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Uvaia (*Eugenia pyriformis* Cambess) fruit: Exploring its antioxidant potential through pressurized liquid extraction

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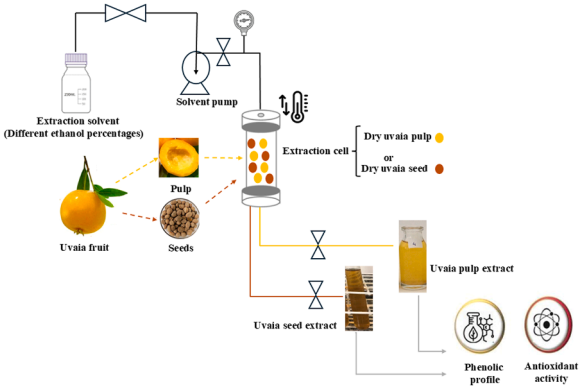
HIGHLIGHTS

- Higher temperatures and ethanol concentration improved phenolic extractions.
- Flavonoid extraction from uvaia pulp was influenced only by solvent composition.
- Higher temperatures and less ethanol enhanced flavonoid extraction from uvaia seed.
- Uvaia seed extracts showed higher antioxidant activity than pulp extracts.

GRAPHICAL ABSTRACT



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ABSTRACT

Uvaia (*Eugenia pyriformis* Cambess) is a Brazilian native fruit with a high concentration of phenolic compounds. In this study, we optimized the extraction of flavonoids and other phenolic compounds from uvaia pulp (U_P) and seed (U_S) by Pressurized Liquid Extraction (PLE) using an ethanolic solution as solvent. Both temperature and ethanol concentration enhanced the extraction of the phenolics from U_P and U_S. The increase in ethanol concentration decreased the yield of flavonoid extraction from U_P. In the case of the U_S, higher temperatures and lower ethanol percentage improved the yield of flavonoid extraction. All extracts showed high antioxidant activity under optimized conditions, especially the U_S extracts (507.40 μM Trolox.g^{-1} of dry U_S). The present findings highlight the effectiveness of PLE in obtaining antioxidant extracts from U_P and U_S, and also contribute to the valorization of uvaia fruit and its residues.

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Nomenclature

U_P	uvaia pulp.
U_S	uvaia seed.
$S_{(P+F)}$	U_S at optimized conditions.

1. Introduction

Brazil is recognized worldwide for its biodiversity, but many native species remain underexplored. One such species is uvaia (*Eugenia pyriformis Cambess*), which belongs to the Myrtaceae family and occurs in the Atlantic Forest extension from the Northeast, Southeast, and South of Brazil at altitudes higher than 800 m [1]. Popularly known as uvaieira, uvaia-do-campo, and uvalha [2], its fruits present a spherical, flat, and piriform shape with an orange or yellow fleshy pulp with an acidic flavor [1,3]. Each fruit presents one to two seeds, which comprise roughly 16 % of the fruit's weight [4]. The processing of uvaia pulp has been increasing due to its aromatic properties. However, the seeds are usually discarded as by-products despite their high nutritional value [4]. Uvaia fruit is rich in macronutrients, fibers, vitamin C, mineral salts, and secondary metabolites, such as phenolic compounds and carotenoids [1, 5]. The fruit contains 39.3 mg of vitamin C, 17.5 mg of yellow flavonoids, and 1.7 mg of carotenoids per 100 g of fruit [6]. Uvaia fresh juice is reported to have 135.14 mg of gallic acid in 100 g of juice [7]. The presence of phenolic compounds and other phytochemicals in uvaia confers potential health benefits to the fruit [1,8] and broad applications in food, cosmetic, and pharmaceutical areas.

Traditional extraction techniques, such as maceration and Soxhlet extraction, are reported in the literature to extract the bioactive compounds from uvaia [6,8,9]. Although these methods are well established, they are questionable from the point of view of sustainability as large amounts of solvents are required and sometimes do not follow regulatory requirements for food applications, not being GRAS (Generally Recognized as Safe) [10]. In addition, these methods usually involve harsh temperatures and long processing times, resulting in substantial bioactive degradation [11], which fosters the development of alternative extraction methods. Unconventional extraction methodologies have been studied to reduce the use of organic solvents and operational time and improve the yields and quality of the obtained extracts [3]. Alternative extraction methods, such as pressurized liquid extraction (PLE), have been reported to recover bioactive compounds from vegetable matrices, presenting superior bioactive stability, extraction efficiency, and selectivity [12].

In the PLE technique, temperature, pressure, and solvent selection are important parameters to be considered for efficient extractions. Higher temperature (50–200 °C) and pressure (30–200 bar) [13] combinations enable short-time extractions and the use of smaller quantities of solvents [14]. These are great advantages of the PLE over conventional extraction techniques and enable eco-friendly extractions [15]. Higher temperatures decrease the solvent viscosity, enabling the deep and more effortless penetration of the solvent in the matrix [16]. In addition, it enables the breakdown of bioactive-matrix linkages [17]. Consequently, the solubilization of the bioactive in the solvent and its diffusion to the surface matrix is facilitated. The use of high pressure facilitates the entry of the solvent into the matrix and thus contributes to a higher extraction yield [12]. The solvent selection must consider its affinity with the target bioactive compound to favor its release from the matrix [17] and reduce the nontarget bioactive extraction.

Several studies in the literature showed the efficiency [18–23] of PLE for the recovery of polyphenols from various fruit matrices (e.g., grape, acai, passion fruit, granadilla, tucumã do Amazonas and, Tahiti lime) utilizing different ethanol: water ratios and temperature ranges. However, to the best of our knowledge, PLE has never been reported before to extract bioactives (phenolics and flavonoids) from uvaia pulp and seeds. Moreover, the effect of temperature and solvent composition of PLE on the antioxidant properties of the extracts is still unknown.

Thus, this study aimed to optimize total phenolic and flavonoid extraction from uvaia fruit (pulp and seeds) through PLE and to understand how the temperature and solvent (water/ethanol ratio) influence the extract composition and its antioxidant activities. Under optimized extraction conditions, the phenolic and carotenoid profiles of the extracts were identified and quantified by High-Performance Liquid Chromatography with a Diode Array Detector (HPLC-DAD) using analytical standards.

2. Materials and methods

2.1. Materials

Uvaia fruit (*Eugenia pyriformis*) was purchased from Sítio do Belo (Paraibuna, São Paulo, Brazil). The chemicals catechin, gallic acid, ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine), Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid) and the phenolic compounds standards (purity > 95.0 %) were acquired from Sigma Aldrich (St. Louis, MO, USA). Carotenoids standards were isolated from natural sources (lutein from spinach, zeaxanthin from goji berry, β-cryptoxanthin from persimmon, and β-carotene from carrot) at Embrapa Food Technology HPLC Laboratory (Rio de Janeiro, Rio de Janeiro, Brazil) with purity > 95.0 %. The ethanol (99.5 % (v/v)) was purchased from Cicla Farma (Serrana, São Paulo, Brazil). The other reagents were of analytical grade, and the solutions were prepared with Ultrapure water (Milli-Q System).

