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Valorization of rambutan (*Nephelium lappaceum* L.) peel: Chemical composition, biological activity, and optimized recovery of anthocyanins

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ABSTRACT

Chemical constituents and bioactive properties of rambutan (*Nephelium lappaceum* L.) peel were characterized and heat-/ultrasound-assisted extractions (HAE/UAE) of anthocyanins were optimized by response surface methodology. Five organic acids, the α-, γ-, and δ-tocopherol isoforms, and twenty-five fatty acids (36.8 % oleic acid) were identified, as well as a phenolic profile composed of ellagitannin derivatives, geraniin isomers, ellagic acid, and delphinidin-*O* derivatives. The extract showed antioxidant activity *via* lipid peroxidation (IC_{50} = 2.79 ± 0.03 μg/mL) and oxidative hemolysis (IC_{50} = 72 ± 2 μg/mL) inhibition, and displayed antibacterial and antifungal properties (MIC ≤ 1 mg/mL). On the other hand, no cytotoxicity was observed in tumor and non-tumor cell lines up to 400 μg/mL. The recovery of anthocyanins was more effective using HAE than UAE, allowing greater yields (16.2 mg/g extract) in just 3 min and using lower ethanol proportions. Overall, rambutan peel could be upcycled into bioactive ingredients and natural colorants for industrial applications.

1. Introduction

The tropical fruit market has been growing worldwide in recent years. The main producers of these fruits are from low-income developing countries, namely from Latin America, Asia, and some African countries. Although the tropical fruit trade represents only 3 % of world agricultural trade, these fruits have high export values, which places them in the third most valuable fruit group (Cádiz-Gurrea et al., 2020). Tropical fruits are sought after by consumers due to their exotic character, unique flavor and aroma, nutritional value, and, in some cases, also for their bioactive properties (Cádiz-Gurrea et al., 2020; Cheok et al., 2018). The supply of this type of fruit is mainly intended for fresh consumption, although processing industries have been established. In this last case, processing can be seen as an alternative to enable the commercialization of low-shelf-life fruits around the world. On the other hand, it can generate a large amount of by-products with no commercial value and be responsible for causing environmental and social impacts (Cheok et al., 2018; Fraga-Corral et al., 2021). To overcover the ecological-economic issues associated with the disposal of such by-products and to recover high value-added compounds with potential for application as bioactive ingredients or colorants in pharmaceutical, cosmetical, and food products, current research has been designed with a focus on the complete reuse or upcycling of agro-industrial food-grade waste and by-products (Cheok et al., 2018). Interestingly, by-products that are normally composed of inedible fruit parts, such as seeds and peels, may have higher levels of bioactive compounds than the edible fruit pulp (Cádiz-Gurrea et al., 2020).

Rambutan (*Nephelium lappaceum* L.) belongs to the Sapindaceae family and is native from Southeast Asia, being also cultivated in other tropical regions, such as America Latina and Australia. This tropical fruit is appreciated for its exotic appearance and the pleasant taste of its transparent whitish pulp (Fig. 1) (Hernández-Hernández et al., 2019;

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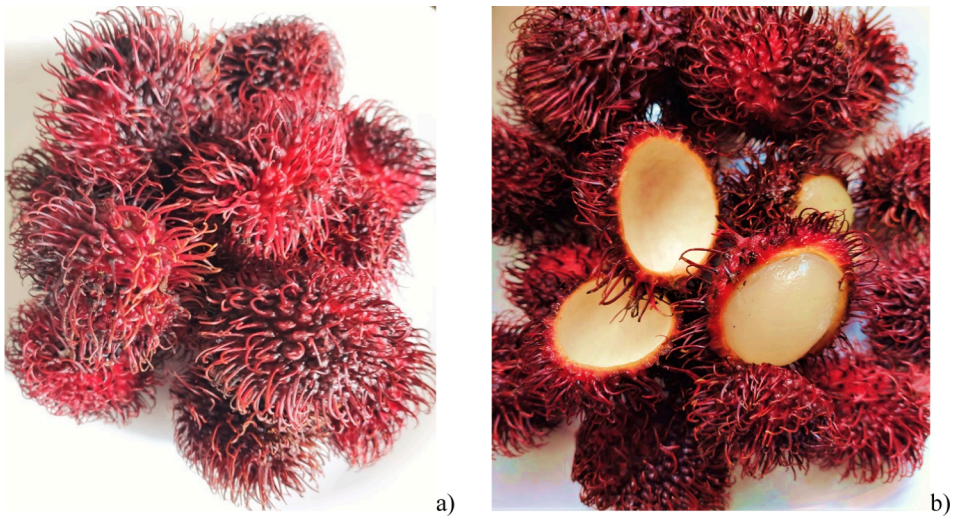

Fig. 1. Rambutan a) whole fruits and b) peels.

Jahurul et al., 2020). In 2018, the global production of rambutan reached 2.5 million tons and this amount is expected to grow by 3.6 % by 2023 (Mota et al., 2020). Rambutan is mostly consumed fresh, but it has also been used to produce liquor, canned goods, syrup, and juice, among others. However, when processing this fruit, its inedible thick epicarp and unique seed, which correspond to 37–62 % and 4–9 % of the whole fruit, respectively, are discarded as useless by-products (Cheok et al., 2018). Rambutan seed has a bitter taste and in Asia it is commonly consumed roasted and used in the formulation of chocolate bleeds (Hernández-Hernández et al., 2019; Jahurul et al., 2020). On the other hand, the fruit peel has not been explored (Hernández et al., 2017; Maran et al., 2017). Despite this, some studies have shown that it is rich in bioactive compounds, namely ellagitannins, and has several bioactivities, showing potential application in the food and pharmaceutical industries, among other sectors (Chollakup et al., 2020; Hernández et al., 2017; Yuvakkumar et al., 2014).

The present work aimed to characterize the rambutan peel composition in organic acids, tocopherols, fatty acids, and phenolic compounds. Furthermore, a hydroethanolic extract was prepared for evaluation of antioxidant activity through cell-based *in vitro* assays, anti-inflammatory activity *via* nitric oxide production inhibition, cytotoxicity in tumor and non-tumor cell lines, and antimicrobial effects against foodborne bacterial and fungal strains. In order to obtain an anthocyanin-rich extract, heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE) of these natural pigments were optimized using response surface methodology (RSM).

2. Material and methods

2.1. Plant material

Red rambutans cultivated in Ilheus/Bahia, North-eastern Brazil, were purchased from "Companhia de Entrepostos e Armazéns Gerais de São Paulo" (CEAGESP, São Paulo, Brazil). Ripe red fruits weighing about 37 ± 4 g and measuring 53 ± 4 mm in length and 42 ± 3 mm in diameter (Fig. 1) were cleaned from foreign materials and the red epicarp (48 ± 3 % of the whole fruit) was separated from the pulp, lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) until complete dryness (24 ± 2 % of dry weight), and reduced to a homogeneous fine powder that was stored at −20 °C until use.

