Samples, chemicals and reagents

6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2 diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Calcium gallate, TPTZ (2,4,6-tripyridyls-triazine) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid, cyanidin 3-glucoside, ellagic acid, gallic acid, rutin, quercetin, p-coumaric acid, chlorogenic acid, myricetin and cyanidin chloride, were the standards used also from Sigma-Aldrich. All reagents/solvents were of analytical or HPLC grade in accordance with the requirement. Milli-Q ultrapure water was used in the required analyzes.

The fruits of Camu-camu were purchased from the company Camu-camu fruits of the Amazon (Vitória do Xingu, Brazil), November/2018 harvest. To determine the composition of the ground fruits, the following physicochemical analyzes were performed: total soluble solids (^{0}brix) using an HI96801 digital refractometer (Hanna Instrument); pH, moisture, ashes, protein (micro Kjeldahl) and lipids and carbohydrates by difference (100 – (moisture + protein + lipids)) [18]; color were determined by the Minolta ® CR400 portable colorimeter.

2.2. Ultrasound assisted extraction (UAE) by experimental design

Camu-camu samples were diluted in water to a concentration of 50 g of solids in 200 mL of solvent [19]. Water was used as solvent, because is ecofriendly and save for future food applications.

An ultrasound system probe was used (750 W and 20 KHz; collective Parmer 750-Watt Ultrasonic Processors). Its amplitude controller allowed the ultrasonic vibrations through the probe tip (titanium 13 mm diameter) to be adjusted to the desired level of 0–40% (0–20 kHz) scale by the controller. The tests had a volume of approximately 250 mL and the probe was inserted directly into the mixture. According to preliminary results, the ultrasound extraction time (X1 = 2.5, 5 and 10 min), amplitude (X2 = 20, 30 and 40%) and temperature (40, 50, and X3 = 60 °C) have been evaluated by experimental design, added of three central points and the response variable was the concentration of phenolic compounds and ascorbic acid, being evaluated by response surface (Statistical program 7.0). All extractions were performed with respectively 5 s of pause and 5 s of pulse and according to preliminary tests and literature results [20]. After extraction, the samples were vacuum filtered and analyzed. The sequential process was described as a flowchart (Fig. 1).

2.3. Reverse osmosis (RO) membrane concentration

The freshly prepared extract in optimized condition and cooled to 25 °C was filtered (0.45 μm) previously and afterwards concentrated under the following conditions: membrane R25A, 500 Da, polyamide, 5 bar pressure and filtration area 3 ft^2. The flow rate was monitored during the process which was performed at room temperature (approximately 25 °C). The process was interrupted when flow stabilized.

The flow was calculated by measuring the weight of the concentrate collected at different time intervals, with the flow data of distilled water in the clean membrane and the stable flow in the foul membrane, it was possible to calculate the percentage of fouling (% F) according to Eq. (1). The volumetric concentration factor (VCF) was determined using feed volume and permeate volume [21].

$$\%F = \frac{(Ji - Jf)}{Ji} \times 100 \quad (1)$$

The concentrate obtained, the initial extracts, and the extract obtained in optimal condition in the ultrasonic extraction were analyzed. Sequential process was described as a flux diagram (Fig. 1).

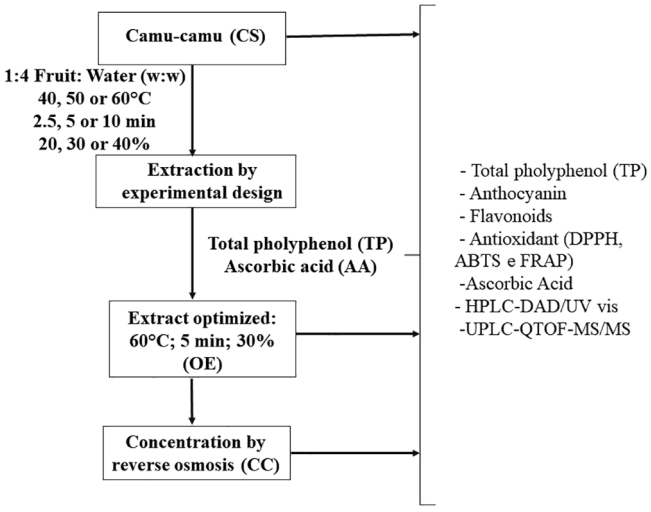

Fig. 1. Sequential process for Camu-camu concentration.

2.4. Bioactive compounds analysis

2.4.1. Total phenolic compounds (TP)

The determination of total phenolic compounds (TP) was performed using the Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3) [22,23]. Absorbance was verified by spectrophotometer at 725 nm after 30 min of incubation at 25 °C. Gallic acid was used as the standard for the calibration curve ($R^2 = 0.99$). Results were expressed as mg of gallic acid equivalent (EAG) g^{-1} of fw.

2.4.2. Vitamin C (VC)

The vitamin C content was quantified by high performance liquid chromatography (HPLC) according to the method of [24] with modifications. A Waters Alliance e2695 HPLC system equipped with a quaternary pump (model waters 2998) and UV-VIS and DAD detectors (set at 254 nm) with automatic sample injection was used. Chromatographic separation was performed using a reverse phase C18 (macherey-nagel) column. The mobile phase used was gradient system water acidified with phosphoric acid (eluent A) and acetonitrile (eluent B) (95:5). The injection volume was 1 µL of sample, and the run flow was 0.4 mL min^{-1} with a total run time of 10 min at room temperature (25 °C). The extracts were filtered (0.45 µL) and injected in duplicate automatically into the column. Quantification was based on the peak area with results expressed in mg/g^{-1} fw applying the standard curve (Y = 4.84eˆ006X-4.02eˆ005, $R^2 = 0.998106$).

2.4.3. Monomeric total anthocyanins

The differential pH method [25] was used to determine total monomeric anthocyanins (TMA) with potassium chloride (KCl) and sodium acetate ($C_2H_3NaO_2$) reagents. Absorbance was verified by spectrophotometer at 520 and 700 nm after 20 min of incubation at 25 °C. Results were expressed as mg cyanidine-3-glycoside.100 g^{-1} fw, According to Eqs. (2) and (3).

$$AT = (ABS520nm - ABS700nm)pH1.0 - (ABS520nm - ABS700nm)pH4.5 \quad (2)$$

$$\text{Anthocyanins} = \frac{(AT \times PM \times df \times 10^3)}{\varepsilon \times \lambda} \quad (3)$$

where PM is the cyanidine-3-glucoside molar mass (449.2 g/mol), df is the dilution factor, 10^3 is used as the g to mg conversion factor, Ɛ is the cyanidine-3-glucoside molar absorption (26,900 L/mol) and ƛ the optical path length of the cuvette (1 cm).

2.4.4. Total flavonoids (TF)

The determination of total flavonoids (TF) was performed by colorimetric assay in absorbance 510 nm using aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$) and sodium hydroxide (NaOH) [26]. The results are expressed in mg of equivalent quercetin (QE) g^{-1} fw.

2.4.5. Antioxidant activity (DPPH, FRAP and ABTS)

The reduction of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyla) was determined by the colorimetric method at 515 nm [27]. The efficiency of the sequestering activity was calculated according to Eq. (4), the results are expressed in mmol of equivalent Trolox (TE) g^{-1} fw.

$$\text{Free radical sequestration efficiency}(\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (4)$$

where:

Control: Absorbance of negative control.
Sample: Mean absorbance of the sample.

The antioxidant activity by ABTS assay was performed using the ABTS reagents (2,2′-azinobis (3-ethylbenzthiazoline sulfonic acid-6)) and persulfate potassium ($K_2S_2O_8$) [28]. Absorbance was verified by spectrophotometer at 734 nm after 6 min of incubation at 25 °C. A calibration curve was prepared using Trolox standard solution, results were expressed as mg of equivalent Trolox (TE) g^{-1}.

For the FRAP method the extracts were mixed with distilled water and the FRAP reagent, then kept for 30 min at 37 °C in a water bath and sent to the spectrophotometer for reading at 595 nm, the results were expressed in mg g^{-1} fw [29].

2.4.6. Bioactive compounds by UHPLC-Q-TOF-MS/MS and HPLC-DAD/UV vis

The CS, OE and CC samples were re-suspended in 500 µL of water/acetonitrile (1:1; v:v) and 3 µL of each sample were injected and analyzed using an ultra-high-performance liquid chromatography (Shimadzu Nexera X2, Japan). The chromatography separation was performed with an Acquity UPLC® CSH C18 column (50 mm × 2.1 mm, 1.7 µm; Waters, USA) with column temperature of 40 °C. The mobile phase was a gradient mixture of solvents; A (H2O with 0.1% formic acid, v:v) and B (acetonitrile) with an optimized linear gradient elution as follows: 5% B 0–1 min, 70% B 1–10 min, 98% B 12–16 min and maintained at 5% B 16–20 min, the final four minutes being intended for reconstitution of the column for the next analysis [30].

Mass spectrometry was performed on a Q-TOF geometry Impact II (Bruker Daltonics Corporation, Germany) high resolution equipped with electrospray ionization (ESI) source. All MS data were acquired by using a sodium formate solution (10 mmol L^{-1} NaOH solution in isopropanol: water (1:1; v:v) solution containing concentrated formic acid) as calibrant to ensure mass accuracy and reproducibility. The ionization source was operated on the positive and negative mode and adjusted to 4500 V with an end plate offset potential of −500 V. The drying gas parameters were adjusted to 8 L min^{-1} at 180 °C and gas pressure of mist at 4 bar. The data were collected in the range of *m/z* 50 to 1300 with 5 Hz of acquisition rate, which the 5 most intense ions were selected for automatic fragmentation (Auto MS/MS). The energies for induced collision dissociation (CID) were 15–40 eV for fragmentation information [30]. The data were acquired by the Hystar Application software version 3.2 and Ot of Control (Bruker Daltonics Corporation, German).

Compounds were quantified by high performance liquid chromatography (HPLC) according to the method of [31] with modifications. Chromatographic separation was performed using a reverse phase C18 (macherey-nagel, 250 × 4.6 mm) column operating at 40 °C. The mobile phase used was 0.1% formic acid in water (v/v) (eluent A) and acetonitrile (eluent B). The injection volume was 1 µL of sample and the flow of the race was 0.9 mL min^{-1} with a total run time of 30 min. The gradient schedule was: 0 min 90% (A), 0–17 min 40% (A), 17–22 min 40% (A), 22–28 min 90% (A) and maintained at 90% (A) until the end of the race at 30 min. UHPLC/DAD analyzes were performed at six different wavelengths: 265 nm for rutin, 275 nm for gallic acid, 310 nm for coumaric acid, 370 nm for myricetin and 520 nm for cyanidin 3-glucoside.

The phenolic compounds were identified by comparing retention time and UV absorption spectra with available standards. The extracts were filtered (0.45 µL) and automatically injected into the column and quantification was based on peak area ($R^2 > 0.99$) with concentrations expressed in mg/100 g^{-1} fw.

2.5. Statistical analysis

All analyzes were performed in triplicate and subjected to analysis of variance and Tukey test for the minimum significant difference ($p < 0.05$) between means using the Sisvar 5.6 statistical program. Calibration curves for antioxidant analyzes were performed using the GraphPrism 5 program.

3. Results and discussion

3.1. Initial fruit characterization

The fruits had on average total soluble solids (TSS) content of 7.20 ± 0.15° brix, pH = 3.24, moisture content of approximately 84.00 ± 0.01, and 0.21 ± 0.03 ash, protein content was 0.81 ± 0.0, and lipids 0.25 ± 0.01 and carbohydrates of 14.94 g/100 g on wet basis. Regarding the color of the whole fruit, Brightness (L) was 52.55 ± 0.68, the tendency for red (a*) was 19.19 ± 1.14 and for yellow (b*) 21.70 ± 0.47.

Ref. [32] characterized the whole fruit of Camu-camu finding pH values = 3.09 and TSS = 7.26 very close to the present study. Ref. [2] also reported similar values for TSS (6.40° Brix), pH (2.44), 0.4 g/100 g protein and 0.2 g/100 g lipid parameters. Ref. [33] also reported similar values for the characterization of *Myrciaria dubia* pulp, and stressed that due to high acidity values, the fruits are not sweet, therefore not widely consumed in *natura*. The authors suggest more studies to develop alternative derivative products to increase sensory acceptance of this fruit.

3.2. Experimental planning – ultrasound assisted extraction

The experimental results of Camu-camu extract for TP and VC (Table 1) ranged from 3.34 to 5.43 mg of GAE/g fw, 5.28 to 6.19 mg/g fw, respectively. Eqs. (4) and (5) demonstrate the degree of significance between variables.

Evaluating p-value, and the response surface (Fig. 2A–F) for the TP extraction (Fig. 2A–C) the three variables: extraction time (X1), amplitude (X2) and temperature (X3) were significant ($p < 0.05$), analyzing the obtained equation ($Y = 4.89 + 0.25\,X1 + 0.25\,X2 - 0.27\,X3 - 0.23\,X1X2 + 0.17\,X1X3 + 0.28\,X2X3$), it is possible to observe interactions with all the three variables, and time and amplitude presented equal and positive influence and temperature presented negative influence. For VC extraction (Fig. 2D–F), only the variable temperature (X3) was significant ($Y = 6.131 + 0.246\,X3$). In combination, the ideal extraction region according to the final results were the following variables: 5 min, 30% amplitude at 60 °C, these conditions were defined considering the booth TP and VC values, and the response surface curvature.