2.2. Raw material preparation, characterization, and proximate composition

Uvaia pulp (UP) was cleaned with water, and the seeds (US) were manually separated from the pulp. Any remaining pulp on the seeds was removed with water. Each was then processed in a blender, freeze-dried (Liotop LP820, São Paulo, Brazil) for 72 h and ground in a knife mill (Marconi, model MA 340, Piracicaba, Brazil) until homogenization. The proximate composition was performed according to the methodologies proposed by the Association of Official Analytical Chemists (AOAC) [24] for proteins (No. 954.01), moisture (No. 934.01), and ash (No. 942.05). Lipids were quantified according to Bligh and Dyer method [25]. Carbohydrate content was calculated by difference. The experiments were conducted in triplicate. The samples were freeze-dried to all other proximate composition analyses except for the moisture. The phenolics and carotenoid profiles in the freeze-dried pulp and seeds were evaluated by High-Performance Liquid Chromatography (HPLC) (Section 2.5.4).

2.3. Experimental design and pressurized liquid extraction

The 2^2 Central Composite Rotational Design (CCRD) was performed with 3 extractions on the central point and 4 extractions on axial points, in a total of 11 assays (extracts). Temperature and ethanol percentage (% v/v) were considered independent variables, while total phenolic compounds and total flavonoid content were the dependent variables evaluated (Table 1). The axial points −1.41 and + 1.41 for ethanol percentage (% v/v) and temperature were based on commonly reported values for phenolic compounds extraction from fruit matrices using PLE [12]. The extractions were conducted in a continuous flow, maintaining a constant pressure of 10 MPa. To obtain the extracts, 1.0 g of U_P or U_S was placed in a 5 cm^3 (inner diameter of 19.6 mm and height of 22.6 cm) extraction cell. The cell was filled with solvent using an HPLC JASCO pump (PU-2080, Tokyo, Japan). The mass flow rate was set at 1.5 g. min^{-1} for all extraction. To ensure this value is constant, the ethanol densities in each extraction were considered to define the volumetric

Table 1
CCRD parameters (temperature and ethanol (% v/v)) used in U_P (uvaia pulp) and U_S (uvaia seed). Description: This table shows the CCRD parameters used for U_P and U_S seed.

Sample	Condition	-1.41	-1	0	+1	+1.41
Pulp	Temperature (°C)	40.0	49.0	70.0	91.0	91.0*
	Ethanol (%v/v)	60.0	66.0	80.0	94.0	100.0
Seeds	Temperature (°C)	40.0	49.0	70.0	91.0	100.0
	Ethanol (%v/v)	50.0	57.0	75.0	93.0	100.0

*Due to experimental limitations, the temperature axial point was performed at 91°C (+1) instead of 100°C (+1.41).

flow rate in the pump. According to preliminary tests, the extraction time was set to 60 min for U_P and 30 min for U_S.

The data were analyzed using Protimiza Experiment Design Software® [26] at a significance level of 5 %. Due to process limitations (section 3.2.1), the temperature axial point for U_P was performed at 91 °C (+1) and 80 % ethanol (0), and this matrix was analyzed as a Custom Design (see Table 4). The software generated mathematical models that defined the optimal extraction conditions. The optimal temperature and ethanol (%) were based on contour curves, as suggested by Rodrigues and Iemma [27]. Three extractions at optimal temperature and ethanol (% v/v) concentration validated this condition.

2.4. Conventional extraction methods

For conventional extraction, 4.0 g of U_P or U_S was mixed with 100.0 mL of ethanol as the solvent, as proposed by Machado et al. [15] and Climaco et al. [28]. Extractions were first performed at 25 °C, lasting 1 h for U_P and 0.5 h for U_S, with an additional extraction time of 8 h for both samples. Furthermore, high temperatures used in PLE optimization were incorporated into the conventional extractions. For U_P, extractions were carried out at both 55 °C and 70 °C for 1 h, followed by an extended extraction for 8 h at each temperature. For U_S, extractions were conducted at 78.5 °C for 0.5 h, followed by 8 h. To carry out the extractions at elevated temperatures, a reflux system was employed to prevent solvent loss. The extracts were evaluated for total phenolic content, total flavonoid content, and antioxidant activity using the methods described in Section 2.5.

2.5. Analysis

2.5.1. Total phenolics content

The total phenolic content was evaluated using the Folin-Ciocalteu method proposed by Machado et al. [15]. A standard calibration curve for acid gallic was built for PLE extracts obtained from U_P (Abs = 0.014 [acid gallic] + 0.04; R^2 = 0.98) (Figure S1-A) and U_S (Abs= 0.014[acid gallic] + 0.02; R^2= 0.99] (Figure S1-B) and from extracts obtained through conventional extraction for U_P and U_S (Abs =0.005[acid gallic] – 0.02; R^2 = 0.96) (Figure S1-C). The absorbance was measured at 760 nm. The results were expressed as milligrams of gallic acid equivalent per gram (mg GAE.g^{-1}) of dry U_P or U_S. The experiments were performed in triplicate.

2.5.2. Total flavonoid content

The total flavonoid content was evaluated according to the methodology proposed by Veggi et al. [29]. A standard calibration curve for catechin was built for PLE extracts obtained from U_P (Abs= 0.002 [catechin] - 0.14; R^2= 0.98] (Figure S2-A) and U_S (Abs= 0.003[catechin] + 0.03; R^2 = 0.99) (Figure S2-B) and for extracts obtained through conventional extraction from U_P and U_S (Abs= 0.003[catechin] - 0.04; R^2= 0.99) (Figure S2-C). The absorbance was measured at 510 nm. The results were expressed as milligrams of catechin equivalent per gram (mg CAE. g^{-1}) of dry U_P or U_S. The concentration factors of pulp extracts obtained through PLE ranged from 2.14 to 3.0, and the experiments were performed in triplicate.

2.5.3. Antioxidant activity

The antioxidant activity of the extracts obtained at optimal extraction conditions was evaluated according to ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging and ferric-reducing antioxidant power (FRAP) assays in a UV–visible spectrophotometer (Hitachi, model U-3010, Tokyo, Japan). All experiments were performed in triplicate.

The ABTS assay was performed as proposed by Rufino et al. [30]. A standard calibration curve for Trolox was built for PLE extracts obtained from U_P (Abs= −0.0002[Trolox] +0.75; R^2=0.99) (Figure S3-A) and U_S (Abs= −0.0003[Trolox] + 0.67; R^2= 0.98) (Figure S3-B) and for extracts obtained through conventional extraction from U_P and U_S (Abs= −0.0012[Trolox] + 0.67; R^2= 0.98] (Figure S3-C). The absorbance was measured at 734 nm.