2.2. Chemical composition analysis

2.2.1. Organic acids

Organic acids were extracted from the dry peel powder (1.5 mg) using 4.5 % *meta*-phosphoric acid (25 mL) as solvent and analyzed by ultra-fast liquid chromatography (UFLC, Shimadzu 20A series, Kyoto, Japan) coupled to a photodiode array detector (PDA). The sample preparation and chromatographic conditions were previously descried by Barros et al. (2013). The detected compounds were quantified by chromatographic comparison of the peak area with calibration curves obtained from the standards of oxalic acid ($y = 1x10^7x + 231891$, $r^2 = 0.9999$, limit of detection (LOD) = 6.3 μg/mL, limit of quantification (LOQ) = 20.8 μg/mL), shikimic ($y = 7 \times 10^7x + 175156$, $r^2 = 0.9999$, LOD = 10.2 μg/mL, LOQ = 56.5 g/mL), ascorbic ($y = 4 \times 10^7x + 1 \times 10^6$, $r^2 = 0.9909$, LOD = 0.29 μg/mL, LOQ = 0.96 μg/mL), citric ($y = 1 \times 10^6x + 10277$, $r^2 = 0.9997$; LOD = 4.4 μg/mL, LOQ = 14.5 μg/mL), and fumaric ($y = 185062x + 117588$, $r^2 = 0.9986$, LOD = 42.5 μg/mL, LOQ = 141.7 μg/mL) acids. The results were expressed in g/100 g of dry weight (dw).

2.2.2. Tocopherols

The extraction of tocopherols from the dried peel powder and their subsequent analysis in a high-performance liquid chromatography system (HPLC, Knauer, Berlin, Germany) connected to a fluorescence detector (FL-2020, Jasco, Japan) were carried out according to the protocol previously described by Barros et al. (2013). Tocol (Matreya, Pleasant Gap, PA, USA) was used as internal standard. Calibration curves used in the quantification process: α-tocopherol ($y = 1.295x$, $r^2 = 0.991$, LOD = 18.06 ng/mL, LOQ = 60.20 ng/mL), γ-tocopherol ($y = 0.567x$, $r^2 = 0.991$, LOD = 14.79 ng/mL, LOQ = 49.32 ng/mL), and δ-tocopherol ($y = 0.678x$, $r^2 = 0.992$, LOD = 20.09 ng/mL, LOQ = 66.95 ng/mL). The results were expressed in mg/100 g dw.

2.2.3. Fatty acids

After obtaining a lipid extract from the dry peel powder by Soxhlet extraction with petroleum ether, the fatty acids were transesterified and the fatty acid methyl esters (FAME) were analyzed by gas–liquid chromatography with flame ionization detection (GC-FID) in a Dani GC 1000 (Milan, Italy) as described by Barros et al. (2013). Fatty acids were identified by comparing the relative retention times of the sample FAME peaks with the standard 47885-U (Sigma-Aldrich, St. Louis, MO, USA). The results were expressed in relative percentage of each fatty acid.

2.2.4. Phenolic compounds

The rambutan peel extracts used in the identification and quantification of phenolic compounds were obtained by solid–liquid extraction with an ethanol/water mixture (80:20, *v/v*), which was acidified with 0.1 % citric acid (1 μM) for the extraction of anthocyanins, as described by Albuquerque, Pinela, et al. (2020). The analysis was made on a Dionex Ultimate 3000 HPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a diode-array detector (DAD) and a mass spectrometer (MS, Thermo Scientific, San Jose, CA, USA) with an electrospray ionization (ESI) source. The technical specifications of the equipment and the chromatographic conditions used in the analysis of the non-anthocyanin and anthocyanin compounds were previously described by Bessada et al. (2016) and Gonçalves et al. (2017), respectively. Data were collected and analyzed using Xcalibur™ software (Thermo Finnigan). The results were expressed as mg/g extract (E) and mg/g dw.

2.3. Bioactivity assessment

2.3.1. Antioxidant activity

Two cell-based assays were used to evaluate the *in vitro* antioxidant activity of the extract, namely the thiobarbituric acid reactive substances (TBARS) formation inhibition assay (Corrêa et al., 2015) and the oxidative hemolysis inhibition assay (OxHLIA) (Lockowandt et al., 2019). Trolox was used as a positive control. The results were expressed as IC_{50} values, which correspond to the extract concentration (μg/mL) that provides 50 % of lipid peroxidation inhibition (in TBARS assay) or required to keep 50 % of the erythrocyte population intact for a 60 min Δt (in OxHLIA).

2.3.2. Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the ability to inhibit the production of nitric oxide (NO) by lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, as described by Corrêa et al. (2015). Dexamethasone was used as a positive control. The results were expressed as EC_{50} values (μg/mL), which correspond to the extract concentration that induces 50 % inhibition of NO production.

2.3.3. Cytotoxic activity

The cytotoxic activity of the extract (1.56–400 μg/mL) was tested in four human tumor cell lines (NCI-H460 - lung carcinoma, MCF-7 - breast carcinoma, HepG2 - hepatocellular carcinoma, and HeLa - cervical carcinoma) and in a non-tumor porcine liver primary cell culture (PLP2) by the sulforhodamine B (SRB) assay (Corrêa et al., 2015). Ellipticine was used as a positive control. The results were expressed as GI_{50} values, which correspond to the extract concentration (μg/mL) required to inhibit 50 % of cell proliferation.

2.3.4. Antimicrobial activity

The antimicrobial activity of the extract was evaluated against the bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (food isolate), *Listeria monocytogenes* (NCTC 7973), *Escherichia coli* (ATCC 35210), *Salmonella enterica* subsp. *enterica* serovar Typhimurium.

(ATCC 13311), and *Enterobacter cloacae* (ATCC 35030), and the microfungi *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum* var. *cyclopium* (food isolate) and *Trichoderma viride* (IAM 5061). The assays were followed as described by Corrêa et al. (2015) and used to determine minimal inhibitory concentrations (MIC, mg/mL) and minimal bactericidal or fungicidal concentrations (MBC and MFC, respectively, mg/mL). All microorganisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Serbia. The commercial food preservatives sodium benzoate (E211) and potassium metabisulfite (E224) were used as positive controls.

2.4. Optimization of anthocyanin extraction using RSM

2.4.1. Experimental design

A central composite rotatable design (CCRD) was implemented to optimize the extraction of anthocyanins from rambutan peel by heat-assisted extraction (HAE) and ultrasound-assisted extraction (UAE) using RSM. As independent variables, time (X_1, *t*), temperature (X_2, *T*), and solvent (X_3, *S*) were used for HAE, and time (X_1, *t*), ultrasonic power (X_2, *P*), and solvent (X_3, *S*) were used for UAE. These independent variables and their range of values were selected based on previous studies (Albuquerque, Pinela, et al, 2020; López et al., 2018; Rocha et al., 2020), as they have been shown to have significant effects on the tested extraction methods. UAE involved a shorter extraction time than HAE, as this unconventional method is commonly reported to be more efficient in mass transfer due to cell wall disruption caused by acoustic cavitation (Das et al., 2022). Furthermore, longer sonication times can overheat the extractive mixture and lead to degradation of thermosensitive constituents (Das et al., 2022). The extraction yield (Y_1: %, *w/w*) and anthocyanin content (Y_2: mg/g extract, E) were the dependent or response variables considered for optimization.

2.4.2. Heat-assisted extraction (HAE)

A carousel system (Carousel Tech, 230–240 V, 50–60 Hz, 825 W, Radleys, Essex, United Kingdom) with stirring was used for HAE. Peel powder (0.6 g) was stirred with 20 mL of solvent (0–100 % ethanol) acidified with 0.1 % citric acid (1 μM) (Albuquerque, Pinela, et al., 2020) and extracted under different times (X_1: 2–90 min), temperatures (X_2: 20–90 °C), and solvent proportions (X_3: 0–100 %), according to the 20 run CCRD matrix. After extraction, the samples were centrifuged and the liquid phase was collected and concentrated in a rotary evaporator to remove ethanol, and the aqueous phase was lyophilized.