In our study, the extraction of compounds was also facilitated by the action of temperature. This is a critical factor that intervenes in the extraction process due to the softening of the tissues, which consequently increases the solubility and diffusion coefficient of the substances, guaranteeing the optimized recovery of the polyphenols. At very extreme temperatures, the yield can be reduced by the thermal and chemical degradation of some compounds, compromising the antioxidant activity [34]. It was also observed in our study that ultrasound contributed to greater extraction of the compounds, along with temperature, improving the phenomena of cavitation, mechanical agitation, process efficiency and facilitating the transport of bioactive compounds from the center of the plant to the broken walls during sonication, in addition to contributing to the reduction of extraction time. [9,35]. The extraction time also had a reasonably significant effect, which mainly influenced CT responses. Operating time is very important during extraction, as it contributes to reducing electricity consumption as the operating time decreases [35].

Previous studies reported that using a combination of parameters such as temperature and time contribute for extraction of phenolic compounds. Ref. [36] used time (30 and 60 min), and temperature (40, 50 and 60 °C) with ultrasonic bath to evaluate bioactive compounds and ascorbic acid of *Myrciaria dubia* nectar, showing a higher availability of those compounds compared with the control sample. Ref. [1] used 30 °C in combination with cloud point technique to extract phenolics from Camu-camu residue. Ref. [37] performed the extraction of Camu-camu seed flour compounds at a temperature of 45 °C with stirring for 45 min and different types of solvents. The aqueous extract was the one with the highest total phenolic content, FRAP, DPPH and inhibition of lipid oxidation.

Mild operating temperatures, between 50 and 60 °C, combined with ultrasound is a process called thermo-sonication, it reduces process time and/or temperature, avoiding product damage and improving compound release. Ref. [38] used probe ultrasound (13 mm diameter), with a frequency of 20 kHz, very similar to this work, to evaluate several parameters among them antioxidant activity of purple cactus pear, and obtained an increase of approximately 41% in phenolic content with 80% amplitude, 25 min extraction, and an increase in antioxidant activity (ABTS, DPPH) until the end of storage (28 days ± 4 °C).

Table 1
Results of total phenolic content and vitamin C for different extraction conditions according to a central composite design.

Run	Extraction conditions			Response	
	Time (min)	Amplitude (%)	Temperature (°C)	TP (mg EAG/g)	VC (mg/g)
1	2.5	20	40	5.02	5.28
2	10	20	40	5.43	5.56
3	2.5	40	40	5.21	5.46
4	10	40	40	5.14	5.90
5	2.5	20	60	3.34	5.68
6	10	20	60	4.88	6.07
7	2.5	40	60	5.09	6.19
8	10	40	60	5.28	6.12
9	5	30	50	4.81	5.89
10	5	30	50	4.74	6.19
11	5	30	50	4.89	6.07

TP- Total phenolic compounds, *VC*- vitamin C.

3.3. Reverse osmosis (RO) membrane concentration

The permeate flow was measured as a function of operating time for the process of reverse osmosis in 3 min intervals until 48 min, and ranged from 15.0 L/(h m^2) at the beginning of the process to 1.8 L/(h m^2), when the solids content reached 4.1° Brix for the EO. There is a reduction in permeate flow as process time increases. This happens because the reverse osmosis membrane used is very dense and almost all the soluble solids are retained, increasing solution osmotic pressure and reducing flow [21].

The membrane fouling is one of the factors that impair the life and performance of reverse osmosis membranes [39], being better a low fouling. The changes in the permeate flow during the reverse osmosis process are mainly due to some factors such as: increases in osmotic pressure, inscriptions on the membrane (microbial adhesion, gel layer formation and solute adhesion) and increase in the viscosity of the membrane fluid that significantly affect the efficiency of the process [17,40]. The membrane fouling level was relatively low (19%), and the concentration factor was 4.1 times that of the feed. Corroborating with data reported in the literature, [14] also used this extraction combination for recovery of phenolic compounds from olive pomace and their concentration by three different polymeric membranes, two nanofiltration (NF90, NF270) and one of reverse osmosis (BW30). The authors reported that the reverse osmosis membrane was considered the most appropriate for future scaled process, as it presented lower fouling index (20%), while the others presented 50 and 60% respectively. The permeate flow was practically constant during the process 15 L/(h m^2), and the final concentrate BW30 presented higher antioxidant activity, concentration of phenolic compounds and flavonoids in 12, 15 and 4%, respectively.

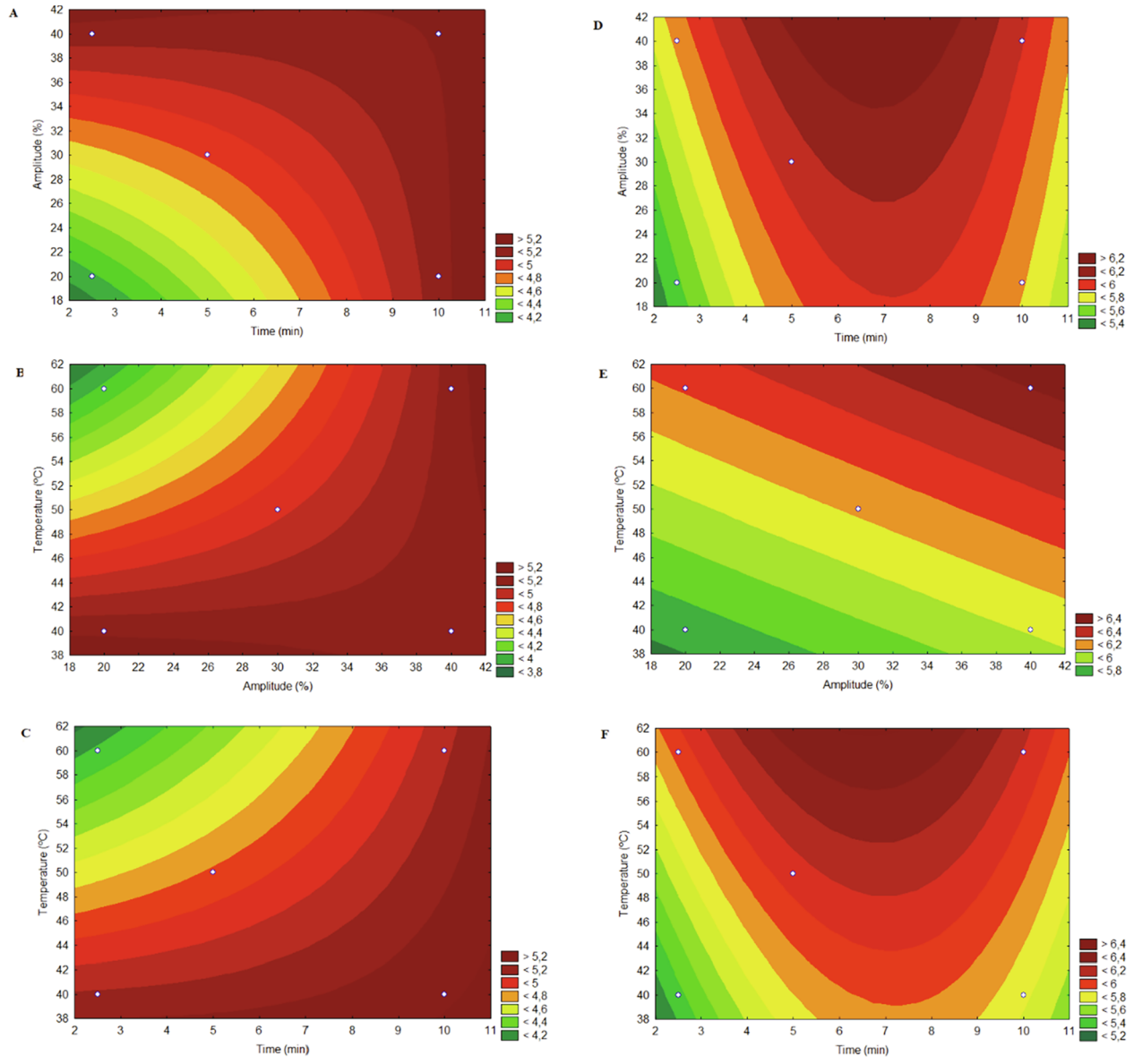

Fig. 2. Response surface between three variables (time, amplitude, and temperature) in the extraction of Total Phenolic compounds (TP mg GAE/g fw, A – C) and vitamin C (VC, mg/g fw, D – F). (A, B, C) TP = 4.89 + 0.25X1 + 0.25X2-0.27X3-0.23X1X2 + 0.17X1X3 + 0.28X2X3 (D, E, F) CV = 6.131 + 0.246X3.

3.4. Bioactive compounds

The levels of total polyphenols were 3.2 times greater (25.7980 mg GAE/g) than the point of optimal extraction (7.9219 mg GAE/g), with significant difference with respect to the other two samples (Table 2).

Camu-camu, has been highlighted as one of the richest dietary fruits due to the high presence of phenolic compounds in its composition and consequently, its antioxidant potential. Ref. [41] evaluated the content of phenolic compounds for different parts of the Camu-camu fruit (pulp, peel and seed), and the results were 0.0866, 0.105 and 3.360 mg/g respectively, lower values than those reported in the present study.

Fidelis et al. [37] studied different solvents (water, propanone and ethano1) for extraction of Camu-camu peel bioactive compounds, and observed better results with DPPH aqueous extract (2838 ± 176 mg AAE/100 g), FRAP (7425 SEA ± 247 mg/100 g) and phenolics (39.23 ± 29 mg GAE/100 g).

The concentrated sample (CC) showed 6.5 times more anthocyanins (66.169 mg of Cyanidin-3-glucoside/100 g) than the sample without extraction (10.228 mg of Cyanidin-3-glucoside/100 g) (Table 3). Souza et al [36] observed that using ultrasound with 30 min and temperature variation of 10, 50 and 60 °C, did not significantly alter total contents of anthocyanins, but the opposite was observed when using 60 min, degrading anthocyanins from Camu-camu nectar. The highest value shown for anthocyanins was of 6.47 mg/100 g of Camu-camu nectar by thermo-sonication. In the present work, extraction with sonication showed greater efficiency in the extraction of anthocyanins (18.368 mg/100 g), compared to that previously presented by [36]. Anthocyanins have been noted for being brightly colored and responsible for much of the red, purple and blue coloration of the fruits.

Reverse osmosis favored flavonoid concentration to almost four times more (28.374 mg TQ/g) than the control (7.295 mg TQ/g) (Table 2). This fact is important because according to literature flavonoids are extremely important phenolics and beneficial to human health, contributing to the reduction of vascular diseases and cancer [36].

All samples were different ($p < 0.05$) for antioxidant activity (DPPH and FRAP), showing that the sequential process, i.e. ultrasound assisted extraction and reverse osmosis favored extraction and concentration of compounds. The ABTS method showed a significant

Table 2
Bioactive compounds from different Camu-camu samples (fresh weight = fw).

	CS	OE	CC
TPC (mg GAE/g)	7.791^{b} ± 0.029	7.922^{b} ± 0.078	25.798^{a} ± 0.293
TMA (mg of cyanidin-3 glucoside/100 g)	10.228^{b} ± 2.066	18.368^{b} ± 1.181	66.169^{a} ± 3.838
TF (mg QE/g)	7.295^{b} ± 0.897	6.902^{b} ± 0.085	28.374^{a} ± 0.3202
ABTS (mmol TE/g)	74.145^{b} ± 0.750	91.157^{b} ± 0.280	216.148^{a} ± 11.700
DPPH (mmol TE/g)	65.538^{c} ± 0.002	126.881^{b} ± 0.290	302.122^{a} ± 1.060
FRAP (mmol TE/g)	222.000^{c} ± 0.562	284.980^{b} ± 0.000	528.667^{a} ± 2.108
Vitamin C (mg/g)	7.569^{b} ± 0.044	7.912^{b} ± 0.156	52.01^{a} ± 0.889
Myricetin (mg/100 g)	0.201^{c} ± 0.000	0.216^{b} ± 0.000	0.307^{a} ± 0.000
Cyanidin-3-glucoside (mg/100 g)	0.622^{c} ± 0.000	0.7820^{b} ± 0.000	2.783^{a} ± 0.001
p- coumaric acid (mg/100 g)	0.067^{c} ± 0.000	0.098^{b} ± 0.000	0.150^{a} ± 0.000
Rutin (mg/100 g)	2.441^{c} ± 0.001	3.035^{b} ± 0.000	9.783^{a} ± 0.002
Gallic acid (mg/100 g)	22.728^{b} ± 0.025	28.501^{b} ± 0.004	97.298^{a} ± 0179

The data are expressed as means ± standard deviations. Means followed by the same letter in the line do not differ significantly from each other by the Tukey test at 5% significance level. TPC = total phenolic compounds; TMA = total monomeric anthocyanins; TF = total flavonoids. GAE = gallic acid equivalent, QE = Quercetin equivalent, TE = Trolox equivalent, CS – control sample; OE – ultrasound optimal extraction; CC – concentrated sample.

difference ($p < 0.05$) only for the concentrated sample (CC), since its value was increased three times (216.148 mmol TE/g) compared to the control sample (CS) (74.145 mmol TE/g).