The FRAP assay was based on the methodology proposed by Clímaco et al. [28] with modifications. FRAP reagent was obtained by a combination of acetate buffer (0.3 M/pH 3.6), TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) (10 mM), and ferric chloride (20 mM) in the proportion of 10:1:1. FRAP reagent was prepared immediately before it's used and heated at 37 °C for 30 min. After that, 2.7 mL of FRAP was added to 90.0 μL of extracts in an appropriate dilution and 270 μL of ultrapure (Milli-Q) water. The test tubes containing the FRAP, extracts, and ultrapure water were homogenized, and after 30 min in the dark, the absorbance was determined at 595 nm. A standard calibration curve for Trolox was built for PLE extracts obtained from U_P and U_S (Abs=0.0013 [Trolox] – 0.01; R^2= 0.99) (Figure S4-A) and for extracts obtained through conventional extraction from U_P and U_S (Abs= 0.0037 + 0.03; R^2=0.98) (Figure S4-B). The results were expressed as μM Trolox.g^{-1} of dry U_P or U_S.

2.5.4. Phenolic compounds profile analysis by HPLC-DAD

High-Performance Liquid Chromatography with a Diode Array Detector (HPLC -DAD) (Waters Corporation, Massachusetts, USA) analysis was performed to evaluate the phenolic profiles from U_P and U_S at optimized extraction conditions and from the freeze-dried matrix.

The phenolic profile from optimized extraction conditions and the freeze-dried matrix (free and hydrolyzed fraction extracted with methanol: water (50: 50)) was carried as proposed by Nascimento et al. [31] in an Alliance Waters™ model 2690/5, with a Waters ™ photodiode array detector model 2996 (270, 310, and 370 nm) and a Thermo Hypersil BDS C_{18} column in series (50 ×4.6 mm×2.4 μm; 100 ×2.6 mm×2.4 μm). The elution was performed using a gradient mode with an aqueous solution of 0.15 % phosphoric acid (95 %) and acetonitrile (5 %). At 12.00 min, the acetonitrile concentration was increased to 12 %, at 18.00 min to 20 %, and at 20.00 min to 50 % acetonitrile. The acetonitrile concentration was maintained at 5 % through to 25.00 min and then returned to the initial condition (5 %). The quantification of phenolic compounds was performed by external standardization, using phenolic acids (ellagic, gallic, syringic, and p-coumaric acid) and flavonoids(isoquercitrin, quercitrin, and quercetin) analytical standards. The quantification was based on the major compound and the available standards.

2.5.5. Carotenoid analysis

To determine the amount of total carotenoid in the freeze-dried matrix, they were first extracted by maceration with acetone and celite as proposed by Rodriguez [32] and then determined by spectrophotometry at 450 nm using a UV-Vis spectrophotometer, UV-1800 model 92 (Shimadzu Corporation, Kyoto, Japan), and petroleum ether as blank. The Beer-Lambert law was used to calculate the total carotenoid content. The carotenoid profile in the freeze-dried matrix and at the optimized extraction conditions was determined as proposed by Pacheco [33] using an HPLC system with a diode array detector, C30 column (S-3 Carotenoid, 4.6 mm × 250 mm), column temperature of

33°C, flow rate of 0.8 mL min^{-1} and running time of 28 min. The elution was performed using gradient mode with methanol (solvent A) and methyl tert-butyl ether (solvent B). The gradient started with 80 % methanol (solvent A) and 20.0 % methyl tert-butyl ether (solvent B). At 0.5 min, solvent B concentration was increased to 25.0 %, at 15.00 min to 85.0 %, and 15.05 min to 90.0 %. The solvent B concentration was maintained at 90.0 % until 16.50 min, returning to 20 % at 16.55 min up to 28 min. The quantification of individual carotenoids was performed by external standardization, using lutein, zeaxanthin, β-cryptoxanthin, and β-carotene analytical standards. Quantification was carried out based on major compounds and the available standards.

2.5.6. Statistical analysis

The results regarding phenolic composition, carotenoid composition, and antioxidant activity for the extracts were statistically evaluated by analysis of variance (ANOVA) with a Tukey test analyzed with a significance level of *p*-value < 0.05. The tests were performed using Sisvar® software (Version 5.6, Universidade Federal de Lavras).

3. Results and discussion

3.1. Raw material characterization and proximate composition

Table 2 shows the proximate composition of U_P and U_S on a dry basis (except for the moisture). U_P and U_S showed high amounts of carbohydrates (over 50.0 %). Usually, seeds present fructans and polysaccharides, such as starch [34], which confers energy for the germination process [35]. The carbohydrates found in the pulp, mainly fructose [34], are responsible for the texture and flavor of the fruit [36]. Also, lipids and proteins were found in both U_P and U_S. In U_P, the lipid content was 5.9 % (w/w), and the protein content was 21.2 % (w/w), which was slightly higher than expected. Previous studies have reported lipid levels in U_P ranging from 0.38 to 2.2 g.100 g^{-1}, and protein levels ranging from 1.69 to 15.82 g.100 g^{-1} [8,9,37,38]. In U_S, the lipid content was 1.7 % (w/w), and the protein content was 9.53 % (w/w), while the literature reports lipid and protein contents in the U_S at 1.50 g.100 g^{-1} and 12.08 g.100 g^{-1}, respectively [34].

In addition to its rich proximate composition in macronutrients, U_P and U_S are alternative sources of bioactive compounds. Overall, the phenolic composition of U_P and U_S are slightly different, but both present ellagic acid as the main compound (Table 3). Another difference between U_P and U_S is the presence of carotenoids in the pulp (13.36 mg.100 g^{-1} of U_P). The carotenoids identified in the U_P were β-cryptoxanthin (4.87 mg.100 g^{-1} of U_P), the major carotenoid found on U_P, followed by β-carotene (1.41 mg.100 g^{-1} of U_P), zeaxanthin (1.03 mg.100.g^{-1} of U_P) and lutein (0.93 mg.100 g^{-1} of U_P) (Table S1 – Supplementary Material). Few studies in the literature explore the carotenoids present in U_P and U_S, and based on the best of our knowledge, none of them explore the seed. Silva et al. [38] reported the presence of 9-*cis*-neoxanthin, *all-trans*-neochrome, *cis*-antheraxanthin, 9-*cis*-violaxanthin, and *all-trans*-zeaxanthin in the U_P. Some different compounds are reported in pulp (rutin, chlorogenic acid, kaempferol, myricetin, caffeic acid, and ferulic acid) and seeds (catechin hexoside, vanillic acid, luteolin hexoside) [34,38,39]. Nutritional and bioactive composition might vary according to cultivation region, harvest time, maturation, soil drought and salinity, pathogens, and light incidence [40–42]. In addition, the analytical techniques used for its identification can also result in divergent results concerning the sensitivity and limitations inherent to each methodology [43]. Apart from this, U_P and U_S have a greater content of ellagic acid than other traditional fruits, such as strawberry (0.0095 mg.g^{-1}), pitanga (0.6 mg.g^{-1}), and jaboticaba (0.14 mg.g^{-1}) [44–46], which makes uvaia fruit a new potential source of this acid. This is interesting due to the potential health benefits of ellagic acid, such as antioxidant, anti-inflammatory, antibacterial, anticancer, and antidiabetic [47].

Table 2
Proximate composition of U_P and U_S.Description: This table shows the proximate composition of Uvaia pulp and seed.