2.4.3. Ultrasound-assisted extraction (UAE)

UAE was caried out using an ultrasonic homogenizer (model CY-500, 20 kHz, Optic Ivymen System, Barcelona, Spain) equipped with a titanium probe as previously described (Albuquerque, Pinela, et al., 2020) and using an ice bath to avoid overheating of extractive mixtures. Peel powder (3 g) was mixed with 100 mL of acidified solvent (0–100 %) and sonicated for a different time (X_1: 2–45 min), power (X_2: 5–500 W), and solvent (X_3: 0–100 % ethanol), according the CCRD matrix. Then, the mixtures were processed as described for HAE.

2.4.4. Analysis of dependent variables

The extraction yields (Y_1: %, *w/w*) were determined gravimetrically and calculated as the ratio between extract weight and dry peel weight, as previously described by Albuquerque, Pinela, et al. (2020). For anthocyanins quantification (Y_2: mg/g extract, E), 10 mg of the lyophilized extracts was redissolved in 2 mL of the same extraction solvent, filtered through 0.22 μm filter disks, and analyzed as described in subsection 2.2.4.

2.5. RSM modelling and statistical analysis

All experiments were performed in triplicate and results were expressed as mean values and standard deviation (SD). Design-Expert software, version 11 (Stat-Ease, Inc., Minneapolis, USA) was used for RSM analysis and to generate the 3D graphs. The significance of the models and their terms was assessed by an analysis of variance (ANOVA) at a 95 % confidence level, and only the significant terms or those necessary for the hierarchy were considered in the models fitting. The lack-of-fit and the coefficients R^2 and R^2_{adj} were used to assess the model-fitting adequacy. To obtain optimal extraction conditions, the numerical optimization criteria were as follows: all independent variables were analyzed in the full range (from −1.68 to 1.68) and had the same importance; the "maximize" option was selected for each response variable; for the global optimum, the same importance was given to Y_1

Table 1
Rambutan peel composition in organic acids, tocopherols, and fatty acids.

	Content
Organic acids (g/100 g dw)	
Oxalic acid	0.55 ± 0.05
Shikimic acid	0.97 ± 0.01
Ascorbic acid	0.371 ± 0.001
Citric acid	0.503 ± 0.002
Fumaric acid	tr
Total organic acids	2.40 ± 0.03
Tocopherols (mg/100 g dw)	
α-tocopherol	0.68 ± 0.02
γ -tocopherol	6.0 ± 0.4
δ-tocopherol	1.05 ± 0.04
Total tocopherols	7.7 ± 0.4
Fatty acids (%)	
Capric acid (C10:0)	0.215 ± 0.004
Undecylic acid (C11:0)	0.176 ± 0.006
Lauric acid (C12:0)	0.379 ± 0.007
Tridecylic acid (C13:0)	0.137 ± 0.004
Myristic acid (C14:0)	0.508 ± 0.01
Palmitic acid (C16:0)	26.1 ± 0.2
Palmitoleic acid (C16:1)	0.610 ± 0.008
Heptadecanoic acid (C17:0)	0.556 ± 0.004
Stearic acid (C18:0)	7.0 ± 0.1
Oleic acid (C18:1n9)	36.8 ± 0.6
Linolelaidic acid (C18:2n6)	0.166 ± 0.008
Linoleic acid (C18:2n6)	16.21 ± 0.01
α-Linolenic acid (C18:3n3)	3.7 ± 0.2
Arachidic acid (C20:0)	0.60 ± 0.02
Eicosenoic acid (C20:1)	1.10 ± 0.01
Arachidonic acid (C20:4n6)	0.55 ± 0.01
Eicosatrienoic acid (C20:3n3)	1.18 ± 0.05
Behenic acid (C22:0)	0.84 ± 0.04
Erucic acid (C22:1)	0.201 ± 0.001
Eicosapentaenoic acid (C20:5n3)	0.289 ± 0.004
cis-13,16-Docosadienoic acid (C22:2)	0.378 ± 0.009
Tricosylic acid (C23:0)	0.264 ± 0.004
Lignoceric acid (C24:0)	1.26 ± 0.05
Nervonic acid (C24:1)	0.438 ± 0.02
Docosahexaenoic acid (C22:6n3)	0.27 ± 0.01
Fatty acid classes (%)	
SFA	38.1 ± 0.4
MUFA	39.1 ± 0.6
PUFA	22.8 ± 0.2
PUFA/SFA	0.598 ± 0.001
n6/n3	3.1 ± 0.1

tr: traces; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated acids.

and Y_2; and optimal solutions with greatest desirability were chosen.

3. Results and discussion

3.1. Chemical composition

3.1.1. Organic acid composition

The rambutan peel was characterized for its composition in organic acids and the results are shown in Table 1. Among the five organic acids detected, shikimic acid was the most abundant (40.4 %), followed by oxalic (23 %) and citric (21 %) acids. Ascorbic acid was quantified in a lower concentration (15 %), while only traces of fumaric acid were detected. In rambutan pulp, citric acid has been described as the major organic acid (0.29–0.68 g/100 g fresh weight (fw), while lower amounts of other organic acids such as lactic (0.10–0.29 g/100 g fw), malic (0.04–0.07 g/100 g fw), tartaric (0.03–0.04 g/100 g fw), and ascorbic (0.01–0.13 g/100 g fw) acids have been described (Chai et al., 2018). In the present study, malic acid was not found in rambutan peel. As expected, the chemical composition of fruits, including the distribution of primary and secondary metabolites, varies depending on the tissue under analysis. In addition, the chemical composition of fruits of the same species can be influenced by agricultural practices and edaphoclimatic factors of the growing sites, such as climatic conditions, soil type and fertility, among others (Monrroy et al., 2020). Thus, the red rambutan fruits analyzed in this work were produced in Northeastern Brazil and, therefore, they may differ in compositional aspects from others grown in different locations.

3.1.2. Tocopherol composition

The rambutan peel composition in tocopherols is shown in Table 1. Three isoforms were detected and identified as α-, γ-, and δ-tocopherols. α-Tocopherol was quantified in the smallest amount (~9%), while γ-tocopherol was the most abundant vitamer (~78 %). In total, this fruit by-product contained 7.7 ± 0.4 mg/100 g dw of total tocopherols. According to the literature, 100 g portions of fresh edible pulp of rambutan contain about 17 μg of γ-tocopherol and 10 μg of α-tocopherol (Isabelle et al., 2010), while the seed contains about 103 μg/100 g of α-tocopherol (Jahurul et al., 2020). When converting the results of the present study to fresh weight (fw), 100 g of rambutan peel yield 0.19 mg of α-tocopherol, a value higher than those described in the literature for other parts of this fruit (Isabelle et al., 2010; Jahurul et al., 2020). To the best of the authors' knowledge, there are no reports on the presence of δ-tocopherol in the pulp.