Ascorbic acid is the most abundant soluble antioxidant in plants, thus authors quantified ascorbic acid in 18 Brazilian tropical fruits, among which Camu-camu is highlighted with 1882 ± 43.2 mg/100 g fresh fruit, followed by Acerola 1357 ± 9.5 mg/100 g fresh fruit and Jabuticaba 238 ± 2.2 mg/100 g fresh fruit, the lowest value found was for Bacuri 2.4 ± 0.3 mg/100 g fresh fruit, [42].

Some processes have been used to obtain Camu-camu products. Pagani et al [17] performed a juice concentration by reverse osmosis (polytetrafluoroethylene membrane, Pall Gelman-TF200) using 0.2 bar, and obtained values very similar to the concentration of acid ascorbic (52.9 mg/g dry matter) found in this work (52.01 mg/g wet matter).

A study evaluating microwave (MW) heating in the processing of Camu-camu juice, achieved better results for ascorbic acid compared with conventional treatment and the highest concentration obtained was 1788 mg/100 g (17.88 mg/g) under conditions of 625 W/30 s [16].

Another study showed different concentrations of ascorbic acid only found in Camu- Camu (pulp and peel separate) from different regions, by UHPLC-DAD. Peel values ranged from 5.98 ± 0.33 to 13.56 ± 0.42 mg/100 g, and pulp 18.05 ± 0.66 to 38.37 ± 2.13 mg/100 g, these values are reported for dry samples. This difference in ascorbic acid content was explained by the variation among lots and the edaphoclimatic conditions. The content of ascorbic acid in fresh pulp (FP) was also evaluated and the highest value was for a Peru sample with 1914. 66 mg/100 g fp. Previous studies have reported values ranging from 845 to 3133 mg/100 g of FP and for certain genotypes a value greater than 6000 mg/100 g of FP has been found [5].

3.5. Bioactive compound identification by UHPLC-Q-TOF-MS/MS

The compounds were identified by UHPLC-Q – TOF-MS/MS and their MS and MS/MS spectra were interpreted by comparing literature spectra and public databases such as KEGG [43], CHEBI [44] and PubChem [45]. A total of 20 compounds were identified in the CS sample, 17 compounds in the OE sample and 17 compounds in the CC

Table 3
Bioactive compounds of different Camu-camu samples.

Compound	Molecular Formula	Exact Mass	CS			OE			CC		
			RT (min)	ESI	Error (ppm)	RT (min)	ESI	Error (ppm)	RT (min)	ESI	Error (ppm)
1 Gallic acid	$C_7H_6O_5$	170.0215	5.31	$[M-H]^-$	1.5	5.27	$[M-H]^-$	2.1	9.89	$[M+H]^+$	−0.6
2 Quercetin	$C_{15}H_{10}O_7$	302.0426	8.96	$[M+H]^+$	−1.0	10.45	$[M-H]^-$	−0.3	8.84	$[M+H]^+$	−0.6
3 Chlorogenic acid	$C_{16}H_{18}O_9$	354.0950	7.51	$[M+H]^+$	3.2	7.81	$[M+H]^+$	−1.6	7.48	$[M+H]^+$	−0.6
4 Ascorbic acid	$C_6H_8O_6$	177.0393	3.26	$[M+H]^+$	−0.4	3.11	$[M+H]^+$	−2.1	3.04	$[M+H]^+$	0.2
5 p-Coumaric acid	$C_9H_8O_3$	164.0473	3.58	$[M+H]^+$	−1.3	3.60	$[M+H]^+$	−3.2	3.57	$[M+H]^+$	−0.1
6 Cyanidin-3-O-glucoside	$C_{21}H_{21}ClO_{11}$	484.0772	3.94	$[M-H]^-$	−5.3	3.83	$[M-H]^-$	−4.7	3.93	$[M-H]^-$	−3.7
7 Myricetin	$C_{15}H_{10}O_8$	318.0375	8.49	$[M+H]^+$	−0.5	8.12	$[M+H]^+$	−1.5	8.44	$[M+H]^+$	0.2
8 Myricetin3″-O-beta-D-glucopyranoside	$C_{21}H_{20}O_{13}$	480.0903	8.14	$[M-H]^-$	4.1	8.07	$[M-H]^-$	3.5	8.04	$[M-H]^-$	−3.8
9 Myricitrin	$C_{21}H_{20}O_{12}$	464.0954	8.49	$[M-H]^-$	3.9	8.52	$[M-H]^-$	4.1	7.41	$[M-H]^-$	3.9
10 Myricetin-3-O-beta-D-xylopyranoside	$C_{20}H_{18}O_{12}$	450.0798	8.38	$[M-H]^-$	4.1	8.46	$[M-H]^-$	4.1	8.30	$[M-H]^-$	4.6
11 Quercetin-3-O-α-D-arabinofuranoside	$C_{20}H_{18}O_{11}$	434.0849	nd	nd		nd	nd		8.87	$[M+H]^+$	−1.8
12 Ellagic acid	$C_{14}H_6O_8$	302.0062	8.72	$[M-H]^-$	2.3	8.69	$[M-H]^-$	5.0	8.69	$[M-H]^-$	4.0
13 Ellagic acid hexose	$C_{20}H_{16}O_{13}$	464.0590	7.75	$[M-H]^-$	5.6	7.75	$[M-H]^-$	3.2	7.69	$[M-H]^-$	3.2
14 Ellagic acid pentoside	$C_{19}H_{14}O_{12}$	434.0485	8.06	$[M-H]^-$	4.5	8.08	$[M-H]^-$	2.4	8.01	$[M-H]^-$	3.7
15 Ellagic acid rhamnoside	$C_{20}H_{16}O_{12}$	448.0641	8.19	$[M-H]^-$	3.6	8.18	$[M-H]^-$	2.0	8.22	$[M-H]^-$	4.5
16 Ellagic acid derivate	$C_{22}H_{18}O_{13}$	490.0747	8.91	$[M-H]^-$	1.5	8.95	$[M-H]^-$	4.4	8.96	$[M-H]^-$	2.9
17 Ellagic acid derivate	$C_{34}H_{40}O_{17}$	720.2265	9.76	$[M-H]^-$	1.1	10.08	$[M-H]^-$	2.0	10.03	$[M-H]^-$	0.9
18 Alnusiin	$C_{41}H_{26}O_{26}$	934.0712	6.90	$[M+H]^+$	−0.4	6.92	$[M+H]^+$	−0.3	6.84	$[M+H]^+$	−2.9
19 Gallocatechin	$C_{15}H_{14}O_7$	306.0739	7.01	$[M-H]^-$	4.0	nd	nd		6.94	$[M-H]^-$	2.0
20 Epigallocatechin 3-gallate	$C_{22}H_{18}O_{11}$	458.0849	8.00	$[M-H]^-$	2.5	nd	nd		8.03	$[M-H]^-$	3.2

*nd = not detect; CS – control sample; OE – ultrasound optimal extraction; CC – concentrated sample.

sample. The compounds identified were manually confirmed, fragmentation spectra verification and mass errors ranged from 0.1 to 5.6 ppm (Table 3). These compounds are belonging to the anthocyanins, ellagitannins, ellagic acid derivatives, flavonols, phenolic acids and proanthocyanidins classes.

Compounds 1, 3, 4 and 5 with $[M-H]^-$ m/z 170.0215, $[M+H]^+$ m/z 354.0950, $[M+H]^+$ m/z 177.0393, $[M+H]^+$ m/z 164.0473 were putatively identified as gallic acid, chlorogenic acid, ascorbic acid and p-coumaric acid, respectively (Table 3) belonging to the class of phenolic acids. Phenolic acids are compounds present mainly in vegetables and fruits, have antioxidant properties and act as antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and vasodilatory constituting important bioactive compounds [46]. The anthocyanian cyanidin-3-O-glucoside (compound 6) was putatively identified at in negative mode and presented the precursor ion $[M-H]^-$ m/z 484.0772. Cyanidin-3-O-glucoside has been previously identified in Camu-camu, as principal anthocyanin that is used as chemical markers [47–49].

The compounds 2, 7, 8, 9 10 and 11 with $[M+H]^+$ m/z 302.0426, $[M+H]^+$ m/z 318.0375, $[M-H]^-$ m/z 480.0903, $[M-H]^-$ m/z 450.0798 and $[M-H]^-$ m/z 434.0849 was identified as quercentin, myricetin, myricetin3″-O-beta-Dglucopyranoside, myricitrin, myricetin-3-O-beta-Dxylopyranoside and quercetin-3-O-α-Darabinofuranoside respectively (Table 3), these compounds are polyphenols belonging to the class of flavonols. Previous studies have shown a relationship between flavonol myricetin and the antimicrobial potential of Camu-camu bark extract [50].

Ellagic acid derivative were the main group of compounds detected in the extracts of Camu-camu. Compounds 12–17 with $[M-H]^-$ m/z 302.0062, $[M-H]^-$ m/z 464.0590, $[M-H]^-$ m/z 434.0485, $[M-H]^-$ m/z 448.0641, $[M-H]^-$ m/z 490.0747, $[M-H]^-$ m/z 720.2265 were identified as ellagic acid, ellagic acid hexose, ellagic acid pentoside, ellagic acid rhamnoside, ellagic acid derivate, ellagic acid derivate, respectively.

Alnusiin (18, $[M+H]^+$ m/z 934.0712) an ellagitanin, was also identified at the first time in Camu-camu, and proanthocyanidins as gallocatechin (19, $[M-H]^-$ m/z 306.0739) previously reported by [48], and epigallocatechin-3-gallate (20, $[M-H]^-$ m/z 458.0849). This latter compound is the most abundant catechin found in green tea. Increasing evidence has shown its beneficiais effect such as antioxidant, antibacterial, anticancer, antiangiogenic, antidiabetic. For these reasons, it has excellent potential for use as functional ingredients to enrich food products [51].

A study with Camu-camu powder also identified most of the phenolic compounds found in the aqueous extract cited above [48]. Another study also quantified some of the phenolic content in Camu-camu pulp like gallic acid, p coumaric, myricetin, and quercetin, but found no significant values for chlorogenic acid [52]. Ref. [1], used cloud point to extract phenolic compounds in Camu-camu agro-industrial residue and detected by HPLC the presence of gallic acid, (+) – catechin, vanillic acid, serum acid, vanillin and quercetin in the coacervated phase, in the diluted phase the components were not identified.

Myricetin, Cianydin-3-glucoside, *p*-coumaric acid, rutin and gallic acid were the major compounds quantified by HPLC/DAD UV vis (Table 2). All samples showed significant difference ($p < 0.05$) among themselves (CS, OE and CC), except for gallic acid. The concentrated sample (CC) when compared with the initial extract (CS) showed 4.0, 4.3- and 4.5-times higher levels of rutin, gallic acid and cyanydin-3-glucoside respectively. Carmo et al [49] evaluated the total phenolic content in different Camu-camu seed extracts. For extraction in 100% water, 18.48 mg/100 g of gallic acid, 4.03 mg/100 g of cyanidyn-3-glucoside and 4.31 mg/100 g ellagic acid were obtained. [37] also reported for aqueous Camu-camu seed extract the quantification of some phenolic compounds such as gallic acid (12.57 mg/100 g), p-coumaric acid (3.42 mg/100 g) and rutin 3.46 (mg/100 g).

4. Conclusion

The combination of ultrasound assisted extraction and concentration by reverse osmosis, is a clean and viable technology for extraction, since it improves availability and concentration of bioactive substances in Camu-camu extract.

The final process resulted in higher level products, when compared to the initial extract (CS), total phenolics were 3.3 times higher; anthocyanins 6.0 times higher, and antioxidant activity on average 4.0 times higher, highlighting vitamin C, 7.0 times higher and cyanidin-3-glucoside 4.5 times higher than the initial extract (CS).

Finally, a Camu-camu (CC) concentrate with high antioxidant activity and rich in phenolic compounds was obtained. This concentrate is functional and feasible for use by food, nutraceuticals and cosmetic industries. Also, a suggestion for future works, this concentrate has potential applications as natural dye as an alternative to the use of synthetic dyes.

Declaration of Competing Interest

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

Acknowledgments

The authors acknowledge the financial support from CAPES scholarship, and CNPQ.

We are grateful for the support provided by the Central Research Support Complex (COMCAP) of the State University of Maringa (UEM).

References

[1] C.E. de Araújo Padilha, J.C.S. de Azevedo, F.C. de Sousa, S.D. de Oliveira, D.F. de S. Souza, J.A. de Oliveira, G.R. de Macedo, E.S. dos Santos, Recovery of polyphenols from camu-camu (Myrciaria dubia H.B.K. McVaugh) depulping residue by cloud point extraction, Chinese J. Chem. Eng. 26 (2018) 2471–2476. https://doi.org/10.1016/j.cjche.2017.10.032.

[2] M.S. Akter, S. Oh, J.B. Eun, M. Ahmed, Nutritional compositions and health promoting phytochemicals of camu-camu (myrciaria dubia) fruit: a review, Food Res. Int. 44 (2011) 1728–1732, https://doi.org/10.1016/j.foodres.2011.03.045.

[3] M.C. Pereira, R.S. Steffens, A. Jablonski, P.F. Hertz, A. De, O. Rios, M. Vizzotto, S.H. Flôres, Characterization and antioxidant potential of Brazilian fruits from the Myrtaceae family, J. Agric. Food Chem. 60 (2012) 3061–3067, https://doi.org/10.1021/jf205263f.