	Composition (% w/w)	
Component	**Pulp**	**Seeds**
Moisture*	90.8 ± 0.3	39.7 ± 1.1
Ash**	4.1 ± 0.1	1.2 ± 0.4
Protein**	21.2 ± 0.2	9.5 ± 0.5
Lipids**	5.9 ± 0.0	1.7 ± 0.4
Carbohydrates**	68.8 ± 0.3	87.6 ± 1.1

*Expressed in wet basis **Expressed in dry basis

Table 3
Profile of phenolic compounds in U_P and U_S.Description: This table shows the phenolic profile for U_P and U_S.

Phenolic compounds*	Pulp		Seeds	
	Free Fraction (mg.g^{-1})	Hydrolyzed fraction (mg.g^{-1})	Free fraction (mg.g^{-1})	Hydrolyzed fraction (mg.g^{-1})
Ellagic acid	0.57 ± 0.01	0.25 ± 0.02	1.01 ± 0.04	0.54 ± 0.02
Gallic acid	ND	0.03 ± 0.00	ND	0.28 ± 0.00
p-coumaric acid	ND	0.01 ± 0.00	ND	0.02 ± 0.00
Isoquercetin	0.05 ± 0.00	ND	ND	ND
Quercetrin	0.27 ± 0.02	0.01 ± 0.00	ND	ND
Quercetin	0.03 ± 0.00	ND	0.03 ± 0.00	ND

*Expressed in mg.g^{-1} in wet basis/ND = Not detected

3.2. Optimization of the extraction process by pressurized liquids

The extractions were performed according to CCRD (Section 2.3). However, one customization was necessary as it was not possible to obtain U_P sample #6 at standard conditions, i.e., extraction at temperature axial point + 1.41 (100 °C) and solvent central point (80.0 % of ethanol) due to the clogging of the tubes during extraction. Some studies in the literature report the presence of soluble pectin in U_P in concentrations ranging from 0.18 % to 1.49 % of galacturonic acid.100 g^{-1} in the uvaia pulp [48,49]. Pectin is a heteropolysaccharide found in plant cell walls and recognized as a thickener and gelling agent [50,51]. At the aforementioned conditions, pectin is capable of solubilizing in the solvent [52]. However, the lower temperature of the outlet tubing (which was not thermally insulated) led to gel formation and tube clogging. In this way, the maximum temperature used in the extraction of U_P was 91.0 °C, as it was the maximum temperature reached without clogging the pipe. Thus, the CCRD for U_P was customized to assure statistical reliability.

Given the conditions evaluated on CCRD, Table 4 shows the total phenolics and total flavonoid content for U_P and U_S extracts. Mathematical models were built (Table S2 – Supplementary Material) to evaluate the effect of temperature and ethanol percentage on the extraction of total phenolics and flavonoids. Only statistically significant coefficients (*p-value* < 0.05) were considered, and Analysis of Variance (ANOVA) was used to evaluate the models (Table 5 and Table S3–Supplementary Material).

The obtained models (Table 5) confirmed that temperature and ethanol percentage influenced U_P and U_S phenolic extractions (Y_1 and Y_3, respectively). According to ANOVA, for both responses, the calculated F (F_{cal}) for regression/residues was higher than the tabulated F (F_{tab}), and the variation explained by the model (R^2) was acceptable within the range of the study. Then, as the generated models were well-fitted to the experimental data, the response surfaces for U_P and U_S phenolic extraction (Fig. 1) were generated.

The temperature exhibited a second-order effect on phenolic extraction from both U_P and U_S. The maximum extraction yield of total phenolics was observed for U_P extracts when the temperature ranged from 55 to 85 °C. For U_S extracts, the better yield on total phenolic

Table 4
Conditions of temperature and ethanolic fraction of the solvent studied on the extractions, codded variables, and the respective phenolic and flavonoid contents of U_P and U_S extracts.Description: This table shows the temperature and ethanolic fraction of the solvent studied in the extractions, coded variables, and the respective phenolic and flavonoid contents of U_P and U_S extracts.

Sample	U_P				U_S			
	Temperature (x_1) (°C)	Ethanol (x_2) (%)	Total phenolic* (Y_1)	Total flavonoid** (Y_2)	Temperature (x_1) (°C)	Ethanol (x_2) (%)	Total phenolic* (Y_3)	Total flavonoid** (Y_4)
1	49.0(−1)	66.0(−1)	15.56	7.91	49.0(−1)	57.0(−1)	21.20	8.54
2	91.0(+1)	66.0(−1)	14.71	7.04	91.0(+1)	57.0(−1)	42.55	17.75
3	49.0(−1)	94.0(+1)	7.47	5.70	49.0(−1)	93.0(+1)	3.87	1.53
4	91.0(+1)	94.0(+1)	4.91	2.34	91.0(+1)	93.0(+1)	23.77	8.24
5	40.0(−1.41)	80.0(0)	10.04	4.16	40.0(−1.41)	75.0(0)	14.51	4.73
6	91.0(+1▲)	80.0(0)	17.1	4.99	100.0(+1.41)	75.0(0)	42.41	15.23
7	70.0(0)	60.0(−1.41)	16.09	6.71	70.0(0)	50.0(−1.41)	38.22	12.81
8	70.0(0)	100.0(+1.41)	5.20	2.19	70.0(0)	100.0(+1.41)	13.57	4.80
9	70.0(0)	80.0(0)	17.15	7.1	70.0(0)	75.0(0)	33.82	11.21
10	70.0(0)	80.0(0)	14.81	5.94	70.0(0)	75.0(0)	33.28	10.10
11	70.0(0)	80.0(0)	15.72	4.73	70.0(0)	75.0(0)	31.53	10.09

*Total phenolic content is expressed as milligrams of gallic acid (mg GAE).g^{-1} of dry U_P or U_S.
**Total flavonoid content is expressed as milligrams of catechin (mg CAE).g^{-1} of dry U_P or U_S.
▲x_1 axial condition (+α) was performed at (+1) instead of (+1.41)

Table 5
Coded models and ANOVA for total phenolics and total flavonoids in U_P and U_S extracts Description: This table shows the coded models and Analysis of Variance (ANOVA) for total phenolics and total flavonoids in Uvaia pulp and seed extracts.