3.1.3. Fatty acid composition

A low amount of fat (2.0 ± 0.5 g/100 g dw) was obtained from rambutan peel, and the fatty acids detected in its composition are listed in Table 2. Twenty-five fatty acids were detected, including twelve saturated fatty acids (SFA, 38.1 %), five monounsaturated fatty acids (MUFA, 39.1 %) and eight polyunsaturated fatty acids (PUFA, 22.8 %). The lipid profile consisted mainly of unsaturated fatty acids (MUFA + PUFA, 61.8 %), similarly to that previously described for rambutan seeds (Araujo et al., 2021). The oleic acid (36.8 %) was the most abundant fatty acids, followed by palmitic (26.1 %) and linolenic (16.21 %) acids. Therefore, this fruit by-product has a fatty acid profile that may be associated with beneficial effects for the maintenance of good health, such as the presence of omega 3 and 6 fatty acids and oleic acid, which has been correlated with the reduction of low-density lipoprotein cholesterol (LDL-C) (Araujo et al., 2021). Moreover, the PUFA/SFA (>0.45) and n6/n3 (<4) ratios can be considered good for cardiovascular health (Araujo et al., 2021; Candela et al., 2011).

3.1.4. Profile in phenolic compounds

The phenolic profile of rambutan peel was characterized by HPLC-DAD-ESI/MS^n and the chromatographic data, namely retention time (Rt, min), maximum absorptions (λ^{max}, nm), pseudomolecular ion ($[M-H]^-$/$[M]^+$), and the main fragment ions in tandem MS^2 are presented in Table 3, as well as the quantification results. Seven phenolic compounds were detected and grouped as non-anthocyanins and anthocyanins, more specifically as ellagic acid derivatives (compounds 1 to 5) and *O*-glycosylated cyanidin derivatives (compounds 6 and 7). Regarding non-anthocyanin compounds, compound 1 showed a $[M-H]^-$ ion at *m/z* 649, released fragment ions at *m/z* 631, *m/z* 613, *m/z* 335, and *m/z* 301, revealing an HHDP moiety. Although it was not possible to elucidate this compound with the data obtained by mass spectrum analysis, the presence of HHDP molecules suggested that it is an ellagitannin derivative. Compound 3 presented a pseudomolecular ion $[M-H]^-$ at *m/z* 953, following MS^2 fragment ions at *m/z* 933, *m/z* 633, *m/z* 463, and *m/z* 301, which are characteristic to the successive loss of two HHDP residues, gallic acid, and hexosyl moiety, respectively. Despite the mass spectrum data, as observed for compound 1, it was not possible to clearly identify this compound with literature data.

Compounds 2 and 4 presented the same pseudomolecular ion at $[M-H]^-$ *m/z* 951, releasing in MS^2 four ion fragments at *m/z* 933, *m/z* 613, *m/z* 462, and *m/z* 301. Both compounds has been reported in rambutan fruit, being tentatively identified as geraniin isomer I (2) and isomer II (4) (Hernández et al., 2017; Palanisamy et al., 2011; Phang et al., 2019; Zhuang et al., 2017). Compound 5 showed a

Table 2
Content of the non-anthocyanin and anthocyanin compounds tentatively identified in rambutan peel extract. The retention time (Rt), wavelengths of maximum absorption in the visible region (λ^{max}), and mass spectral data are presented.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$/ $[M]^+$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Type of identification/ Reference	Content (mg/g)	
							Extract (E)	Dry weight (dw)
Non-anthocyanin compounds								
1	6.26	278	649/-	631(100), 613(32), 479 (10), 335(12), 301(10)	Ellagitannin derivative I	DAD-MS^n	4.4 ± 0.2	1.53 ± 0.08
2	10.01	277	951/-	933(100), 613(5), 462(5), 301(10)	Geraniin isomer I	Hernández et al. (2017), Zhuang et al. (2017), Palanisamy et al. (2011)	6.322 ± 0.001	2.209 ± 0.001
3	14.23	270	953/-	933(100), 633(12), 463 (21), 301(45)	Ellagitannin derivative II	DAD-MS^n	4.4 ± 0.3	1.5 ± 0.1
4	17.18	254/ 361	951/-	933(100), 613(5), 462(5), 301(10)	Geraniin isomer II	Hernández et al. (2017), Zhuang et al. (2017), Palanisamy et al. (2011)	7.7 ± 0.6	2.7 ± 0.2
5	17.95	255/ 366	301/-	284(32), 257(100), 185 (32), 173(10), 157(5), 145 (7)	Ellagic acid	DAD-MS^n/Hernández et al. (2017), Zhuang et al. (2017)	8.779 ± 0.001	3.067 ± 0.001
					Total non-anthocyanin compounds		31.6 ± 0.5	11.0 ± 0.2
Anthocyanin compounds								
6	13.26	515	-/783	303(100)	Delphinidin-*O* derivative	DAD-MS^n	4.98 ± 0.02	1.52 ± 0.08
7	15.66	515	-/783	303(100)	Delphinidin-*O* derivative	DAD-MS^n	6.59 ± 0.06	2.209 ± 0.001
					Total anthocyanin compounds		11.57 ± 0.08	4.13 ± 0.03

Standards used in quantification: ellagic acid ($y = 26719x - 317255$, $r^2 = 0.9986$; LOD = 0.41 μg/mL, LOQ = 1.24 μg/mL) for peaks 1–5; and cyanidin-3-*O*-glucoside ($y = 134578x - 3 \times 10^6$, $r^2 = 0.9956$, LOD = 0.25 μg/mL, LOQ = 0.83 μg/mL) for peaks 6 and 7.

Table 3
Antimicrobial activity of rambutan peel extract and positive controls.

	Peel extract		E211		E224	
Antibacterial activity (mg/mL)	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria						
Staphylococcus aureus	1.00	2.00	4.00	4.00	1.00	1.00
Bacillus cereus	1.00	2.00	0.50	0.50	2.00	4.00
Listeria monocytogenes	1.00	2.00	1.00	2.00	0.50	1.00
Gram-negative bacteria						
Escherichia coli	1.00	2.00	1.00	2.00	0.50	1.00
Salmonella Typhimurium	1.00	2.00	1.00	2.00	1.00	1.00
Enterobacter cloacae	0.50	1.00	2.00	4.00	0.50	0.50
Antifungal activity (mg/mL)	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus fumigatus	1.00	2.00	1.00	2.00	1.00	1.00
Aspergillus versicolor	0.25	0.50	2.00	2.00	1.00	1.00
Aspergillus niger	0.50	1.00	1.00	2.00	1.00	1.00
Penicillium funiculosum	0.50	1.00	1.00	2.00	0.50	0.50
Penicillium verrucosum var. *cyclopium*	0.50	1.00	2.00	4.00	1.00	1.00
Trichoderma viride	0.50	1.00	1.00	2.00	0.50	0.50

MIC: minimal inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration.

pseudomolecular ion $[M-H]^-$ at *m/z* 301 and characteristic MS^2 fragments coherently with the available standard of ellagic acid; the information previous described by Hernández et al. (2017) and Zhuang et al. (2017) for rambutan peel was also used in the identification of this compound.

According to the literature, hydrolysable tannins are the main class of compounds found in rambutan fruit, and the main compound identified in its peel are geraniin, corilagin, and ellagic acid (Hernández et al., 2017; Thitilertdecha et al., 2010). However, corilagin was not detected in the samples of the present study.