[4] M.C.E.V. Schiassi, V.R. de Souza, A.M.T. Lago, L.G. Campos, F. Queiroz, Fruits from the Brazilian Cerrado region: physico-chemical characterization, bioactive compounds, antioxidant activities, and sensory evaluation, Food Chem. 245 (2018) 305–311, https://doi.org/10.1016/j.foodchem.2017.10.104.

[5] E.C.E. Cunha-Santos, J. Viganó, D.A. Neves, J. Martínez, H.T. Godoy, Vitamin C in camu-camu [Myrciaria dubia (H.B.K.) McVaugh]: evaluation of extraction and analytical methods, Food Res. Int. 115 (2019) 160–166, https://doi.org/10.1016/j.foodres.2018.08.031.

[6] S.W. Kim, M.J. Ko, M.S. Chung, Extraction of the flavonol quercetin from onion waste by combined treatment with intense pulsed light and subcritical water extraction, J. Clean. Prod. 231 (2019) 1192–1199, https://doi.org/10.1016/j.jclepro.2019.05.280.

[7] M. Castro-Puyana, M.L. Marina, M. Plaza, Water as green extraction solvent: Principles and reasons for its use, Curr. Opin. Green Sustain. Chem. 5 (2017) 31–36, https://doi.org/10.1016/j.cogsc.2017.03.009.

[8] 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.

[9] F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560, https://doi.org/10.1016/j.ultsonch.2016.06.035.

[10] E.J. Klein, K.A. Santos, F. Palú, M.G.A. Vieira, E.A. da Silva, Use of supercritical CO2 and ultrasound-assisted extractions to obtain A/B-amyrin-rich extracts from uvaia leaves (Eugenia pyriformis Cambess.), J. Supercrit. Fluids. 137 (2018) 1–8. https://doi.org/10.1016/j.supflu.2018.02.019.

[11] G.D. Arend, W.T. Adorno, K. Rezzadori, M. Di Luccio, V.C. Chaves, F.H. Reginatto, J.C.C. Petrus, Concentration of phenolic compounds from strawberry (Fragaria X ananassa Duch) juice by nanofiltration membrane, J. Food Eng. 201 (2017) 36–41,

https://doi.org/10.1016/j.jfoodeng.2017.01.014.
[12] C. Conidi, A. Cassano, F. Caiazzo, E. Drioli, Separation and purification of phenolic compounds from pomegranate juice by ultrafiltration and nanofiltration membranes, J. Food Eng. 195 (2017) 1–13, https://doi.org/10.1016/j.jfoodeng.2016.09.017.
[13] W. Kujawski, A. Sobolewska, K. Jarzynka, C. Güell, M. Ferrando, J. Warczok, Application of osmotic membrane distillation process in red grape juice concentration, J. Food Eng. 116 (2013) 801–808, https://doi.org/10.1016/j.jfoodeng.2013.01.033.
[14] M. Antónia Nunes, S. Pawlowski, A.S.G. Costa, R.C. Alves, M.B.P.P. Oliveira, S. Velizarov, Valorization of olive pomace by a green integrated approach applying sustainable extraction and membrane-assisted concentration, Sci. Total Environ. 652 (2019) 40–47, https://doi.org/10.1016/j.scitotenv.2018.10.204.
[15] P.O. Bagci, M. Akbas, H.A. Gulec, U. Bagci, Coupling reverse osmosis and osmotic distillation for clarified pomegranate juice concentration: Use of plasma modified reverse osmosis membranes for improved performance, Innov. Food Sci. Emerg. Technol. 52 (2019) 213–220, https://doi.org/10.1016/j.ifset.2018.12.013.
[16] S. de Souza Comapa, L.M.S. Carvalho, C.V. Lamarão, F. das C. do A. Souza, J.P.L. Aguiar, L.S. da Silva, J.M. Mar, E.A. Sanches, F.F. dos Santos, J. de Araújo Bezerra, P.H. Campelo, Microwave processing of camu-camu juices: Physicochemical and microbiological parameters, J. Food Process. Preserv. 43 (2019) 1–11. https://doi.org/10.1111/jfpp.13989.
[17] A.L.R. Souza, M.M. Pagani, M. Dornier, F.S. Gomes, R.V. Tonon, L.M.C. Cabral, Concentration of camu-camu juice by the coupling of reverse osmosis and osmotic evaporation processes, J. Food Eng. 119 (2013) 7–12, https://doi.org/10.1016/j.jfoodeng.2013.05.004.
[18] AOAC, Official method of Analysis, 18th ed., Washington, 2005.
[19] C.W.I. Haminiuk, M.S.V. Plata-Oviedo, G. de Mattos, S.T. Carpes, I.G. Branco, Extraction and quantification of phenolic acids and flavonols from Eugenia pyriformis using different solvents, J. Food Sci. Technol. 51 (2014) 2862–2866, https://doi.org/10.1007/s13197-012-0759-z.
[20] Z. Pan, W. Qu, H. Ma, G.G. Atungulu, T.H. McHugh, Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel, Ultrason. Sonochem. 19 (2012) 365–372, https://doi.org/10.1016/j.ultsonch.2011.05.015.
[21] F.C. Cianci, L.F.M. Silva, L.M.C. Cabral, V.M. Matta, Clarificação e concentração de suco de caju por processos com membranas, Ciência e Tecnol. Aliment. 25 (2005) 579–583, https://doi.org/10.1590/s0101-20612005000300030.
[22] V.L. Singleton, J.A. Rossi Jr, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, Am. J. Enol. Vitic. 16 (3) (1965) 144–158.
[23] W.S. Pierpoint, The extraction of enzymes from plant tissues rich in phenolic compounds, Methods Mol. Biol. 244 (2004) 65–74.
[24] L.C. Neves, V.X. da Silva, J.A. Pontis, A. Flach, S.R. Roberto, Bioactive compounds and antioxidant activity in pre-harvest camu-camu [Myrciaria dubia (H.B.K.), Mc Vaugh] fruits, Sci. Hortic. (Amsterdam) 186 (2015) 223–229, https://doi.org/10.1016/j.scienta.2015.02.031.
[25] W.R. Lee J, Durst RW, Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study., J. AOAC Int. 88(5) (2005) 1269–1278.
[26] M. Alothman, R. Bhat, A.A. Karim, Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents, Food Chem. 115 (2009) 785–788, https://doi.org/10.1016/j.foodchem.2008.12.005.
[27] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D. Hawkins Byrne, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, J. Food Compos. Anal. 19 (2006) 669–675. https://doi.org/10.1016/j.jfca.2006.01.003.
[28] N. Nenadis, L.F. Wang, M. Tsimidou, H.Y. Zhang, Estimation of scavenging activity of phenolic compounds using the ABTS.+ assay, J. Agric. Food Chem. 52 (2004) 4669–4674, https://doi.org/10.1021/jf0400056.
[29] R. Pulido, L. Bravo, F. Saura-Calixto, Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay, J. Agric. Food Chem. 48 (2000) 3396–3402, https://doi.org/10.1021/jf9913458.
[30] T. Luiza, C. Oldoni, N. Merlin, M. Karling, S. Teresinha, S. Matias, D. Alencar, R. Gustavo, F. Morales, E. Aparecido, E. Jorge, Bioguided extraction of phenolic compounds and UHPLC-ESI-Q-TOF-MS/MS characterization of extracts of Moringa oleifera leaves collected in Brazil, Food Res. Int. 125 (2019) 108647, , https://doi.org/10.1016/j.foodres.2019.108647.
[31] F. Giusti, G. Caprioli, M. Ricciutelli, S. Vittori, G. Sagratini, Determination of fourteen polyphenols in pulses by high performance liquid chromatography-diode array detection (HPLC-DAD) and correlation study with antioxidant activity and colour, Food Chem. 221 (2017) 689–697, https://doi.org/10.1016/j.foodchem.2016.11.118.
[32] M.L. Grigio, E.A. Chagas, B. Rathinasabapathi, P. Cardoso, A. Raniely, S. Thiely, M. Sobral, R. Rodrigues, Qualitative evaluation and biocompounds present in different parts of camu-camu (Myrciaria dubia) fruit, 11 (2017) 124–129. https://doi.org/10.5897/AJFS2016.1574.
[33] A. Fujita, V.B. Souza, L.D. Daza, C.S. Fávaro-Trindade, D. Granato, M.I. Genovese, Effects of Spray-Drying Parameters on In Vitro Functional Properties of Camu-Camu (Myrciaria dubia Mc. Vaugh): A Typical Amazonian Fruit, J. Food Sci. 82 1083–1091 (2017), https://doi.org/10.1111/1750-3841.13668.
[34] N. Ilaiyaraja, K.R. Likhith, G.R. Sharath Babu, F. Khanum, Optimisation of extraction of bioactive compounds from Feronia limonia (wood apple) fruit using response surface methodology (RSM), Food Chem. 173 (2015) 348–354, https://doi.org/10.1016/j.foodchem.2014.10.035.
[35] S. Chakraborty, R. Uppaluri, C. Das, na of, Institution of Chemical Engineers, 2020. https://doi.org/10.1016/j.fbp.2020.01.003.
[36] F. das C. do Amaral Souza, L. Gomes Sanders Moura, K. de Oliveira Bezerra, J. Paiva Lopes Aguiar, J. Moreira Mar, E.A. Sanches, F.F. dos Santos, A.M. Bakry, B. Nicolau Paulino, P.H. Campelo, Thermosonication applied on camu–camu nectars processing: Effect on bioactive compounds and quality parameters, Food Bioprod. Process. 116 (2019) 212–218. https://doi.org/10.1016/j.fbp.2019.06.003.
[37] M. Fidelis, J.S. Santos, G.B. Escher, M. Vieira do Carmo, L. Azevedo, M. Cristina da Silva, P. Putnik, D. Granato, In vitro antioxidant and antihypertensive compounds from camu-camu (Myrciaria dubia McVaugh, Myrtaceae) seed coat: A multivariate structure-activity study, Food Chem. Toxicol. 120 (2018) 479–490. https://doi.org/10.1016/j.fct.2018.07.043.
[38] N. Del Socorro Cruz-Cansino, E. Ramírez-Moreno, J.E. León-Rivera, L. Delgado-Olivares, E. Alanís-García, J.A. Ariza-Ortega, J. De Jesús Manríquez-Torres, D.P. Jaramillo-Bustos, Shelf life, physicochemical, microbiological and antioxidant properties of purple cactus pear (Opuntia ficus indica) juice after thermoultrasound treatment, Ultrason. Sonochem. 27 (2015) 277–286, https://doi.org/10.1016/j.ultsonch.2015.05.040.
[39] L. dos S. Sousa, B.V. Cabral, G.S. Madrona, V.L. Cardoso, M.H.M. Reis, Purification of polyphenols from green tea leaves by ultrasound assisted ultrafiltration process, Sep. Purif. Technol. 168 (2016) 188–198. https://doi.org/10.1016/j.seppur.2016.05.029.
[40] N.K. Rastogi, Reverse Osmosis and Forward Osmosis for the Concentration of Fruit Juices, Elsevier Inc., 2018. https://doi.org/10.1016/B978-0-12-802230-6.00013-8.
[41] E. Arellano-Acuña, I. Rojas-Zavaleta, L. Paucar-Menacho, Camu-camu (Myrciaria dubia): Tropical fruit of excellent func- tional properties that help to improve the quality of life, Sci. Agropecu. 7 (2016) 433–443 https://doi.org/10.17268/sci.agropecu.2016.04.08.
[42] Maria do Socorro M. Rufino, Ricardo E. Alves, Edy S. de Brito, Jara Pérez-Jiménez, Fulgencio Saura-Calixto, Jorge Mancini-Filho, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil, Food Chemistry 121 (4) (2010) 996–1002, https://doi.org/10.1016/j.foodchem.2010.01.037.
[43] H. Ogata, S. Goto, K. Sato, W. Fujibuchi, H. Bono, M. Kanehisa, KEGG: Kyoto encyclopedia of genes and genomes, Nucleic Acids Res. 27 (1999) 29–34, https://doi.org/10.1093/nar/27.1.29.
[44] K. Degtyarenko, P. De matos, M. Ennis, J. Hastings, M. Zbinden, A. Mcnaught, R. Alcántara, M. Darsow, M. Guedj, M. Ashburner, ChEBI: A database and ontology for chemical entities of biological interest, Nucleic Acids Res. 36 (2008) 344–350. https://doi.org/10.1093/nar/gkm791.
[45] S. Kim, P.A. Thiessen, E.E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B.A. Shoemaker, J. Wang, B. Yu, J. Zhang, S.H. Bryant, PubChem substance and compound databases, Nucleic Acids Res. 44 (2016) D1202–D1213, https://doi.org/10.1093/nar/gkv951.
[46] L. Zhang, Y. Li, Y. Liang, K. Liang, F. Zhang, T. Xu, M. Wang, H. Song, X. Liu, B. Lu, Determination of phenolic acid profiles by HPLC-MS in vegetables commonly consumed in China, Food Chem. 276 (2019) 538–546, https://doi.org/10.1016/j.foodchem.2018.10.074.
[47] C.F. Zanatta, E. Cuevas, F.O. Bobbio, P. Winterhalter, A.Z. Mercadante, Determination of anthocyanins from Camu-camu (Myrciaria dubia) by HPLC-PDA, HPLC-MS, and NMR, J. Agric. Food Chem. 53 (2005) 9531–9535, https://doi.org/10.1021/jf051357v.
[48] D. Fracassetti, C. Costa, L. Moulay, F.A. Tomás-Barberán, Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (Myrciaria dubia), Food Chem. 139 (2013) 578–588, https://doi.org/10.1016/j.foodchem.2013.01.121.
[49] M.A.V. Do Carmo, M. Fidelis, C.G. Pressete, M.J. Marques, A.M. Castro-Gamero, T. Myoda, D. Granato, L. Azevedo, Hydroalcoholic Myrciaria dubia (camu-camu) seed extracts prevent chromosome damage and act as antioxidant and cytotoxic agents, Food Res. Int. 125 (2019) 108551, https://doi.org/10.1016/j.foodres.2019.108551.
[50] N. Conceição, B.R. Albuquerque, C. Pereira, R.C.G. Corrêa, C.B. Lopes, R.C. Calhelha, M.J. Alves, L. Barros, I.C.F.R. Ferreira, By-Products of Camu-Camu [Myrciaria dubia (Kunth) McVaugh] as Promising Sources of Bioactive High Added-Value Food Ingredients: Functionalization of Yogurts, Molecules. 25 (2019) 1–17, https://doi.org/10.3390/molecules25010070.
[51] W. Chen, W. Wang, X. Ma, R. Lv, R. Balaso Watharkar, T. Ding, X. Ye, D. Liu, Effect of pH-shifting treatment on structural and functional properties of whey protein isolate and its interaction with (−)-epigallocatechin-3-gallate, Food Chem. 274 (2019) 234–241, https://doi.org/10.1016/j.foodchem.2018.08.106.
[52] G.A. Bataglion, F.M.A. Da Silva, M.N. Eberlin, H.H.F. Koolen, Determination of the phenolic composition from Brazilian tropical fruits by UHPLC-MS/MS, Food Chem. 180 (2015) 280–287, https://doi.org/10.1016/j.foodchem.2015.02.059.