	Response	Equation	F_{cal}	F_{tab}	R^2 (%)
U_P extracts	TPC	$Y_1 = 16.43 - 2.58x_1^2 - 4.17x_2 - 3x_2^2$	23.73	4.35	91.05
	TFC	$Y_2 = 5.35 - 1.67x_2$	14.49	5.12	61.85
U_S extracts	TPC	$Y_3 = 32.88 + 10.09x_1 - 3.29x_1^2 - 8.87x_2 - 4.57x_2^2$	57.3	4.53	97.45
	TFC	$Y_4 = 9.55 + 3.85\ x_1 - 3.48\ x_2$	77.93	4.46	95.12

R^2, coefficient of determination; F_{cal}, calculated F-factor; F_{tab}, tabulated F-factor at 5 % significance; x_1 and x_2, coded independent variables (temperature and ethanol percentage, respectively). TPC: total phenolic content, TFC: total flavonoid content.

extraction was at higher temperature ranges (80–100 °C) when compared to U_P. When elevated temperatures are applied to PLE extractions, high amounts of total phenolic compounds are expected in the extracts [53]. When high pressure and high temperatures are combined, there is an increase in mass transfer, solvent diffusivity, and solute solubility, which enables interactions within the cell that reduce the surface tension of the solvent [28]. This reduction, aligned with the reduction of solvent viscosity caused by high temperatures, makes its penetration into the matrix easier [20]. Also, high temperatures allow plant tissue to soften, weakening interactions such as Van der Waals, hydrogen bonds, and dipole attraction between bioactive compounds and the cell membrane [18,54]. However, prolonged exposure to temperature might also lead to phenolic degradation [54]. The longer extraction time for U_P could be responsible for the lower optimal temperature ranges for phenolic extraction compared to the U_S. In addition, although the phenolic profile from both U_P and U_S does not differ, the last one has higher amounts of ellagic acid that possesses great thermal stability [55] and thus may increase the U_S temperature extraction. The composition of the matrix and the presence of other thermal-sensitive compounds can also affect phenolic extraction [56]. Also, phenolic oxidation and reactivity increase at high temperatures [57]. The exposure of protein hydrophobic groups and phenolic reactivity can enhance the protein-phenolic interactions and the phenolic binding to the matrix, decreasing phenolic extraction [58]. On the other hand, the high phenolic content in U_S extracts at high temperatures could be associated with the release of insoluble phenolic acids that are linked with lignin [53].

The influence of solvent on extractions is related to the capacity of the solvent to solubilize the phenolics, i.e., the ability of the solvent to form hydrogen bonds and solvate the phenolics, releasing it from the matrix [41]. The ethanol percentage in the solvent influences the phenolic yield in both U_P and U_S, indicating that solvent polarity impacts the efficiency of phenolic extraction. In the U_P extracts, the optimal phenolic yield is achieved with a solvent ethanol content between 60.0 % and 80.0 % (lower polarity), while in the U_S extracts, it is optimal between 50.0 % and 70.0 %. This can be explained by the differences in the phenolic profile found in the U_P and U_S, as shown in Table 3. Gallic and ellagic acid are the main phenolics in the U_S (Table 3), known to possess moderate to high polarity [59] and thus are best extracted in a more polar solvent. On the contrary, U_P has quercetin, isoquercitrin, and quercitrin, which are well extracted at an ethanol percentage of around 70.0 % [60], meaning they are well extracted in a less polar solvent.

For total flavonoid extraction from the U_P, only the linear coefficient for solvent was significant ($p < 0.05$). Despite F_{cal} for regression/residues being higher than F_{tab}, the R^2 was not considered satisfactory, and the model did not fit well the experimental data. Although it cannot be used for prediction, there is a tendency observed for ethanol percentage on total flavonoid content in U_P extracts. As the percentage of ethanol increases, the amount of flavonoids in the U_P extracts decreases, i.e. the ethanol percentage in the solvent negatively affects the extraction. Conversely, the temperature did not influence the total flavonoid extraction from the pulp within the range evaluated.

Regarding the U_S (Y_4), temperature and ethanol percentage in the solvent have a linear influence on the total flavonoid extraction (Table 5). Since F_{cal} for regression/residues was higher than F_{tab}, and the R^2 was satisfactory, the generated model was well fitted to experimental data, and a response surface was generated (Fig. 2-A). In the U_S, the increase in temperature and the decrease in ethanol percentage were favorable to flavonoid extraction yield. Huaman-Castilla et al. [61] observed the same effect of temperature and ethanol percentage on quercetin, kaempferol, and resveratrol extraction from Carmènére grape

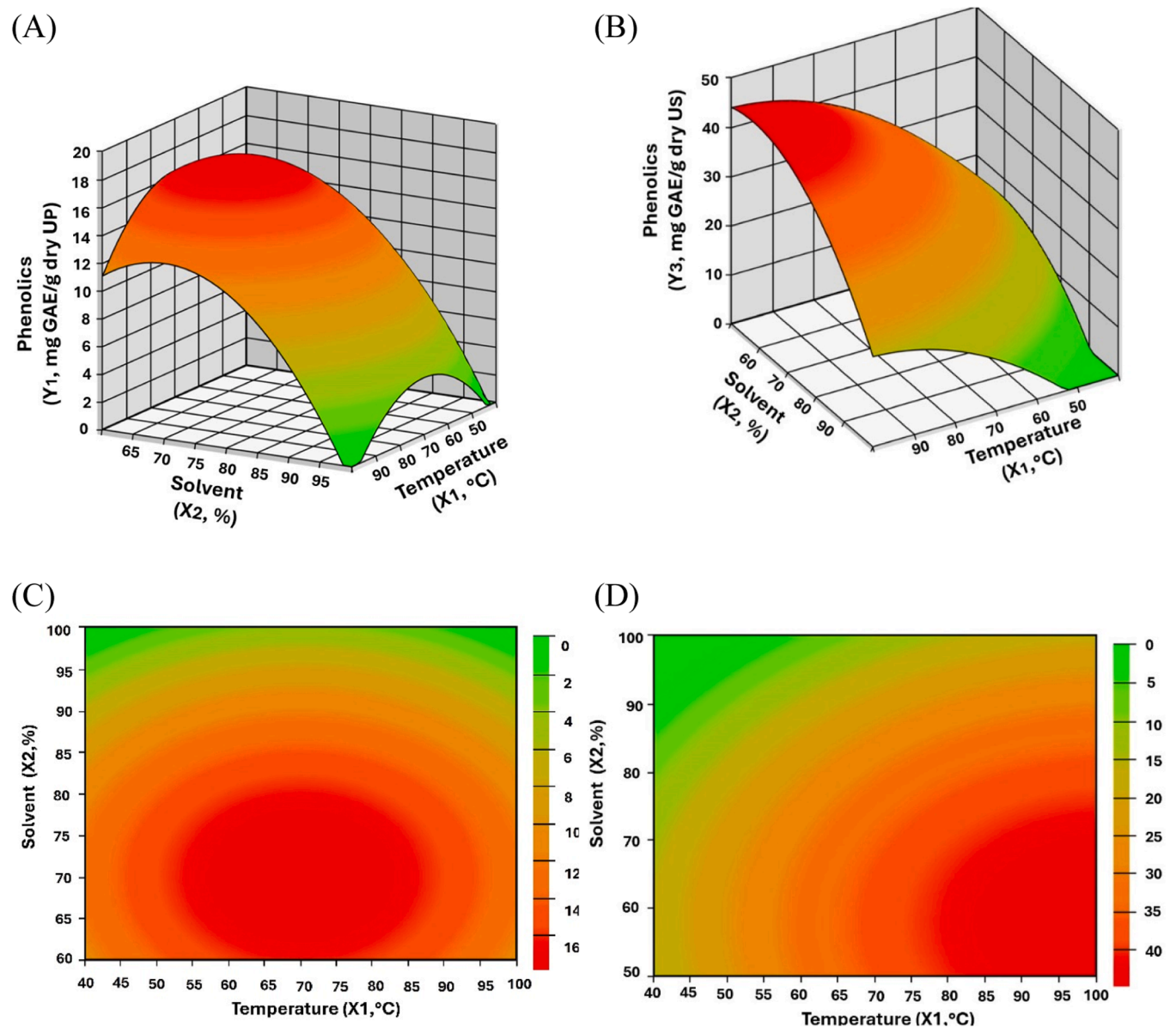

Fig. 1. Response surface and contour curves for phenolic content on extracts of U_P and U_S under different temperature and ethanol percentage conditions. (A) and (B) are the surface responses for U_P and U_S, respectively. (C) and (D) are the contour curves for U_P and U_S, respectively. In contour curves, the right axis means the content of total phenolics (mg GAE.g^{-1} dry U_P or U_S). Description: This figure shows the response surface and contour curves for both U_P and U_S phenolic extraction.

pomace.