Concerning the total of non-anthocyanin phenolic compounds, rambutan peel extract showed a composition rich in ellagitannin derivatives, highlighting the high amount of geraniin isomers I and II, which totalized about 14 and 4.9 mg/g of extract and dry peel, respectively. Moreover, ellagic acid was found in reasonable quantities, and low amounts of unidentified ellagitannins I and II (compounds 1 and 3, respectively) were quantified. In total, the rambutan peel had an amount of 31.6 and 11 mg of non-anthocyanin phenolic compounds, namely ellagitannin derivatives, per gram of extract and dry peel, respectively. The profile of phenolic compounds of rambutan peel can be highly influenced by the edaphoclimatic conditions of the plant growth sites (Hernández et al., 2017), but also by the extraction methods. For example, Mexican rambutan shown a higher amount of total phenolics (582 mg gallic acid equivalent (GAE)/g dw) (Hernández et al., 2017) than rambutan cultivated in other regions, such as Vietnam (91.7–171.1 mg GAE/g dw) (Phuong, Li, Van Camp, et al., 2020), Panama (340 mg GAE/g dw) (Monrroy et al., 2020), and India (552.64 mg GAE/100 g dw) (Maran et al., 2017). However, these results cannot be compared with those of the present work as they were quantified using different methods. To the best of the authors' knowledge, there are no data in the literature reporting the phenolic composition of rambutan cultivated in Brazil.

Regarding anthocyanins, two compounds (6 and 7) were detected with the same pseudomolecular ion $[M]^+$ at *m/z* 783 and a unique fragment ion at *m/z* 303, which corresponded to delphinidin aglycone, being tentatively identified as a delphinidin-*O* derivative. To the best of the authors' knowledge, the presence of this compound in rambutan peel extract is not described in the literature. As shown in Table 2, the extract had a total amount of anthocyanins of 11.57 ± 0.08 mg/g E, which was equivalent to the extraction of 4.13 ± 0.03 mg/g dw.

Studies on the characterization of anthocyanins in rambutan fruit are scarce. Hernández et al. (2017) reported pelargonidin in purified aqueous extract of rambutan peel cultivated in Mexico. Sun et al. (2011) determined the total amount of anthocyanins in purified hydroethanolic extract of rambutan peel by the pH differential method and, according to the authors, a total of 1.81 mg of anthocyanins per gram of fresh peel was quantified. Ultrasound-assisted extraction has been proposed and optimized by Maran et al. (2017) to obtain phenolic compounds, including anthocyanin, from rambutan peel grown in India. According to these authors, it was possible to obtain about 1.026 mg/g dw of total anthocyanins when sonicating the rambutan peel at 20 W for 20 min at a temperature of 50 °C using non-acidified aqueous solvent. On the other hand, a low concentration (0.7 mg/g dw) of anthocyanin was detected in

an ethanolic extract obtained by macerating rambutan peel from Panama (Monrroy et al., 2020). It is worth noting that the results of the mentioned studies were obtained through spectrophotometric methods, which may explain the quantitative differences.

3.2. Bioactive properties

3.2.1. Antioxidant activity

The antioxidant activity of rambutan peel extract was evaluated by the cell-based TBARS and OxHLIA assays, which assess the extract capacity to inhibit lipid peroxidation (by monitoring the formation of malondialdehyde and other TBARS) and to delay oxidative hemolysis, respectively. Regarding the ability to inhibit lipid peroxidation, the peel extract showed higher antioxidant activity ($IC_{50} = 2.79 \pm 0.03$ μg/mL) than the positive control Trolox ($IC_{50} = 5.8 \pm 0.6$ μg/mL). On the other hand, the extract concentration required to protect 50 % of the sheep erythrocyte population for a 60 min Δt ($IC_{50} = 72 \pm 2$ μg/mL) was higher than that of the positive control ($IC_{50} = 21.8 \pm 0.2$ μg/mL). These results are somehow promising when compared with other natural extracts obtained from fruits/fruit by-products, such as red raspberry (*Rubus idaeus* L.) (IC_{50} values of 28–48 μg/mL for TBARS and 449–520 μg/mL for OxHLIA) (Rocha et al., 2020), passion fruit (*Passiflora edulis* Sins) peel (IC_{50} values of 115–136 μg/mL for TBARS and 78–144 μg/mL for OxHLIA) (Ghada et al., 2020), mulberry (*Morus nigra* L.) seeds (IC_{50} values 23 ± 2 μg/mL for TBARS and 46.0 ± 0.8 μg/mL for OxHLIA), and grape (*Vitis vinifera* L.) seeds (IC_{50} values 168 ± 3 μg/mL for TBARS and 70 ± 1 μg/mL for OxHLIA) (Gómez-Mejía et al., 2021).

The antioxidant proprieties of rambutan peel have been reported in some studies. For example, rambutan peel extract has been added in edible films based on pectin extracted from *Citrus junos* pomace and blending of cassava starch and whey protein to increase the antioxidant activity of these films (Chollakup et al., 2020; Go & Song, 2020). Thitilertdecha et al. (2010), after purification and isolation of phenolic compounds from rambutan peel, concluded that geraniin is the main responsible for the antioxidant activity of rambutan peel extract, followed by ellagic acid and corilagin. Besides, the antioxidant activity of this ellagitannin was much higher than that of butylated hydroxytoluene (BHT) in lipid peroxidation (77–186 folds) and $DPPH^{\bullet}$ (42–87 folds) assays. Moreover, the study by Sun et al. (2011) showed that the purified anthocyanin extract from rambutan peel was able to inhibit the linoleic acid oxidation at least 4 days. It also showed a higher reducing power than BHT in the concentration range from 50 to 500 μg/mL. Therefore, the antioxidant activity of the hydroethanolic extract prepared in this study can be explained by the set of phenolic compounds found in it. According to Monrroy et al. (2020), the antioxidant activity of the rambutan peel can be related to phenolic acids and non-anthocyanin flavonoids, while a weak correlation was found for anthocyanins.

3.2.2. Anti-inflammatory and cytotoxic activities

Regarding the extract ability to inhibit the NO production by LPS-stimulated RAW 264.7 cells, no activity was observed for the maximum concentration tested (400 μg/mL). On the other hand, Li et al. (2018) reported that 400 μg/mL of rambutan peel extract obtained by microwave-assisted extraction with 80.8 % ethanol was able to reduce the NO production by 40.2 %. Another study using an aqueous extract obtained from the Sri Lankan variety of rambutan showed that the anti-inflammatory activity *via* NO scavenging is concentration dependent, having increased from 4.1 % to 31.7 % with concentrations ranging from 100 to 500 μg/mL (Uduwela et al., 2019). Therefore, the anti-inflammatory activity of rambutan peel can be achieved if used in high concentrations. On the other hand, the ellagitannin geraniin, the major phenolic compound found in the extract of this fruit by-product, has already shown anti-inflammatory activity against LPS-induced acute lung injury in mice by inhibiting NF-κB and activating Nrf2 signaling pathways (Zhu et al., 2017).

The rambutan peel extract was also screened for its ability to inhibit the proliferation of human tumor cell lines, as well as for its hepatotoxic effect on a porcine liver primary cell line. As observed for anti-inflammatory activity, the maximal extract concentration tested (400 μg/mL) did not show anti-proliferative activity in any of the tested cell lines. According to the literature, geraniin has a potential hepaprotective effect and therapeutic action against metabolism dysfunction in rats when daily oral-administered for 4 weeks (at 25 mg/kg body weight) (Cheng et al., 2020). Moreover, the LD_{50} values capable of causing acute and subacute toxicity by the oral administration of hydroethanolic rambutan peel extract in rats were higher than 5.0 and 2.5 g/kg body weight, respectively, which implies that such therapeutic doses are possibly not harmful to the body (Li et al., 2020).