LWT - Food Science and Technology 138 (2021) 110785

Contents lists available at ScienceDirect

LWT

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

Uvaia (*Eugenia pyriformis Cambess)* residue as a source of antioxidants: An approach to ecofriendly extraction

Letícia Misturini Rodrigues [a,*], Edilson Bruno Romanini [a], Evandro Silva [b], Eduardo Jorge Pilau [b], Silvio Cláudio Da Costa [c], Grasiele Scaramal Madrona [d]

[a] Department of Food Science, State University of Maringá, Av. Colombo 5790, CEP 87020-900, Maringá, PR, Brazil
[b] Laboratory of Biomolecules and Mass Spectrometry, Department of Chemistry, State University of Maringá, 5790, Av. Colombo 5790, CEP 87020-900, Maringá, PR, Brazil
[c] Department of Biochemistry, State University of Maringá, Av. Colombo 5790, CEP 87020-900, Maringá, PR, Brazil
[d] Department of Food Engineering, State University of Maringa, Av. Colombo 5790, CEP 87020-900, Maringa, PR, Brazil

ARTICLE INFO

Keywords:
Eugenia pyriformis Cambess
Phenolic compounds
Antioxidant activity
Flavonoids
UHPLC-QTOF-MS/MS

ABSTRACT

Aqueous ultrasound assisted extraction, followed by membrane concentration, was first used to concentrate the bioactive compounds of Uvaia residue (seed and peel). The extract, obtained under ideal conditions of 40 °C, 2.5 min, and 40% of amplitude, was concentrated using reverse osmosis, and presented relatively low fouling (39%). At the end of the sequential processes, a concentrate (CF) was obtained with an increase of 6.2 times for phenolics (332.22 mg GAE/100 g), and 7.8 times for total flavonoids (1300.18 mg/100 g) compared to the initial extract (RS). A significant increase was observed by HPLC-DAD/UV–vis for the concentration of compounds. Fifteen compounds were putatively identified by UHPLC-QTOF-MS/MS. The obtained concentrate was produced naturally, which can be used as a source of antioxidants in the development of functional food formulations, nutraceuticals or also by pharmaceutical industries.

1. Introduction

Uvaia (*Eugenia pyriformis Cambess*) is also known as Uvalha, Uvaia do mato, or Uvalheira, and belongs to the Myrtaceae family. This plant is native to the Atlantic forest and can also be found in the states of São Paulo, and Rio Grande do Sul- BR. The size of the round, juicy but sour fruits varies from 2.0 to 2.4 cm. The peel color varies from yellow to orange and may have a smooth or velvety texture. Its thin peel makes the fruit highly susceptible to mechanical damage (Jacomino, da Silva, de Freitas & Morais, 2018; de Paulo Farias, Neri-Numa, de Araújo, & Pastore, 2020). The weight of the fruit is about 8.5 g, with the seeds corresponding to approximately 16% of the total weight of the fruit (de Paulo Farias et al., 2020; Klein, Santos, Palú, Vieira, & da Silva, 2018). Several phenolic compounds have been isolated from Uvaia, among which are the flavonoids (querecetin, kaempferol, and rutin) and phenolic acids (gallic, chlorogenic, and caffeic acids) (Windson, Haminiuk, & Plata-oviedo, 2014).

Food, pharmaceutical, and cosmetic industries have focused their attention on polyphenols that are found in the pulp, peels, and leaves of vegetables as these compounds exhibit high antioxidant activities and are hence effective against reactive oxygen species. In addition, the antiviral, antibacterial, and antifungal activities of these molecules in fruits help in the defense mechanism of cells wherein they minimize the damage caused by free radicals (Sganzerla et al., 2018).

Hence, the extraction of solid samples using solvents has been performed to obtain products with added value. Among the various extraction techniques (maceration and soxhlet) employed, ecofriendly extraction techniques are being widely used currently, such as microwave-assisted extraction, ultrasound-assisted extraction (UAE), subcritical extraction, and extraction using supercritical carbon dioxide. The use of ultrasonic waves to extract unstable and thermolabile photoconstituents has been shown to be the best alternative. The efficiency of sonication extraction is mainly because of the action of cavitation, and mechanical and thermal effects that occur during the flow of ultrasonic waves, which facilitate mass transfer, cell wall degradation, and reduction in fragment size (Mahindrakar & Rathod, 2020; Zhang et al., 2015). In addition, this unconventional method reduces extraction time, temperature, and solvent consumption, favoring higher extraction

* Corresponding author. CP 87020-900, Maringá, Paraná, Brazil.
E-mail addresses: leticia_misturini@hotmail.com, leticia_misturini@hotmail.com (L.M. Rodrigues), brunoromanini84@gmail.com (E.B. Romanini), evandroas20@gmail.com (E. Silva), epilau@gmail.com (E.J. Pilau), sccosta@uem.br (S.C. Da Costa), grasiele@yahoo.com (G.S. Madrona).

https://doi.org/10.1016/j.lwt.2020.110785
Received 21 August 2020; Received in revised form 24 November 2020; Accepted 15 December 2020
Available online 17 December 2020

efficiency, and reduced energy consumption compared to conventional techniques (Fu, Belwal, Cravotto, & Luo, 2020; Ordoñez-santos, Martínez-girón, & Rodríguez-rodríguez, 2019).

In association with the UAE technique, reverse osmosis (RO) membrane separation can be applied to concentrate bioactive compounds in fruit juices, resulting in the conservation of nutritional and sensory characteristics. This is a promising technique as it promotes mild dehydration that consequently increases the content of total soluble solids (TSS) and bioactive compounds (Gunathilake, Yu, & Rupasinghe, 2014). The process of separation/concentration by RO is based on pressure; the higher the concentration of the product, the greater the increase in its osmotic pressure (Bagci, Akbas, Gulec, & Bagci, 2019).

A few studies have reported alternatives for reusing Uvaia fruit residues. Klein et al. (2018) evaluated two techniques (supercritical fluid extraction [SFE] and ultrasound-assisted extraction [UAE]) for extracting compounds from Uvaia leaves. Other studies evaluated the fruit pulp (da Silva et al., 2019, 2018; Pereira et al., 2012); the recovery of *Eugenia pyriformis* phenolic compounds using different solvents (Windson et al., 2014); and the nutritional, physicochemical, and antimicrobial characteristics of Uvaia pulp in three different extracts (Sganzerla et al., 2018).

The present study is the first to use a combination of two technologies (UAE and RO) to extract and concentrate bioactive compounds from Uvaia residues. Thus, the main objective was to investigate the use of clean technologies for the aqueous extraction of bioactive compounds from Uvaia peel and seed by UAE, followed by concentration by RO, and characterization of all the products identified.

2. Materials and methods

2.1. Samples, chemicals, and reagents

The solutions were prepared using analytical reagents and Milli-Q water for the required analyzes. Reagents for antioxidants (Trolox, TPTZ, DPPH, Folin-Ciocalteu reagent), were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ascorbic acid, gallic acid, ellagic acid, quercetin, rutin, p-coumaric acid, chlorogenic acid, and myricetin were the standards used that were also obtained from Sigma-Aldrich. All reagents and solvents used were of analytical or HPLC grade.

2.2. Residue sample and chemical analyses

Uvaia residues (peel and seed) were purchased from Sitio do Belo (Paraibuna, SP, Brazil) from the November 2018 harvest and frozen (−18 °C) until use.

To determine the residue composition, the samples were ground together, and the following physicochemical analyses were performed: total soluble solids (° brix) using an HI96801 digital refractometer (Hanna Instruments); pH, moisture, ash and protein content (micro-Kjeldahl method), lipid and carbohydrate content by difference (AOAC, 2005); and color parameters using the Minolta® CR400 portable colorimeter. All analyses were performed in triplicate.

2.3. Phenolic compounds extraction

The residue (50 g) was diluted in water (200 mL) (Windson et al., 2014) to obtain a denominated fresh extract (RS). A central composite design was used based on the factors defined after the preliminary tests and according to the literature (Rodrigues et al., 2020). Table 1 details the sonication time (X1 = 2.5, 6.25, and 10 min), frequency (X2 = 20, 30, and 40%), and temperature (X3 = 40, 60, and 50 °C) with eight factorial points and three repetitions in the central points. The response variable was the concentration of phenolic compounds that was determined based on the response surface (Statistic 7.0 program).

Extraction was performed by probe ultrasound with a power of 750 W and a frequency of 20 KHz (Collective Parmer 750-Watt Ultrasonic Processors). Using the automatic amplitude integrated in the equipment, amplitude variation for extraction at the desired level (0–40%, 0–20 kHz) could be performed, allowing the adjustment of ultrasonic vibrations through the probe tip (titanium, 13 mm) that was inserted directly into a 250 mL sample used per test.

Table 1
Results of total phenolic content at different extraction conditions according to a central composite design.

Run	Extraction conditions		Response	
	Time (min)	Amplitude (%)	Temperature (°C)	TPC (mg/100 g)
1	2.5	20	40	56.556
2	10	20	40	58.489
3	2.5	40	40	57.489
4	10	40	40	57.311
5	2.5	20	60	58.356
6	10	20	60	56.556
7	2.5	40	60	58.867
8	10	40	60	58.556
9	6.25	30	50	56.333
10	6.25	30	50	56.289
11	6.25	30	50	56.622

TPC - total phenolic compounds.

All extractions were pulsated (5 s pause and 5 s pulse) according to preliminary tests and literature (Pan, Qu, Ma, Atungulu, & McHugh, 2012; Rodrigues et al., 2020).

2.4. Reverse osmosis

The extract obtained under the optimized conditions was cooled to 25 °C and transferred to a filtration system (0.45 μm) to remove larger particles. In sequence, the extract was concentrated using an R25A membrane, 500 Da, polyamide, pressure of 5 bar, and filtration area of 3 sq. feet. The process was conducted at room temperature (25 °C), and the flow rate was monitored until stabilization. Then, the process was stopped, and the final concentrate was obtained (CF).

The flow was calculated using the weight of the concentrate collected and measured at different time intervals, with the flow data of distilled water in the clean membrane and the stable flow in the dirty membrane. Thus, the percentage of fouling (% F) was calculated according to equation (1), using the data collected during the concentration process. The volumetric concentration factor (VCF) was determined using the feed and permeate volumes (Cianci, Silva, Cabral, & Matta, 2005).

$$\%F = \frac{(Ji - Jf)}{Ji} X100 \quad \text{Eq. (1)}$$

In sequence, the fresh extract (RS), the extract obtained by ultrasonic extraction (RE), and the final concentrate (CF) were analyzed. The sequential process is described in Fig. 1.

2.5. Bioactive compounds

2.5.1. Total phenolic compounds

Total phenolic compounds (TPC) were determined using the Folin-Ciocalteu reagent method, as described previously (Singleton & Rossi, 1965). The obtained values were compared with the gallic acid calibration curve ($R^2 = 0.99$). Results were expressed in milligrams of gallic acid equivalent (mg GAE) · 100 g^{-1} of fresh residue.