The process optimization was based on surface responses (Figs. 1 and 2). The extraction of phenolics from UP (Fig. 1-C) showed an optimal extraction region within 55°C to 85°C and 60 % and 80 % ethanol. The lowest solvent percentage within the optimal extraction range of phenolics was chosen to validate the model (condition A: 70 °C and 60.0 % of ethanol) had seen the total flavonoids extraction tended to be higher at lower ethanol concentrations. Additionally, aiming to work with the lowest optimal temperature, a second condition was also chosen for validation (condition B: 55 °C and 70.0 % ethanol). These ranges also consider ethanol consumption and energy use, aiming for a cost-effective process.

For the U_S, the maximum extraction area for total phenolics ranged from 80–100 °C and 50–70.0 % of solvent (Fig. 1-D). For total flavonoids, the optimal temperature ranged from 90 to 100 °C, and the ethanol percentage ranged from 50 % to 60.0 %. So, the process optimization ($S_{(P+F)}$) was performed for U_S extracts at 90.0 °C and 50.0 % solvent.

Table 6 shows predicted and experimental data for total phenolic and flavonoid content at the optimized conditions. For U_P, the error between the total phenolic in the predicted model and the observed values for the extracts at optimized conditions was around 13 %, confirming that the predicted phenolic extraction model was acceptable on the evaluated temperature range and ethanol percentage in the solvent. The extractions performed in the optimized conditions show similar values to those obtained before for the total flavonoids in the U_P extracts. Although two conditions were chosen to optimize the process, either the total phenolic or total flavonoid content was statistically the same at these conditions (Table 6). This indicates the process flexibility and demonstrates that either with higher ethanol and lower temperature or with lower ethanol and higher temperature (within the optimized range for phenolic extraction), the same levels of phenolics and flavonoids can be achieved. The U_P extracts obtained under optimized conditions showed higher levels of total phenolics and flavonoids than those produced through conventional extraction methods under the proposed conditions (Table 7). At these extractions, the total phenolic and flavonoids ranged

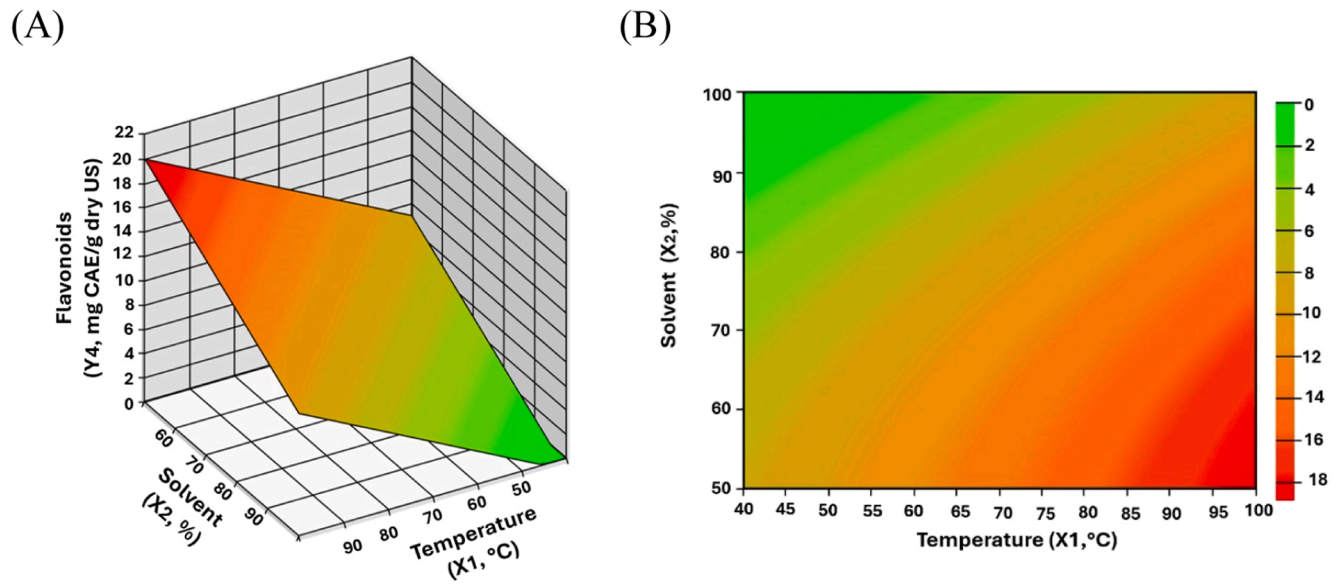

Fig. 2. Response surface (A) and (B) contour curves for U_S flavonoid extractions as a function of temperature and ethanol percentage. In contour curves, the right axis means the content of flavonoids (mg CAE.g^{-1} dry U_S). Description: This figure shows the response surface and contour curves for U_S flavonoid extraction.

Table 6
Total phenolic (TPC) and flavonoid content (TFC) in extracts obtained under optimized conditions. Description: Predicted and experimental values for total phenolic and flavonoid content in extracts obtained under optimized conditions.

Optimized condition*	Model prediction		Experimental values		Relative error	
	TPC^X	TFC^Y	TPC^X	TFC^Y	TPC^X	TFC^Y
A (70°C; 60 %)	16.32	-	18.81 ± 0.34^a	5.48 ± 0.82^a	13.2 %	-
B (55°C;70 %)	16.59	-	18.05 ± 1.26^a	5.11 ± 0.45^a	8.1 %	-
S_{P+F}(90°C;50 %)	42.91	18.14	34.43 ± 1.92^b	22.03 ± 1.27^b	−24.6 %	−28.9 %

*Condition: extraction temperature; ethanol concentration.
^{X}TPC: Total Phenolic Content expressed as milligrams of gallic acid (mg GAE).g-1 of dry U_P or U_S
^{Y}TFC: Total Flavonoid Content expressed as milligrams of catechin (mg CAE).g-1 of dry U_P or U_S
a,b: In a column, different letters indicate statistically different samples for $p < 0.05$

Table 7
Total phenolic (TPC) and flavonoid content (TFC) content and antioxidant activity for pulp and seed extracts obtained through conventional extraction methods. Description: Total phenolic, total flavonoid content, and antioxidant activities for UP and US extracts obtained through conventional extractions.