3.2.3. Antimicrobial activity

The results of the antimicrobial activity of rambutan peel extract are shown in Table 3. It exerted bacteriostatic and bactericidal effects against all tested strains. For some bacteria, lower concentrations of rambutan peel extract than control compounds were required, as in the case of *S. aureus* (MIC of 1 and 4 mg/mL for peel extract and E211, respectively), *B. cereus* (MIC of 1 and 2 mg/mL for peel extract and E224, respectively), and *E. cloacae* (MIC of 0.5 and 2 mg/mL for peel extract and E211, respectively). For other bacteria, the efficiency of the extract was similar to that of the synthetic food preservatives, except for E224, which was more active against *L. monocytogenes* and *S.* Typhimurium, while a lower concentration of E211 was necessary to inhibit the growth of *B. cereus*. These results are in line with those found in the literature. Phuong, Le, Van Camp, et al. (2020) achieved similar results using a hydromethanolic extract of rambutan peel against *S. aureus* and *L. monocytogenes*. These authors also described antibacterial action against *Salmonella enteritidis*, *Pseudomonas aeruginosa,* and *Vibrio campbellii*. In another study, the addition of rambutan peel ethanolic extract to an edible active film based on whey protein and cassava starch potentiated the reduction in the growth of *B. cereus*, *E. coli,* and *S. aureus* when applied to Salami (Chollakup et al., 2020). Moreover, the addition of zinc oxide nanocrystal to rambutan extract inhibited the growth of *S. aureus* and *E. coli* in cotton fabrics up to twenty washing cycles (Yuvakkumar et al., 2014). Therefore, this fruit by-product can be an interesting source of natural preservatives against several microorganisms; however, more studies are needed to determine which are the main phytochemicals responsible for the antimicrobial effect and to understand the mechanism of action of these compounds/extracts in different food matrices and other products, such as cosmetics, textiles, and therapeutical agents.

Regarding the antifungal activity, the peel extract showed all MIC lower than or equal to those of the synthetic preservatives (Table 3). It had fungicidal activity even at low concentrations against all tested microorganisms (MFC from 0.5 to 2 mg/mL). In a previous study, a methanolic extract of rambutan peel showed low inhibitory activity against *A. niger* (Phuong, Le, Van Camp, et al., 2020), which did not agree with the result of this work, where the extract had antifungal activity at a lower concentrations than the tested preservatives.

In addition to antimicrobial activity, other authors have attributed antiviral activity to rambutan peel, more specifically to geraniin, against dengue virus type-2 (Abdul Ahmad et al., 2019), herpes simplex, and hepatitis B virus (Fraga-Corral et al., 2021). Shikimic acid, the major organic acid detected in the samples of this study, also has been described with potential for the synthesis of antiviral drugs (Priyanka Singh et al., 2020); and its synergistic effect with other phenolic compounds such as geraniin (Hernández-Hernández et al., 2019) may potentiate the antiviral properties of rambutan peel extracts.

3.3. Optimized extraction of anthocyanins

To date, although there are few studies on the optimized extraction of bioactive compounds from rambutan peel (Maran et al., 2017; Phuong, Le, Dang, et al., 2020), there are no data on the optimized

Table 4
Extraction yield (Y_1) and anthocyanins content (Y_2) obtained experimentally with the CCRD used for HAE and UAE. For each response variable, the predicted values are presented between brackets. The estimated model coefficients, statistical data, and model-predicted optimal extraction conditions that maximize each response variable are also presented.

Run	Coded values			HAE		UAE	
	X_1	X_2	X_3	Y_1 (%, *w/w*)	Y_2 (mg/g E)	Y_1 (%, *w/w*)	Y_2 (mg/g E)
1	−1	−1	−1	19.07 (19.67)	7.40 (7.10)	19.97 (20.88)	6.82 (6.61)
2	1	−1	−1	22.38 (22.47)	8.20 (7.58)	18.92 (19.32)	7.47 (7.26)
3	−1	1	−1	25.98 (24.14)	13.95 (12.66)	24.28 (25.16)	6.28 (6.37)
4	1	1	−1	27.09 (26.93)	8.27 (7.61)	27.54 (28.29)	7.43 (7.02)
5	−1	−1	1	22.11 (21.10)	7.17 (7.36)	13.97 (12.31)	5.08 (5.12)
6	1	−1	1	25.90 (23.98)	10.52 (11.56)	9.62 (10.75)	5.13 (5.77)
7	−1	1	1	26.12 (25.56)	6.77 (6.91)	25.41 (24.10)	6.80 (7.22)
8	1	1	1	27.77 (28.36)	5.75 (5.58)	26.13 (27.23)	7.50 (7.87)
9	−1.68	0	0	21.75 (23.79)	9.32 (8.65)	25.87 (26.52)	8.70 (8.13)
10	1.68	0	0	27.25 (28.49)	8.75 (7.94)	29.90 (27.84)	9.83 (9.23)
11	0	−1.68	0	22.91 (22.39)	9.34 (8.47)	15.19 (14.99)	7.53 (7.90)
12	0	1.68	0	30.63 (29.98)	7.99 (8.12)	33.02 (32.44)	9.23 (9.47)
13	0	0	−1.68	18.67 (18.93)	7.34 (9.04)	18.71 (17.23)	2.69 (3.22)
14	0	0	1.68	20.06 (21.32)	7.90 (7.55)	8.42 (9.13)	3.46 (2.48)
15	0	0	0	25.38 (26.14)	7.71 (8.30)	28.17 (27.18)	9.45 (8.68)
16	0	0	0	26.18 (26.14)	7.06 (8.30)	26.30 (27.18)	8.11 (8.68)
17	0	0	0	27.06 (26.14)	7.46 (8.30)	26.00 (27.18)	8.97 (8.68)
18	0	0	0	25.23 (26.14)	8.25 (8.30)	28.85 (27.18)	8.79 (8.68)
19	0	0	0	26.13 (26.14)	8.05 (8.30)	27.35 (27.18)	7.91 (8.68)
20	0	0	0	26.11 (26.14)	8.49 (8.30)	25.67 (27.18)	8.77 (8.68)
Estimated model coefficients							
Intercept	b_0	23.39		8.30		27.18	8.68
Linear term	b_1	2.35***		−0.36 ns		0.39 ns	0.33*
	b_2	3.75***		−0.18 ns		5.19***	0.46**
	b_3	1.19*		−0.74 ns		−2.41***	−0.16 ns
Quadratic terms	b_{11}	ns		ns		ns	ns
	b_{22}	ns		ns		−1.22**	ns
	b_{33}	−6.01***		ns		−4.95***	−2.03***
Interaction terms	b_{12}	ns		−3.91***		1.17*	ns
	b_{13}	ns		2.62**		ns	ns
	b_{23}	ns		−4.25***		1.88***	0.58**
Statistical data							
Significance of the model		<0.0001		0.0004		<0.0001	<0.0001
Lack-of-fit		0.0751		0.0758		0.3395	0.7441
Coefficient of determination (R^2)		0.8939		0.8118		0.9712	0.9396
Adjusted coefficient of determination (R^2_{adj})		0.8656		0.7249		0.9544	0.9180
Optimal individual conditions							
X_1: time (min)		90.0		2.6		45.0	45.0
X_2: temperature (°C) or power (W)		90.0		89.4		500.0	500.0
X_3: solvent (%)		55.0		19.5		52.1	55.6
Optimal response:		32.3±1.1		16.8±0.9		36.4±1.5	10.1±0.6
Optimal global conditions							
X_1: time (min)		2.0				45.0	
X_2: temperature (°C) or power (W)		90				500	
X_3: solvent (%)		32				54.3	
Optimal response:		26.33±1.1		15.2±0.9		36.4±1.5	10.1±0.6