2.5.2. Total carotenoids

The carotenoid analysis was performed according to Lichtenthaler (Lichtenthaler, 1987), wherein a 2 mL sample of the extract was mixed with 18 mL of 80% acetone. The mixture was then filtered using a filter paper in the absence of light and read sequentially on a spectrophotometer at an absorbance of 647, 663, and 470 nm. The concentration was calculated according to the following equation.

$$C_{(x+c)}\ (\mu g/mL) = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198 \quad \text{Eq. (2)}$$

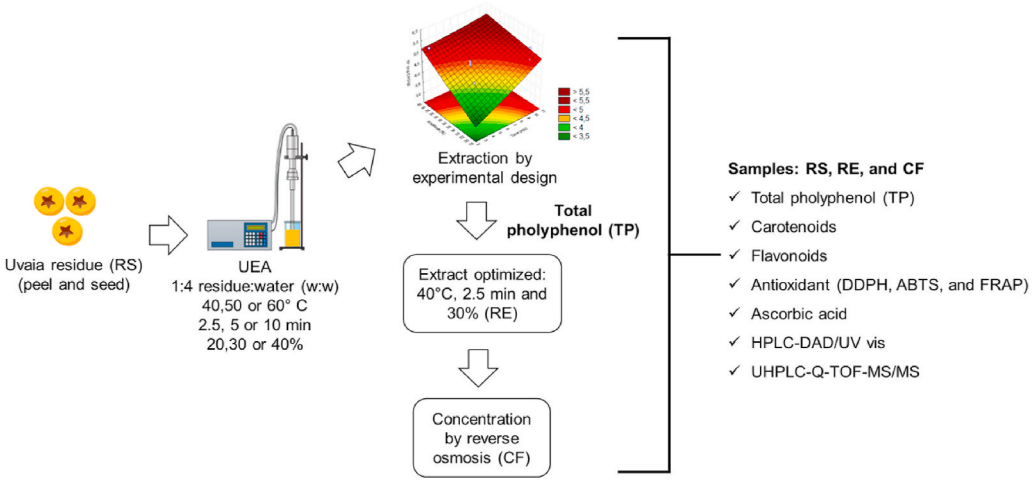

Fig. 1. Sequential process for Uvaia residue concentration.

Where Ca (μg/mL) = 12.25 A663–2.79 A647; Cb (μg/mL) = 21.50 A646–5.10 A663.

Carotenoid concentration was expressed in μg/100 g of fr. All analyses were performed in triplicate.

2.5.3. Total flavonoids

Total flavonoids (TF) were determined by performing a spectrophotometric assay (Alothman, Bhatm & Karim, 2009). The results are expressed in mg of quercetin equivalent (QE) · 100 g^{-1} fr.

2.5.4. Vitamin C

Vitamin C (VC) content was determined by high-performance liquid chromatography, as described previously (Rodrigues et al., 2020). The quantification was based on the peak area with results expressed in mg/g^{-1} fr by applying the standard curve (Y = 4.84eˆ006X-4.02eˆ005, R^2 = 0.998106).

2.6. Antioxidant capacity (ABTS, DPPH and FRAP)

Antioxidant capacity was determined by using three methods and measuring the free radical-scavenging capacity.

Antioxidant activity was determined using the ABTS (2,2′ azinobis (3 ethylbenzothiazoline-6-sulfonic acid) and a previously reported colorimetric assay (Nenadis, Wang, Tsimidou, & Zhang, 2004).

Reduction in the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyla) was measured according to the colorimetric method at 515 nm (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006).

For performing the ferric reducing antioxidant power (FRAP) assay, the extracts were mixed with distilled water and the FRAP reagent, incubated for 30 min at 37 °C in a water bath, and spectrophotometric analysis was performed at 595 nm (Pulido, Bravo, & Saura-Calixto, 2000).

The results were expressed in mmol of Trolox equivalent (TE). g^{-1} fr. All analyses were performed in triplicate.

2.7. Chemical characterization of bioactive compounds by UHPLC-Q-TOF-MS/MS and quantification by HPLC-DAD/UV vis

Samples of RS, RE, and CF extracts were resuspended in 500 μL water:acetonitrile (1:1; v-v) and 3 μL of each extract were injected and analyzed using ultra-high performance liquid chromatography (Shimadzu, Nexera X2, Japan) coupled to a hybrid quadrupole time-of-flight high-resolution mass spectrometer (Impact II, Bruker Daltonics Corporation, Germany) that was equipped with an electrospray ionization source. Chromatographic separation conditions were determined using an Acquity UPLC® BEH C18 column packed with 135 Å pore and 1.7 μm particle size, and a 2.1 × 100 mm column (Waters, USA) at a flow rate of 0.250 mL min^{-1}. The gradient mixture of solvents A (H_2O with 0.1% formic acid; v:v) and B (acetonitrile with 0.1% formic acid; v-v) was as follows: 5% B 0–1 min, 70% B 1–5 min, 98% B 5–15 min, and maintained at 5% B 16–20 min at 40 °C. The mass spectrometer was calibrated using a solution of sodium formate (10 mmol L^{-1}; isopropanol: water; 1:1; v-v) containing 50 μL concentrated formic acid. The capillary voltage was operated in positive ionization mode, set at 4500, with an end plate offset potential of −500 V. The dry gas parameters were set to 8 L min^{-1} at 180 °C with a nebulization gas pressure of 4 bar. Data were collected from m/z 50 to 1300 with an acquisition rate of 5 Hz, and the 5 ions of interest were selected by auto MS/MS scan fragmentation (de Almeida et al., 2018).

Compounds were quantified by HPLC-DAD/UV Vis, as described previously (Rodrigues et al., 2020).

2.8. Statistical analysis

All analyzes were subject to analysis of variance and Tukey test ($p < 0.05$) using the Sisvar 5.6 statistical program, and the standard curves for the antioxidant tests were plotted using the GraphPad Prism 5 program. The results were reported as mean ± standard deviation, and an experimental design was developed using Statistic 7.0.

3. Results and discussion

3.1. Residue initial characterization

The residues exhibited on an average, a total soluble solid (TSS) content of 2.70 ± 0.058° Brix, pH = 3.93, moisture content of approximately 76.0 ± 1.000 and 0.31 ± 0.015 ash, protein content of 2.66 ± 0.006, lipid content of 0.35 ± 0.006, and carbohydrate content of 20.99 g/100. Regarding the color of the whole fruit, brightness (L) was 58.84 ± 0.666, the tendency for red (a*) was 2.83 ± 0.706, and that for yellow (b*) was 39.05 ± 0.641.

A previous study evaluated the physicochemical characteristics, as well as the antioxidant potential of three fruits (namely yellow guava, Guabiroba, and Uvaia) that belong to the Myrtaceae family. To evaluate Uvaia, the authors used the pulp and peel as raw material and discarded the seeds, and obtained the values of 0.52 for the lipid content, 15.82 g/100 g dry matter for the protein content, 94.50 for the moisture content, and 0.23 g/100 g fresh matter for the ash content (Pereira et al., 2012). Some of these values are similar to those obtained in the present study,

although the sample (peel and seed) used was not the same.

3.2. Experimental design

The experimental results of total phenolic content (Table 1) of the Uvaia residue were considered as the response variable of the experiment. The values ranged from 56.289 to 58.867 mg of EAG/100 g of fr.

The three independent variables and the corresponding levels were as follows: sonication time (min) 2.5, 6.25, and 10 (X1), ultrasound amplitude (%) 20, 30, and 40 (X2), and temperature (°C) 40, 50, and 60 (X3). Fig. 2 (A-C) shows the response surface according to the effect of the variables. For the extraction of TP (Fig. 2 (A-C)), both time (in a linear way) and the interaction between X1 and X2 were not significant. On the other hand, time in the quadratic form was significant. The others (X2 and X3) were significant, and the interaction between amplitude (X2) and temperature (X3) had a positive effect, contrary to the effect of time (X1) and temperature (X3). The best UAE conditions extracting for TPC were defined using the ANOVA results and the critical values indicated by software Static 7.0 as follows: extraction time of 2.5 min, ultrasound amplitude of 40%, and extraction temperature of 40 °C.

Our study is the first to evaluate the extraction of Uvaia residue according to the cited conditions, and the extraction of phenolic compounds from the residue was facilitated by the action of temperature and ultrasound amplitude. Corroborated by literature, a previous study reported that this fact could be explained by the UAE technique (the ultrasonic waves act as a cavitation agent) that can be responsible for cell wall rupture; thus, favoring the penetration of the solvent and increasing the extraction rate. Temperature also significantly affected the extraction of these compounds, as it controls solubility and the mass transfer rate of target compounds in the solvent and during cavitation (Mahindrakar & Rathod, 2020).

Another similar study evaluated different extraction techniques, types of solvents, extraction time, and ultrasonic power for quantifying total phenolic and flavonoid content and antioxidant activity of *Mucuna macrocarpa* beans. For process optimization, ultrasound with water as a solvent yielded the best results. For the extraction of phenolic compounds, the best process conditions were 27.30 W of power for 12.57 min. The authors stated that the results obtained by the above optimization demonstrate the effectiveness of the ultrasonic treatment (Aware, Patil, Vyavahare, Gurme, & Jadhav, 2019).

The UAE extraction conducted previously (Chakraborty, Uppaluri, & Das, 2020) indicated that pulsed mode sonication was more efficient than the conventional one for the aqueous extraction of antioxidants, and total phenolic and soluble proteins from bitter gourd *Momordica charantia*, and response variables were addressed as a function of temperature variation and extraction time.

3.3. Concentration by reverse osmosis

In the RO, the permeate flow was evaluated as a function of operating time in 5 min intervals until 30 min, and ranged from 25.0 L/(h.m^2) to 14.0 L/(h.m^2), when the solids content reached 3.4° Brix for the CF.

The permeate flow was reduced during the process. According to the literature, this occurs because of concentration polarization (solute formation) and incrustations on the membrane surface, such as microbial adhesion, gel layer formation, and solute adhesion, which affect efficiency (Rastogi, 2018). Despite some restrictions for the use of RO, the advantages should be highlighted, such as obtaining high quality concentrates, low operating temperatures, operational simplicity, and energy efficiency, mainly resulting in the concentration of nutritional compounds (Gunathilake, 2020; Rastogi, 2018).

In the present study, the concentration factor was 7.02 times that of the feed, and membrane fouling level was relatively low (39%), which is

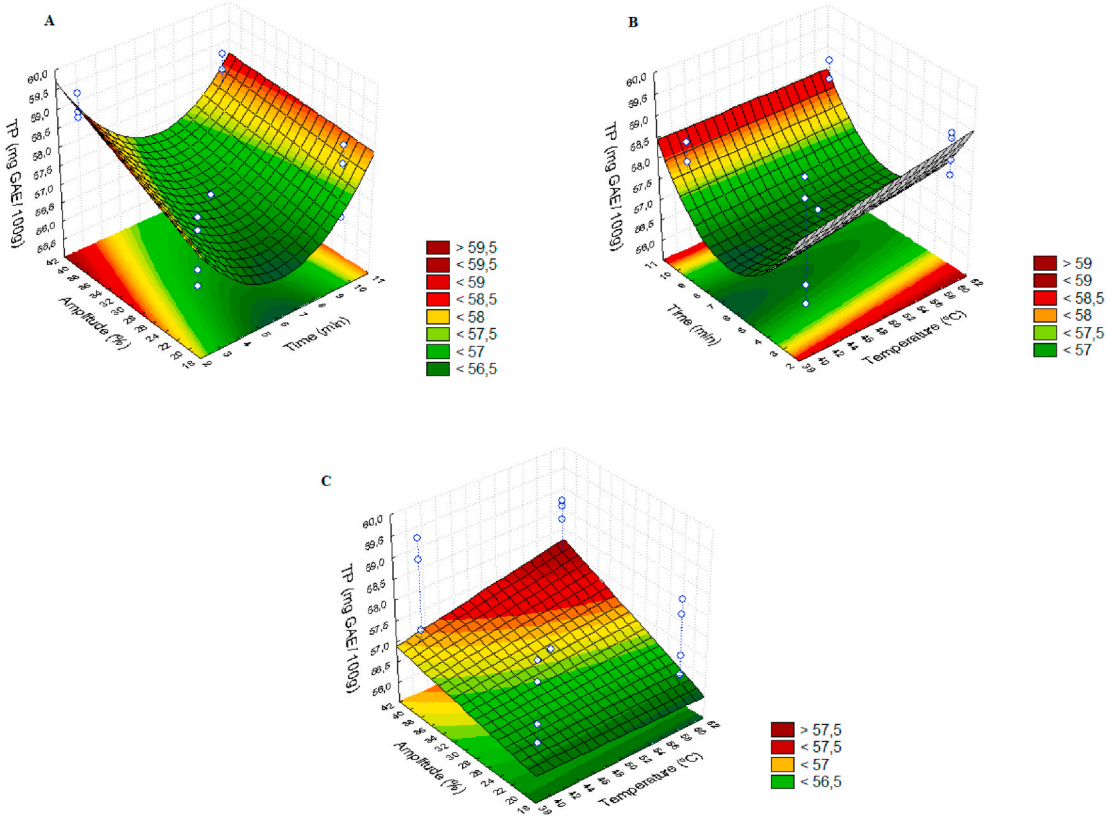

Fig. 2. Response surface between three variables (time, amplitude, and temperature) in the extraction of Total Phenolic compounds (TP mg GAE/100 g ff, A - C). (A, B, C) TP = $1.19 \times 1^2 + 0.27$ X2 + 0.40 X3 – $0.37 \times 1 \times 3 + 0.42 \times 2 \times 3$.

a positive point because fouling is a limiting factor in the membrane process. A previous study reported that membrane encrustation is usually caused by the deposition of colloidal particles, inorganic and organic compounds, and microbes on the surface, which affect membrane quality and reduce their utility (Jiang, Li, & Ladewig, 2017; Rastogi, 2018; Sousa, Cabral, Madrona, Cardoso & Reis, 2016).