	Extraction Parameters				Antioxidant activity (μM Trolox.g^{-1} of dry sample)	
Pulp extracts	**Temperature (°C)**	**Time (h)**	**TPC^X**	**TFC^Y**	**ABTS**	**FRAP**
	25	1	2.87 ± 0.20^{ab}	1.86 ± 0.05^a	12.57 ± 0.84^a	11.92 ± 0.16^a
	25	8	4.65 ± 0.02^c	1.78 ± 0.10^b	13.11 ± 1.48^a	11.37 ± 0.13^b
	55	1	3.07 ± 0.06^c	1.38 ± 0.02^c	11.66 ± 0.10^a	9.22 ± 0.20^c
	55	8	7.17 ± 0.11^e	2.23 ± 0.15^a	11.72 ± 0.25^a	20.04 ± 0.37^d
	70	1	4.00 ± 0.21^{bf}	1.52 ± 0.02^c	14.93 ± 1.22^a	10.94 ± 0.10^b
	70	8	7.99 ± 0.15^g	2.46 ± 0.04^{ad}	37.19 ± 0.53^b	21.12 ± 0.33^e
Seed extracts	25	0.5	2.72 ± 0.05^d	2.54 ± 0.08^d	14.96 ± 0.48^a	13.06 ± 0.21^f
	25	8	3.27 ± 0.10^{ad}	5.24 ± 0.05^e	16.19 ± 0.36^a	17.50 ± 0.09^g
	78.5	0.5	1.72 ± 0.04^h	6.18 ± 0.18^f	4.53 ± 6.26^c	23.70 ± 0.49^h
	78.5	8	1.35 ± 0.06^h	6.27 ± 0.22^f	6.79 ± 0.10^c	37.49 ± 0.12^i

^{X}TPC: total phenolic content is expressed as milligrams of gallic acid (mg GAE).g^{-1} of dry U_P or U_S.
^{Y}TFC: Total flavonoid content is expressed as milligrams of catechin (mg CAE).g^{-1} of dry U_P or U_S
a-i: In a column, different letters indicate statistically different samples for $p < 0.05$.
^{X}TPC: total phenolic content is expressed as milligrams of gallic acid (mg GAE).g^{-1} of dry U_P or U_S.
^{Y}TFC: Total flavonoid content is expressed as milligrams of catechin (mg CAE).g^{-1} of dry U_P or U_S
a-i: In a column, different letters indicate statistically different samples for $p < 0.05$

from 2.87 to 7.99 mg of gallic acid (mg GAE),.g^{-1} of uvaia pulp, and 1.38–2.46 mg of catechin (mg CAE),.g^{-1}, respectively. Silva et al. [38], Haminiuk et al. [39], Silva et al. [62], and Stafussa et al. [63] also obtained uvaia pulp extracts through conventional extraction methods. The total phenolic and flavonoids ranged from 0.25 to 4.83 mg of gallic acid (mg GAE).g^{-1} of uvaia pulp and 0.01–0.38 mg of catechin (mg CAE).g^{-1}, respectively, in those methods.

For U_S extracts, the differences between the predicted model and the

values observed of optimized conditions can be assigned to variations inherent to the equipment used on the U_S extractions. Such variations are attributed to fluctuations in temperature when extractions were carried out at temperatures close to 100 °C. It is well known that the PLE process is highly influenced by changes in temperature conditions, and small variations are acceptable for this type of process [21]. Although these variations occurred, PLE also efficiently obtained phenolic compounds and flavonoids from uvaia seed. In conventional extraction methods at proposed conditions, the total phenolic and flavonoids range from 1.35 to 3.37 mg of gallic acid (mg GAE),.g-1 of uvaia pulp, and 2.54–6.27 mg of catechin (mg CAE).g^{-1}, respectively (Table 7). Although the antioxidant activity increased with the total phenolic content in conventional extractions, ABTS values did not differ statistically for pulp extracts at 55 °C for 1 and 8 h. These results indicate that the phenolics exhibit a low propensity to donate hydrogen and capture the radical ABTS [28]. However, under these conditions, the antioxidant activity increased according to FRAP assay.

3.3. Compounds characterization and antioxidant activity

Table 8 shows the phenolic compounds and carotenoids identified in optimized conditions for U_P extracts (A and B) and U_S extracts ($S_{(P+F)}$). Although *p*-coumaric acid was not identified in the extracts at optimized conditions for both U_P and U_S, it was present in the hydrolyzed fraction of both U_P and U_S. This suggested that the conditions used during extractions (temperature and ethanol concentration) were insufficient to break the chemical bonds between *p*-coumaric acid and the matrix. Syringic acid is hydroxybenzoic acid present in complex structures of fruits such as lignin [63]. It was observed on PLE extracts (Table 8) but was absent on the raw materials (Table 3) as it was probably degraded under the harsh acid and alkaline conditions applied to their extraction [64,65]. Compared to conventional extraction, the high temperature and the use of ethanol percentage on the solvent during PLE extractions may have contributed to the onset of lignin depolymerization and disruption of lignin-phenolic bonds [66,67]. This degradation produces aldehydes that are subsequently transformed into phenolic acids associated with lignin, such as syringic acid, which is derived from syringaldehyde [66]. The same tendency was observed by Bagatini et al. [68] when comparing the phenolic profile of *Eugenia uniflora L.* leaf obtained by PLE and aqueous infusion. Also, quercetrin was found in U_S extracts and not in the seeds. The presence of ethanol in the optimized conditions for the U_S could partially contribute to the quercitrin extraction since this glycosylate form of quercetin [69] is soluble in ethanol [70]. Other studies, including those by Farias et al. [34,71] and Sganzerla et al. [37], identified variations in the phenolic profile. These differences may be related to the analytical technique used to evaluate the phenolic profile and to variations in the maturation stage of the fruits, as previously mentioned. In addition to phenolics, the carotenoids were identified at optimized conditions for U_P extracts. Overall, the carotenoids present in both U_P-optimized conditions were similar, except for the presence of β-carotene and the higher amounts of β-cryptoxanthin in extracts obtained at condition B. Moreover, the extracts at condition B (55 °C/70.0 % of ethanol) tended to present slightly higher amounts of carotenoid fractions than condition A (70 °C/ 60.0 % of ethanol) (Table 8). Although lutein, zeaxanthin, and β-cryptoxanthin present in the extracts have polar hydroxyl groups in their structure, their chain length [72,73] may have conferred greater solubility in the solvent (ethanol 70.0 %) used on the optimized condition B than flavonoids (ethanol 60.0 %). The absence of β-carotene in condition A is the main difference in the carotenoid profile found in the extracts. β-carotene is known for its sensitivity, with thermal degradation and oxidation the main causes of its losses [74]. In fact, condition A used a higher temperature extraction (70.0 °C) when compared to that used in condition B (55.0 °C), which may have led to β-carotene losses during the PLE extractions. The destabilization of sensitive bioactive compounds can still occur despite PLE extractions involving milder temperatures and shorter extraction times than conventional extraction methods [75]. Thus, some level of loss is expected during the process.