Natural values for HAE, X_1: 2 min (-1.68), 20 min (-1), 45 min (0), 72 min (1), and 90 min (1.68)), X_2: 20 °C (-1.68), 34 °C (-1), 55 °C (0), 76 °C (1), and 90 °C (1.68), and X_3: 0 % (-1.68), 20 % (-1), 50 % (0), 80 % (1), and 100 % ethanol *v/v* (1.68)); and for UAE, X_1: 2 min (-1.68), 11 min (-1), 23.5 min (0), 36 min (1), and 45 min (1.68), X_2: 5 W (-1.68), 105 W (-1), 252.5 W (0), 400 W (1), and 500 W (1.68), and X_3: 0 % (-1.68), 20 % (-1), 50 % (0), 80 % (1), and 100 % ethanol *v/v* (1.68). For *b*, the subscripts 1, 2, and 3 stand for time, temperature/power, and solvent, respectively. The model coefficients were predicted at a 95 % confidence level. Significant effect at:
ns: not significant ($p > 0.05$).
* $p < 0.05$;
** $p < 0.01$;
*** $p < 0.001$.

extraction of anthocyanins. Therefore, this study aimed to determine the best conditions to recover these natural pigments from rambutan peel using generally recognized as safe (GRAS) solvents allowed in the food industry and two extraction methods, HAE and UAE.

3.3.1. Experimental data and model validation

The CCRD involved 20 experimental runs for each extraction method and the results are presented in Table 4. For HAE, the response Y_1 range from 18.67 % (with run 13) to 30.63 % (with run 12), while, for UAE, the minimal and maximal responses for Y_1 were 8.42 % (run 14) and 33.02 % (with run 12), respectively. For Y_2, HAE yielded better results (5.75–13.95 mg/g E, with runs 8 and 3, respectively) than UAE (2.69–9.83 mg/g E, with runs 13 and 10, respectively). These results suggested that the extraction of anthocyanins from rambutan peel is complex and, therefore, mathematical models were developed to better described the effect of the independent variables on the target responses. For this, the experimental results were fitted to a second-order polynomial equation and ANOVA was performed to determine the significance of the models and their coefficients (Table 4). All models were significant ($p < 0.05$) and the lack-of-fit was not significant ($p > 0.05$). The R^2 and R^2_{adj} range from 0.8118 to 0.9712 and from 0.7249 to 0.9544, respectively, thus indicating that the variability of each response can be explained by the independent variables involved in the extraction processes.

3.3.2. Effect on extraction yield

As illustrated in Fig. 2, both HAE and UAE methods induced a similar behavior on the extraction yield (Y_1). For HAE, the extraction rate was promoted by the positive linear effects of temperature ($b_1 = 3.75$) and time ($b_2 = 2.35$), which means that extraction is intensified by an increase in these variables. The strong quadratic effect (b_{33} = -6.01) caused by the solvent was also evident in the 3D graphs combining the effects of this variables with time ($X_3 \times X_1$) or temperature ($X_3 \times X_2$). Therefore, it was possible to obtain better extraction yields using medium ethanol proportions combined with longer times and higher temperatures (Table 4). For UAE, the most pronounced linear effects were those of ultrasonic power ($b_2 = 5.19$), followed by solvent ($b_3 = 2.41$). The strong quadratic effect (b_{33} = -4.95) of the solvent was also noticeable on the surface of the two 3D graphs illustrating this variable (Fig. 2). In addition, the extraction trends also had interaction effects of power with the other two process variables (Table 4), which justified the use of RSM. Thus, the application of high ultrasonic power or temperature for longer times tended to maximize this response variable, as did an intermediate ethanol proportion. These results can be justified by the greater mass transfer promoted by these extraction intensification factors, heat and ultrasonic waves, which promote cell rupture and the consequent release of solutes into the solvent. However, different