In another study, authors used RO to concentrate bioactive compounds from the Camu-camu fruit, and the results proved the efficiency of the process, with low fouling index (19%) and permeate flow from 15.0 to 1.8 L/(h.m^2) for 48 min, resulting in a high concentration of bioactive compounds (Rodrigues et al., 2020). Another study also used aqueous extraction to extract sugars and phenolic compounds from carob kibbles and subsequently concentrated the sample by RO and dianofiltration. A concentrate was obtained with 4.0 times the amount of compounds studied using the RO membrane (Almanasrah et al., 2015), which is lower than that obtained in our study (concentrated extract of 7.02 times).

3.4. Bioactive compounds

The levels of TPC in the CF were 6.1 times higher (332.225 mg GAE/100 g) than the ultrasound extraction point (54.645 mg GAE/100 g), with a significant difference with respect to the other two samples (Table 2), thus making water a good choice for an ecofriendly phenolic compound extraction.

The literature reports different solvents used for the extraction of compounds from fruits (pulp and residue) and vegetables. Corroborating our study, the authors evaluated three different extracts (water, methanol, and ethanol) from 21 food residues from four different classes (fruits, vegetables, oilseeds, and drinks) and evaluated antioxidant activity by DPPH, as well as contents of phenols, flavonoids, and ascorbic acid. For polyphenol extraction, water was the most efficient, whereas ethanol extraction was more efficient for antioxidant activity. Among the fruit class, for TPC, an aqueous extract of pineapple peel (68.3 mg gallic acid/g), and plum pomace (51.8 mg gallic acid/g) was the highest, whereas the lowest was that for watermelon rind (15.1 mg gallic acid/g) The levels of total phenolic compounds in the concentrated sample (CF), were 6.1 times higher (332.225 mg GAE/100 g) than the ultrasound extraction point (54.645 mg GAE/100 g), with significant difference with respect to the other two samples (Table 2), being water a good choice for an ecofriendly phenolic compounds extraction (Kuppusamy, Venkateswarlu, & Megharaj, 2020).

Table 2
Bioactive compounds from different Uvaia residue samples (fr).

	RS	RE	CF
TPC (mg GAE/100 g)	52.951^{b} ± 0.391	54.645^{b} ± 0.146	332.225^{a} ± 3.520
Carotenoids (μg/100 g)	0.000^{b} ± 0.000	7.203^{b} ± 1.616	358.059^{a} ± 40.660
TF (mg QE/100 g)	167.267^{c} ± 20.285	360.741^{b} ± 19.217	1300.179^{a} ± 8.541
ABTS (mmol TE/g)	14.219^{b} ± 0.449	15.729^{b} ± 0.000	52.068^{a} ± 0.721
DPPH (mmol TE/g)	9.602^{b} ± 0.055	9.602^{b} ± 0.055	50.431^{a} ± 0.291
FRAP (mmol TE/g)	20.787^{b} ± 0.131	22.203^{b} ± 0.028	136.761^{a} ± 0.580
Vitamin C (mg/100 g)	3.540^{b} ± 0.028	3.360^{b} ± 0.000	4.420^{a} ± 0.141
Myricetin (mg/100 g)	0.191^{c} ± 0.000	0.205^{b} ± 0.000	0.323^{a} ± 0.002
Chlorogenic acid (mg/100 g)	0.182^{c} ± 0.000	0.254^{b} ± 0.000	0.654^{a} ± 0.000
Ellagic acid (mg/100 g)	1.859^{c} ± 0.000	2.466^{b} ± 0.000	9.407^{a} ± 0.000
p-coumaric acid (mg/100 g)	0.073^{a} ± 0.000	0.088^{a} ± 0.000	0.093^{a} ± 0.001
Quercetin (mg/100 g)	0.000^{b} ± 0.000	0.000^{b} ± 0.000	0.083^{a} ± 0.000
Rutin (mg/100 g)	0.112^{c} ± 0.000	0.228^{b} ± 0.000	2.243^{a} ± 0.000
Gallic acid (mg/100 g)	2.043^{b} ± 0.000	2.160^{b} ± 0.001	8.119^{a} ± 0.001

The data are expressed as means ± standard deviations. Means followed by the same letter in the lines do not differ significantly from each other by the Tukey test at a 5% significance level. RS = control sample, RE = ultrasound optimal extraction, CF = concentrated sample, TPC = total phenolic compounds, TF = total flavonoids, GAE = gallic acid equivalent, QE = quercetin equivalent, TE = Trolox equivalent.

Another study evaluated the extracts of peel and seeds from kinnow, litchi, grape and banana that were obtained by extraction with 70% water:methanol. Considering TPC, grape seed extract had the highest value (37.4 mg GAE/g-dw), and the lowest value was found for Kinnow seed (3.68 mg GAE/g-dw). The authors highlight that fruit residues are potential sources of antioxidants that can be used in the food and pharmaceutical industries (Babbar, Oberoi, Uppal, & Patil, 2011).

Regarding Uvaia, Lopes et al. (2018) evaluated the potential of *Eugenia uvalha Cambess* juice, and reported a value of 135.14 mg GAE/100 g for TPC, which is lower than that found in the present study for the CF. This difference can be explained by the use of juice and not residues.

The CF showed 49.5 times more carotenoids (358.059 μg/100 g) than the optimal extraction sample (RE) (7.203 μg/100 g) (Table 3). According to Zillo, Silva, Zanatta, and Spoto (2015) Uvaia has high carotenoid content, and in their study, they determined these levels using fresh Uvaia and frozen pulp (0.91 and 0.366 μg/g, respectively). A recent study also reported high levels of carotenoids for six Uvaia (*Eugenia pyriformis Cambess*) varieties (Common, Rugosa, Doce de Patos de Minas, Pêra, Rugosa Doce, and Dura), and the quantification for Common was 137.30 mg beta-carotene 100 g^{-1} fw (da Silva et al., 2019).

For flavonoids, RO favored the concentration of these compounds to almost 7.8 times more (1300.179 mg QE/100 g) than the control (167.267 mg QE/100 g), showing a significant difference ($p < 0.05$) between the three samples (Table 2). A study analyzed aqueous extracts of fruit residues and highlighted higher contents of flavonoids in pineapple peel (15.3 mg quercetin/g), watermelon rind (12.6 mg quercetin/g), and butternut peel (11.5 mg quercetin/g) (Kuppusamy et al., 2020).

For the analysis of antioxidant activity by ABTS, DPPH, and FRAP methods, the samples showed a significant difference ($p < 0.05$) only for the CF. The highest increase was observed in FRAP analysis, where the CF was 6.5 times greater (136.761 mmol TE/g) than the control sample (RS) (20.787 mmol TE/g).

Considering the levels of phenolic compounds quantified by HPLC/UV DAD vis (Table 2), there was greater recovery and concentration of rutin, ellagic acid, gallic acid, and chlorogenic acid. Except for gallic acid and myricetin, the three samples showed a significant difference ($p < 0.05$) between them (RS, RE, and CF). The concentration was increased by 20.0, 5.0, and 4.0 times for rutin, ellagic acid, gallic acid compared to the RS. Vitamin C could also be recovered from the residues and concentrated, and its level was 1.2 times (4.420 mg/100 g of fr.) in the CF, showing a significant difference compared to RS and RE. *P*-coumaric acid did not vary between samples, and only traces of quercetin were found in the CF.

In a study performed by Windson et al. (2014), the purée of Uvaia (*Eugenia pyriformis*) was used to evaluate the extraction of phenolic compounds and quantify the compounds by high-performance liquid chromatography (HPLC-DAD/UV) using different solvents. For aqueous extraction, levels of gallic acid, chlorogenic acid, p-coumaric acid, and rutin were (255.8; 33.4; 2.9; 0.83 mg/kg of FR), respectively. These values are different from the ones reported in the present study, probably because the authors used a purée of Uvaia.

In a recent study, different extraction techniques (UAE, soxhlet, and stirred batch) were evaluated to obtain bioactive compounds from *Syzygium cumini* kernel powder (SCSKP). In addition to performing TPC, TFC, and IC_{50} analyses, the study evaluated catechin and gallic acid yield by HPLC, and the highest levels of these two compounds was obtained by UAE (54.5 mg/g of gallic acid) (Mahindrakar & Rathod, 2020).

Table 3
Bioactive compounds of different Uvaia samples.

ID	Compound identify	RT (min)	Molecular Formula	$[M+H]^+$ Measuared	$[M+H]^+$ Accuracy	Error (ppm)	Sample			Class
							RS	RE	CF	
1	Ascorbic Acid	1.19	$C_6H_8O_6$	177.0394	177.0394	0.2	✓	✓	✓	Phenolic acids
2	p-Coumaric acid	1.23	$C_9H_8O_3$	165.0540	165.0546	−3.6	✓	✓	✓	Phenolic acids
3	Gallic acid	1.97	$C_7H_6O_5$	171.0288	171.0288	0.0	✓	✓	✓	Phenolic acids
4	Chlorogenic acid	3.95	$C_{16}H_{18}O_9$	355.1028	355.1024	1.2	✓	✓	✓	Phenolic acids
5	Dihydrokaempferol	3.62	$C_{15}H_{12}O_6$	289.0705	289.0707	−0.6	nd	nd	✓	Flavanonol
6	Epicatechin	3.63	$C_{15}H_{14}O_6$	291.0853	291.0863	−3.4	✓	✓	✓	Flavan-3-ol
7	Taxifolin	3.86	$C_{15}H_{12}O_7$	305.0647	305.0655	−2.6	✓	✓	✓	Flavanonol
8	Rutin	3.87	$C_{27}H_{30}O_{16}$	611.1584	611.1606	−3.6	✓	✓	✓	Flavonol
9	Myricetin	3.97	$C_{15}H_{10}O_8$	319.0449	319.0448	0.3	✓	✓	✓	Flavonol
10	Quercetin	4.00	$C_{15}H_{11}O_7$	303.0485	303.0499	−4.6	✓	✓	✓	Flavonol
11	Isoquercitin	4.00	$C_{21}H_{20}O_{12}$	465.1029	465.1027	0.4	✓	✓	✓	Flavonol
12	Quercetin-3-O-pentoside	4.17	$C_{20}H_{18}O_{11}$	435.0916	435.0922	−1.4	✓	✓	✓	Flavonol
13	Quercitrin	4.22	$C_{21}H_{20}O_{11}$	449.1076	449.1078	−0.4	✓	✓	✓	Flavonol
14	Kaempferol 3-O-alpha-L-rhamnoside	4.49	$C_{21}H_{20}O_{10}$	433.1121	433.1129	−1.8	nd	nd	✓	Flavonol
15	Bellidifolin-8-O-glucoside	3.83	$C_{20}H_{20}O_{11}$	437.1064	437.1078	−3.2	✓	✓	✓	Xanthone

*nd = not detect; RT-retention time; RS - control sample; RE - ultrasound optimal extraction; CF - concentrated sample.

3.5. Bioactive compound identification by UHPLC-Q-TOF-MS/MS

The compounds were putatively identified by interpreting the MS and MS/MS spectra results and using public mass spectra libraries such as GNPS, PubChem, and CheBI. Fifteen compounds were putatively identified that belonged to the following chemical classes: phenolic acids, flavonoids (flavonol, flavan-3-ol, and flavanonol), and xanthone, with a mass error range of 0–3.6 ppm (Table 3).

Four phenolic acids were detected in the Uvaia residue samples (RS, RF, and CF). Compound 1 with a retention time of 1.19 min showed the precursor ion $[M+H]^+$ *m/z* 177.0394 and was putatively identified as ascorbic acid (vitamin C). Compounds 2 (rt: 1.23 min), 3 (rt: 1.97 min), and 4 (rt: 3.95 min) showed precursor ions $[M+H]^+$ *m/z* 165.0540; $[M+H]^+$ *m/z* 171.0288 e; and $[M+H]^+$ *m/z* 355.1028, and were putatively identified as p-coumaric acid, gallic acid, and e-chlorogenic acid, respectively, belonging to the class of carboxylic acids. These compounds are antioxidants that can be used for the treatment of some chronic diseases related to oxidative stress as they exhibit anti-inflammatory, antibacterial, antiproliferative, and anticarcinogenic activities (de la Rosa, Moreno-Escamilla, Rodrigo-García, & Alvarez-Parrilla, 2019).

Ten flavonoids (5–14, Table 3) were predominantly detected in the samples, seven flavonol 8 (rt:3.87 min), 9 (rt: 3.97 min), 10 (rt: 4.00 min), 11 (rt: 4.00 min), 12 (rt:4.17 min), 13 (rt: 4.22 min) and 14 (rt: 4.49 min) and precursor ions $[M+H]^+$ *m/z* 611.1584, $[M+H]^+$ *m/z* 319.0449, $[M+H]^+$ *m/z* 303.0485, $[M+H]^+$ *m/z* 465.1029, $[M+H]^+$ *m/z* 435.0916, $[M+H]^+$ *m/z* 449.1076, and $[M+H]^+$ *m/z* 433.1121 were putatively identified as rutin, myricetin, quercetin, isoquercitrin, quercetin-3-O-pentoside, quercitrin, and kaempferol-3-O-alpha-L-rhamnoside respectively. Two flavanonol 5 (rt:3.62 min) and 7 (rt: 3.86 min) with $[M+H]^+$ *m/z* 289.0705 and $[M+H]^+$ *m/z* 305.0647 were putatively identified as dihydrokaempferol and taxifolin respectively and one flavan-3-ol 6 (rt: 3.63 min) with $[M+H]^+$ *m/z* 291.0853 was putatively identified as epicatechin.