Both carotenoids and phenolic compounds are recognized for their antioxidant activity, which is related to their ability to react with free radicals [76]. All extracts showed antioxidant activity (Table 9) due to the presence of phenolic acids (ellagic, gallic syringic acid), carotenoids (in the case of extracts at conditions A and B), and the presence of flavonoids (isoquercitrin, quercitrin, and quercetin). The ellagic acid antioxidant activity is related to the presence of four hydroxyl groups and two lactones, which enables this molecule to scavenge the reactive oxygen species (ROS) through the transference of phenolic-H [77,78]. Phenolic acids have a phenol moiety in their structure that is largely reactive, but the main mechanism related to their antioxidant activity is as a hydrogen donator [78]. Flavonoids' antioxidant activity consists of their capacity to donate the hydrogen atom from hydroxyl groups and the conjugate structure, enabling good resonance and ROS stabilization [79]. Different from the phenolic compounds cited, the antioxidant activity of the carotenoids is not attributed to their capacity to donate hydrogens, but they are good quenchers of singlet oxygen and peroxyl radical due to the presence of a highly conjugated isoprenyl chain [79]. According to the data shown in Table 9, $S_{(P+F)}$ showed higher antioxidant activity, while the antioxidant activities for extracts at conditions A and B were statistically the same. This result is in accordance with the results presented in Section 3.2, which showed higher total phenolics and total flavonoid content for U_S extracts. The higher antioxidant activity of $S_{(P+F)}$ could be related to the high levels of ellagic and gallic acid

Table 8
Phenolics and carotenoids profile of the U_P (A and B) and U_S (S_{S+F}) extracts at optimized conditions. Description: This table describes the profile of the phenolics and carotenoids of the extracts obtained from U_P and U_S under optimized conditions.

Bioactive class	Bioactive compound	A	B	$S_{(P+F)}$
Phenolics (μg. mL^{-1})	Gallic Acid	2.3 ± 0.00[a]	2.2 ± 0.00[a]	7.3 ± 0.00[b]
	Syringic Acid	0.3 ± 0.00[a]	0.3 ± 0.00[a]	1.2 ± 0.00[b]
	Ellagic Acid	14.1 ± 0.00[a]	14.2 ± 0.00[a]	26.6 ± 0.00[b]
	Isoquercetin	2.2 ± 0.00[a]	0.4 ± 0.00[a]	ND
	Quercetin	0.2 ± 0.00[a]	0.2 ± 0.00[a]	0.7 ± 0.00[b]
	Quercetrin	1.9 ± 0.00[a]	1.9 ± 0.00[a]	0.1 ± 0.00[b]
Carotenoids (μg. mL^{-1})	Lutein	0.09 ± 0.03[a]	0.11 ± 0.01[a]	ND
	Zeaxanthin	0.18 ± 0.01[a]	0.24 ± 0.03[a]	ND
	β-cryptoxanthin	0.04 ± 0.01[b]	0.13 ± 0.02[a]	ND
	β -carotene	ND	0.02 ± 0.00[a]	ND

*In a line, different letters indicate samples statically different for $p < 0.05$
A:70 °C, 60.0 % of ethanol; B: 55 °C, 70.0 % of ethanol; $S_{(S+F)}$: U_S seed extracts at optimized conditions.
ND: not detected.

Table 9
Antioxidant activity (μM Trolox.$g^{-1}$of dry sample) for U_P and U_S extracts at optimized conditions. Description: This table shows the antioxidant activity (μM Trolox.$g^{-1}$of dry sample) for U_P and U_S extracts at optimized conditions.

Sample	FRAP (μM Trolox.$g^{-1}$of dry sample)	ABTS (μM Trolox.$g^{-1}$of dry sample)
A	231.41 ± 8.95[a]	72.54 ± 3.45[a]
B	189.72 ± 8.44[a]	56.54 ± 4.22[a]
$S_{(P+F)}$	389.00 ± 27.66[b]	507.40 ± 26.80[b]

*In a column, different letters indicate samples statically different for $p < 0.05$
A: U_P extracts at 70 °C and 60.0 % of ethanol; B: U_P extracts at 55 °C and 70.0 % of ethanol; $S_{(P+F)}$: U_S extracts at optimized conditions.

when compared to the amounts of these components in extracts at conditions A and B. Ellagic acid is a potent antioxidant compared to ascorbic acid and α-tocopherol [77]. Also, due to the hydroxyl groups on meta positions (carbon 3 and 5), gallic acid has higher antioxidant activity than syringic acid [80]. Apart from the differences in extract composition, the distinct mechanism of action of the ABTS and FRAP methods lead to different results. The ABTS method measures the scavenging activity of antioxidants in the extracts towards a stable free radical ($ABTS^{\bullet+}$ cation), reflecting the antioxidant capacity to donate a hydrogen atom and thus can capture hydrophilic compounds, such as phenolic acids [28]. In contrast, FRAP assesses the capacity of the antioxidant present in the extract to donate an electron, reducing the complex ferric tripyridyltriazine complex (Fe(III)-TPTZ) to ferrous complex (Fe(II)-TPTZ) [81], being this method suitable to recovery flavonoids.

4. Conclusion

Uvaia fruit has an excellent nutritional composition, with the pulp containing higher levels of macronutrients than the seeds. The bioactive composition was similar between the pulp and seed extracts, except for the absence of carotenoids in the last one. The bioactives found in higher concentrations were gallic and ellagic acid. The predicted extraction models showed that both temperature and solvent (ethanol) percentages of PLE influenced total phenolic extraction from U_P and total phenolic and total flavonoid extraction from the seeds. The optimized conditions were observed at 55 ºC and 70.0 % ethanol for the pulp and 50.0 % ethanol and 90 ºC for the seeds. The presence of ethanol improved the extraction, and the high temperatures enhanced the breakdown of the linkages between bioactive and U_P and U_S matrix. Conversely, high solvent concentrations negatively affected the extraction of total flavonoids. The extracts obtained from U_S showed higher amounts of total phenolic and total flavonoid content, resulting in higher antioxidant activity for the seeds. The results of this study indicate that Pressurized Liquid Extraction with ethanol and water efficiently extracts phenolics and flavonoids from U_P and U_S, and is a promising alternative to traditional extraction methods. Moreover, the high antioxidant activity of U_S extracts can contribute to developing new products in cosmetics, food, and pharmaceutical areas and to the valorization of the fruit.

CRediT authorship contribution statement

Manuela Cristina Pessanha de Araújo Santiago: Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Renata Galhardo Borguini:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Ana Clara Troya Raineri Fiocco:** Investigation. **Cristiane Conte Paim de Andrade:** Writing – review & editing, Validation, Methodology. **Luiz Henrique Fasolin:** Writing – review & editing, Supervision, Resources, Conceptualization. **Carolina Siqueira Franco Picone:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Rafael Augusto Juliato:** Investigation, Formal analysis. **Isadora de Souza Lopes:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.supflu.2024.106498.

Data Availability

Data will be made available on request.

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