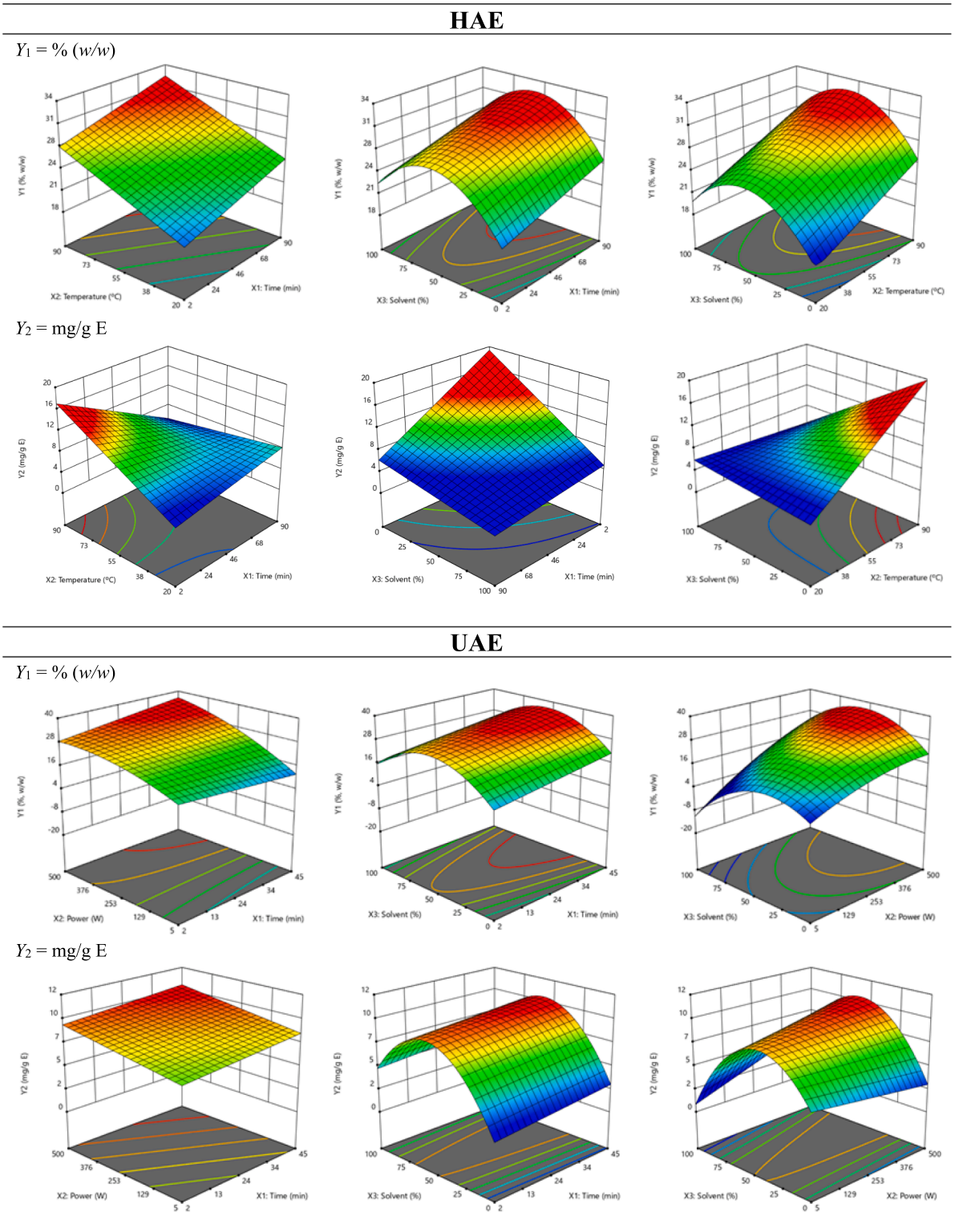

Fig. 2. Response surface graphs illustrating the combined effects of the independent variables (X_1: time, X_2: temperature/power, and X_3: solvent) on extraction yield (Y_1) and anthocyanin content (Y_2) obtained from rambutan peel. In each graph, red-colored areas indicate regions of maximum response. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phytoconstituents can be extracted, including phenolic compounds but also carbohydrates, organic acids, and proteins, which are not the target compounds of this optimization study but are present in plant matrices and, therefore, in the obtained extracts.

Under the optimal processing conditions presented in Table 4, it was possible to achieve the best model-predicted yield of 36.4 % with UAE, when processing the sample at 500 W for 45 min, using 52 % ethanol, while HAE yielded 32.2 % and involved 88 min stirring at 90 °C with 55 % ethanol. These extraction yields were slightly higher than that (31.0 %) obtained with the conventional extraction performed to obtain the

extract used in the anthocyanin characterization, which can be explained by the intensification factors used in HAE and UAE.

3.3.3. Effect on anthocyanin extraction

The anthocyanin extraction process followed a different trend from that observed for Y_1. According to the model coefficient shown in Table 4, obtaining an anthocyanin-rich extract (Y_2) using HAE depended on the interaction between all independent variables. Additionally, the combination of a long processing time and high temperature had a negative effect, as did a high ethanol proportion. On the other hand, the interaction between time and solvent has a positive effect on extraction. As illustrated in Fig. 2, the maximum anthocyanin concentration in the peel extract was obtained at high temperature (89.4 °C) during a shorter time (2.6 min) and using a low ethanol proportion (19.5 %). Under these optimal processing conditions, the maximum response for Y_2 was 16.8 mg/g E (Table 4). This extraction process can thus be selective for anthocyanins, as the shorter time and the lower ethanol proportion needed for Y_2, compared to Y_1, promote the recovery of these pigments, but are not sufficient for the extraction of other plant matrix constituents towards the extract weight. Selectivity for anthocyanins as a function of the applied processing conditions has already been observed in other studies (Rocha et al., 2020).

In turn, UAE was less efficient than HAE for anthocyanin recovery. The highest anthocyanin extraction was observed at the extremes of time and power, and medium ethanol proportion. These conditions were thus necessary to promote mass transfer, probably caused by disruption of plant cell walls and vacuoles *via* acoustic cavitation (Das et al., 2022). Moreover, unlike HAE which involved stirring the extractive mixture, the agitation that occurs in UAE is causes by the acoustic pressure as a function of the applied ultrasonic power. Therefore, runs involving low sonication power had low agitation and consequently low mass transfer and lower yields. This observation may also explain the observed selectivity for the recovery of anthocyanins by HAE not verified for UAE. When applying the individual optimal UAE conditions in Table 4, the maximum yield of anthocyanins was 10.1 mg/g E, 1.66-fold lower than the maximal response obtained with HAE. The value was also lower than that (11.57 mg/g E) obtained with the extraction method used in the preparation of the extract used in the anthocyanin characterization (Table 2).

3.3.4. Global optimal extraction conditions

The optimal conditions that simultaneously maximize the two response variables (Y_1 and Y_2) are shown in Table 4. For HAE, these conditions were $X_1 = 2.0$ min, $X_2 = 90$ °C and $X_3 = 24.3$ % ethanol and yielded $Y_1 = 25.3$ % (*w/w*) and $Y_2 = 16.3$ mg/g E. For UAE, the optimal conditions were $X_1 = 45$ min, $X_2 = 500$ W, and $X_3 = 54.3$ %, yielding $Y_1 = 36.4$ % (*w/w*) and $Y_2 = 10.1$ mg/g E. Overall, UAE was more efficient to increase the extraction yield (extract weight); however, it led to a lower amount of anthocyanin in the extract, which suggested that the extract obtained by UAE was less pure in anthocyanins than that obtained by HAE. A low selectivity of UAE for anthocyanins was also observed in previous studies (Albuquerque, Pinela, et al., 2020; López et al., 2018; Rocha et al., 2020). In the studied by Monrroy et al. (2020), the higher anthocyanin extraction from rambutan peel using an ultrasonic bath (20 min) and boiling (10 min) with 60 % ethanol was 0.57 mg/g against 0.35 mg/g, respectively. However, it is worth noting that boiling for 10 min may have been too long and led to the degradation of these pigments.

Regarding optimized extractions and the standard extraction method reported in subsection 2.2.4 (Albuquerque, Pereira, et al., 2020), the UAE had lower extraction capacity, since its maximum extraction value was 0.87-fold less than that obtained with 80 % ethanol for 1 h extraction plus 1 h re-extraction. On the other hand, the HAE efficiency was 1.40-fold high.

4. Conclusion

Rambutan peel presented different classes of bioactive compounds, including organic acids, tocopherols, fatty acids, procyanidins, and anthocyanins. To the best of the authors' knowledge, this is the first study reporting its composition in organic acids, tocopherols, and fatty acids. The rambutan peel extract showed high antioxidant activity, as well as antibacterial and antifungal properties. On the other hand, no cytotoxic effects on tumor and non-tumor cell lines were observed. Furthermore, two extraction methods for the recovery of anthocyanins from this by-product were optimized using an RSM-coupled five-level, three-factor experimental design. HAE was more efficient to obtain a higher anthocyanin content than UAE. Still, future studies may be interesting to assess the impact of other independent variables on the extraction processes, such as solid/liquid ratio and sample particle size, in order to make them more efficient. Overall, rambutan peel could be exploited to obtain natural colorants and other bioactive compounds as a strategy to support the circular bioeconomy and to reduce the environmental impact of the fruit processing industry.

CRediT authorship contribution statement

Bianca R. Albuquerque: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft. **José Pinela:** Methodology, Validation, Writing – review & editing. **Maria Inês Dias:** Investigation, Validation, Writing – review & editing. **Carla Pereira:** Investigation, Validation, Writing – original draft. **Jovana Petrović:** Investigation, Validation, Writing – review & editing. **Marina Soković:** Resources, Validation, Writing – review & editing. **Ricardo C. Calhelha:** Investigation, Validation. **M. Beatriz P.P. Oliveira:** Conceptualization, Supervision, Writing – review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Supervision, Writing – review & editing. **Lillian Barros:** Conceptualization, Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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