Flavonoids in general, have antioxidant activity due to the inhibitory effects of the production of free radicals and due to the scavenging activity of reactive species. These compounds can be found in fruits, vegetables, legumes, tea, etc. Specific parts of these foods contain higher levels of flavonoids such as the peel of some fruits. There is strong scientific evidence that demonstrates that the bioactive constituents (flavonoids, carotenoids, vitamins) of vegetables and fruits provide nutritional benefits for consumers. Thus, flavonoids have stood out for having an impact on immune cells and inflammatory processes (Maleki, Crespo, & Cabanillas, 2019).

Xanthone compound 15 (rt: 3.83 min) with $[M+H]^+$ *m/z* 437.1064 was putatively identified as bellidifolin-8-O-glucoside. It is important to highlight that for the Uvaia residue; this was the first report found. A previous study reported that this important class exhibits antioxidant, antimicrobial, anticancer, and anti-inflammatory activities, and xanthone exhibits antidiabetic activity (Santos, Freitas, & Fernandes, 2018).

4. Conclusion

The present study provided some important conclusions. First, the results showed that water used as a non-toxic solvent was efficient for extracting bioactive compounds from the Uvaia residue. In addition, the combination of ultrasound-assisted extraction and concentration by reverse osmosis, which are considered clean techniques, were extremely feasible, improving the bioavailability of the compounds as well as the concentration of these substances. Hence, the residue (peel and seed) used as raw material is highly promising for the extraction of bioactive compounds.

At the end of the process, a product with higher antioxidant levels was obtained compared to the initial extract (RS), with 6.2 times higher polyphenol content; 7.8 times higher flavonoid content, and 5.1 times higher antioxidant activity, highlighting levels of rutin (20.0 times higher), ellagic acid (5.0 times) and gallic acid (4.0 times higher). The final concentrate obtained (CF), with a high content of phenolic compounds and high antioxidant activity, was produced naturally and can be used to develop functional food formulations and nutraceuticals or used in the pharmaceutical industry.

CRediT authorship contribution statement

Letícia Misturini Rodrigues: Writing - original draft, Formal analysis, conceived and designed the study, collected and interpreted the test data, drafted and revised the manuscript, collected and analyzed the test data. **Edilson Bruno Romanini:** helped with the experiment. **Evandro Silva:** Formal analysis, collected and analyzed the test data. **Eduardo Jorge Pilau:** evised the manuscript. **Silvio Cláudio Da Costa:** evised the manuscript. **Grasiele Scaramal Madrona:** Writing - original draft, Formal analysis, conceived and designed the study, collected and interpreted the test data, drafted and revised the manuscript.

Declaration of competing interest

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

Acknowledgements

The authors acknowledge the financial support from CAPES scholarship, and CNPQ. We are grateful for the support provided by the Central Research Support Complex (COMCAP) of the State University of Maringa (UEM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110785.

Appendices

$$\%F = \frac{(Ji - Jf)}{Ji} X100 \quad \text{Eq. (A.1)}$$

$$C_{(x+c)}\ (\mu g/mL) = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198 \quad \text{Eq. (A.2)}$$

References

Almanasrah, M., Brazinha, C., Kallioinen, M., Duarte, L. C., Roseiro, L. B., Bogel-lukasik, R., et al. (2015). Nanofiltration and reverse osmosis as a platform for production of natural botanic extracts : The case study of carob by-products. *Separation and Purification Technology, 149*, 389–397. https://doi.org/10.1016/j.seppur.2015.06.008

de Almeida, R. T. R., do Prado, R. M., Porto, C., dos Santos, G. T., Huws, S. A., & Pilau, E. J. (2018). Exploring the rumen fluid metabolome using liquid chromatography-high-resolution mass spectrometry and Molecular Networking. *Scientific Reports, 8*(1), 4–11. https://doi.org/10.1038/s41598-018-36196-4

Alothman, M., Bhat, R., & Karim, A. A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry, 115*(3), 785–788. https://doi.org/10.1016/j.foodchem.2008.12.005

Aoac. (2005). *Official method of analysis* (18th ed.).

Aware, C. B., Patil, R. R., Vyavahare, G. D., Gurme, S. T., & Jadhav, J. P. (2019). Ultrasound-assisted aqueous extraction of phenolic, flavonoid compounds and antioxidant activity of Mucuna macrocarpa beans: Response surface methodology optimization. *Journal of the American College of Nutrition, 38*(4), 364–372. https://doi.org/10.1080/07315724.2018.1524315

Babbar, N., Oberoi, H. S., Uppal, D. S., & Patil, R. T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International, 44*(1), 391–396. https://doi.org/10.1016/j.foodres.2010.10.001

Bagci, P. O., Akbas, M., Gulec, H. A., & Bagci, U. (2019). Coupling reverse osmosis and osmotic distillation for clarified pomegranate juice concentration: Use of plasma modified reverse osmosis membranes for improved performance. *Innovative Food Science & Emerging Technologies, 52*(December 2018), 213–220. https://doi.org/10.1016/j.ifset.2018.12.013

Chakraborty, S., Uppaluri, R., & Das, C. (2020). Na of. In food and bioproducts processing. Institution of chemical engineers. https://doi.org/10.1016/j.fbp.2020.01.003.

Cianci, F. C., Silva, L. F. M., Cabral, L. M. C., & Matta, V. M. (2005). Clarificação e concentração de suco de caju por processos com membranas. *Ciência e Tecnologia de Alimentos, 25*(3), 579–583. https://doi.org/10.1590/s0101-20612005000300030

Fu, X., Belwal, T., Cravotto, G., & Luo, Z. (2020). Sono-physical and sono-chemical effects of ultrasound: Primary applications in extraction and freezing operations and influence on food components. *Ultrasonics Sonochemistry, 60*(104726). https://doi.org/10.1016/j.ultsonch.2019.104726

Gunathilake, K. D. P. P. (2020). Emerging technologies available for the enhancement of bioactives concentration in functional beverages. In *Biotechnological progress and beverage consumption*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-816678-9.00002-3.

Gunathilake, K. D. P. P., Yu, L. J., & Rupasinghe, H. P. V. (2014). Reverse osmosis as a potential technique to improve antioxidant properties of fruit juices used for functional beverages. *Food Chemistry, 148*, 335–341. https://doi.org/10.1016/j.foodchem.2013.10.061

Jacomino, A. P., da Silva, A. P. G., Freitas, T. P., Morais, V. S. P. Uvaia— Eugenia pyriformis Cambess. 435–438. https://doi.org/10.1016/B978-0-12-803138-4.00058-7.

Jiang, S., Li, Y., & Ladewig, B. P. (2017). A review of reverse osmosis membrane fouling and control strategies. *The Science of the Total Environment, 595*, 567–583. https://doi.org/10.1016/j.scitotenv.2017.03.235

Klein, E. J., Santos, K. A., Palú, F., Vieira, M. G. A., & da Silva, E. A. (2018). Use of supercritical CO2 and ultrasound-assisted extractions to obtain A/B-amyrin-rich extracts from uvaia leaves (Eugenia pyriformis Cambess.). *The Journal of Supercritical Fluids, 137*(January), 1–8. https://doi.org/10.1016/j.supflu.2018.02.019

Kuppusamy, S., Venkateswarlu, K., & Megharaj, M. (2020). Examining the polyphenol content, antioxidant activity and fatty acid composition of twenty - one different wastes of fruits, vegetables, oilseeds and beverages. *SN Applied Sciences, 673*, 1–13. https://doi.org/10.1007/s42452-020-2441-9

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*(C), 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Lopes, J. M. M., Lage, N. N., Guerra, J. F. C., Silva, M., Bonomo, L. F., Paulino, A. H. S., et al. (2018). A preliminary exploration of the potential of Eugenia uvalha Cambess juice intake to counter oxidative stress. *Food Research International, 105*(May 2017), 563–569. https://doi.org/10.1016/j.foodres.2017.11.067

Mahindrakar, K. V., & Rathod, V. K. (2020). *Jo ur na l P re of. Chemical Engineering & Processing: Process Intensification, 107841*. https://doi.org/10.1016/j.cep.2020.107841

Maleki, S. J., Crespo, J. F., & Cabanillas, B. (2019). Anti-inflammatory effects of flavonoids. *Food Chemistry, 125124*, 1–11. https://doi.org/10.1016/j.foodchem.2019.125124, 299(July).

Nenadis, N., Wang, L. F., Tsimidou, M., & Zhang, H. Y. (2004). Estimation of scavenging activity of phenolic compounds using the ABTS.+ assay. *Journal of Agricultural and Food Chemistry, 52*(15), 4669–4674. https://doi.org/10.1021/jf0400056

Ordoñez-santos, L. E., Martínez-girón, J., & Rodríguez-rodríguez, D. X. (2019). *Extraction of total carotenoids from peach palm fruit (Bactris gasipaes) peel by means of ultrasound application and vegetable oil • Extracción de carotenoides totales a partir de epicarpio de chontaduro (Bactris gasipaes) mediante aplicación de ultra.* (Vol. 86, pp. 98–103), 209.

Pan, Z., Qu, W., Ma, H., Atungulu, G. G., & McHugh, T. H. (2012). Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel. *Ultrasonics Sonochemistry, 19*(2), 365–372. https://doi.org/10.1016/j.ultsonch.2011.05.015

de Paulo Farias, D., Neri-Numa, I. A., de Araújo, F. F., & Pastore, G. M. (2020). A critical review of some fruit trees from the Myrtaceae family as promising sources for food applications with functional claims. *Food Chemistry, 306*(October 2019). https://doi.org/10.1016/j.foodchem.2019.125630, 125630.

Pereira, M. C., Steffens, R. S., Jablonski, A., Hertz, P. F., De O. Rios, A., Vizzotto, M., et al. (2012). Characterization and antioxidant potential of Brazilian fruits from the Myrtaceae family. *Journal of Agricultural and Food Chemistry, 60*(12), 3061–3067. https://doi.org/10.1021/jf205263f

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*(8), 3396–3402. https://doi.org/10.1021/jf9913458

Rastogi, N. K. (2018). Reverse osmosis and forward osmosis for the concentration of fruit juices. In *Fruit juices: Extraction, composition, quality and analysis*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-802230-6.00013-8.

Rodrigues, L. M., Romanini, E. B., Silva, E., Pilau, E. J., da Costa, S. C., & Madrona, G. S. (2020). Camu-camu bioactive compounds extraction by ecofriendly sequential processes (ultrasound assisted extraction and reverse osmosis). *Ultrasonics Sonochemistry, 64(December 2019), 105017*. https://doi.org/10.1016/j.ultsonch.2020.105017

de la Rosa, L. A., Moreno-Escamilla, J. O., Rodrigo-García, J., & Alvarez-Parrilla, E. (2019). Phenolic compounds. In *Postharvest physiology and biochemistry of fruits and vegetables*. Elsevier Inc. https://doi.org/10.1016/b978-0-12-813278-4.00012-9.

Santos, C. M. M., Freitas, M., & Fernandes, E. (2018). A comprehensive review on xanthone derivatives as α-glucosidase inhibitors. *European Journal of Medicinal Chemistry, 157*, 1460–1479. https://doi.org/10.1016/j.ejmech.2018.07.073

Sganzerla, W. C., Beling, P. C., Ferrareze, J. P., Komatsu, R. A., Nunes, M. R., & e de Lima Veeck, A. P. (2018). Nutritional, physicochemical and antimicrobial properties of uvaia pulp (Eugenia pyriformis Cambess). *Communications in Plant Sciences*, 1–7. https://doi.org/10.26814/cps2018001

da Silva, A. P. G., Spricigo, P. C., Purgatto, E., de Alencar, S. M., Sartori, S. F., & Jacomino, A. P. (2019). Chemical composition, nutritional value and bioactive compounds in six uvaia accessions. *Food Chemistry, 294*(May), 547–556. https://doi.org/10.1016/j.foodchem.2019.04.121

da Silva, A. P. G., Tokairini, T., de, O., de Alencar, S. M., & Jacomino, A. P. (2018). *Characteristics of the fruits of two uvaia populations grown in Salesópolis* (Vol. 40). SP, Brazil: Revista Brasileira de Fruticultura. https://doi.org/10.1590/0100-29452018511, 2.

Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.

Sousa, L., dos, S., Cabral, B. V., Madrona, G. S., Cardoso, V. L., & Reis, M. H. M. (2016). Purification of polyphenols from green tea leaves by ultrasound assisted ultrafiltration process. *Separation and Purification Technology, 168*, 188–198. https://doi.org/10.1016/j.seppur.2016.05.029

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003

Windson, C., Haminiuk, I., & Plata-oviedo, M. S. V. (2014). *Extraction and quantification of phenolic acids and flavonols from Eugenia pyriformis using different solvents., 51* (October), 2862–2866. https://doi.org/10.1007/s13197-012-0759-z

Zhang, Q. A., Shen, H., Fan, X. H., Shen, Y., Wang, X., & Song, Y. (2015). Changes of gallic acid mediated by ultrasound in a model extraction solution. *Ultrasonics Sonochemistry, 22*, 149–154. https://doi.org/10.1016/j.ultsonch.2014.06.010

Zillo, R., Silva, P., Zanatta, S., & Spoto, M. (2015). Parâmetros físico-químicos e sensoriais de polpa de uvaia (Eugenia Pyriformis) submetidas à pasteurização. *Bioenergia Em Revista: Dialogos, 4*(2) (ISSN: 2236-9171).