



Green extraction of anthocyanin from red cabbage waste using acid whey as a promising bio-based solvent

Jaine Oliveira^a, Laís Benvenuti^a, Bianca R. Albuquerque^{c,e}, Tiane C. Finimundy^{c,d}, Filipa Mandim^{c,d}, Tânia C.S.P. Pires^{c,d}, Carla Pereira^{c,d}, Rúbia Carvalho Gomes Corrêa^{b,c}, Lillian Barros^{c,d}, Acácio Antonio Ferreira Zielinski^{a,*}

^a Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil

^b Postgraduate Program in Clean Technologies, Cesumar University - UNICESUMAR, Maringá, PR 87050-390, Brazil

^c Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^d Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

^e REQUIMTE—Science Chemical Department, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

ARTICLE INFO

Keywords:

Green extraction
Bio-based solvent
Dairy industry
By-product valorization
Pressurized liquid extraction
Ultrasound-assisted extraction

ABSTRACT

The use of acid whey as a biological-based solvent may be an innovative and sustainable strategy to add value to agro-industrial by-products. This study assessed acid whey as a solvent for extracting anthocyanins from discarded red cabbage leaves. Methods such as Heating and Stirring Extraction (HSE), Ultrasound-Assisted Extraction (UAE), and Pressurized Liquid Extraction (PLE) were compared using acid whey or acidified ethanol as solvents. The yield of anthocyanin extract from acid whey is approximately 7 % lower compared to that obtained from acidified ethanol. All extracts exhibited *in vitro* antioxidant activity. Anthocyanin-rich extracts from red cabbage leaves revealed the predominant presence of cyanidin-3-O-(feruloyl)-sophoroside-5-O-glucoside (949 *m/z*). No cytotoxic effects were observed in the tested extracts, underscoring their safety and potential for industrial applications. Furthermore, incorporating powdered extracts into marshmallows yielded promising results as a natural colorant, emphasizing their versatility. These findings underscore acid whey role as a sustainable biobased solvent for extracting anthocyanins from red cabbage waste.

Industrial relevance: The use of acid whey as a solvent for extracting anthocyanins from purple cabbage leaves is a valuable alternative in the agroindustry. This practice adds value to by-products and promotes sustainability. Emerging technologies, such as ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE), enhance the efficiency of this process. Analysis shows that acid whey is efficient and environmentally friendly, yielding extracts with antioxidant properties. These extracts can also serve as natural colorants, meeting the demand for natural ingredients. Thus, acid whey enhances sustainability in agroindustrial production.

1. Introduction

Current food systems typically produce by-products known as “agro-industrial waste”. It includes waste from the meat and dairy processing industries, such as acid whey, as well as peels, seeds, and cakes made from pressing fruits and vegetables to extract juices and oils (Menchik, Zuber, Zuber, & Moraru, 2019; Reguengo, Salgado, Sivieri, & Maróstica Júnior, 2022). However, the sustainable management of food waste is a high-priority global issue aimed at improving food security and conserving natural resources and ecosystems (de Albuquerque, Corrêa,

de Lima Sampaio, & Barros, 2023).

Acid whey, the most produced effluent by the dairy sector, hurts the environment because of its high Biochemical oxygen demand (BOD) content (31,900 to 62,400 mg/L) (Menchik et al., 2019). Dairy industries typically underutilize acid whey due to its challenging characteristics, including high acidity (pH 4.0–4.5) and high mineral content (ash 0.42–1.13 wt. weight) (Menchik et al., 2019). Consequently, acid whey leads to an overload for the dairy sector, which has not yet developed a sustainable method to value this biowaste (Kumar Awasthi et al., 2022).

* Corresponding author.

E-mail address: acacio.zielinski@ufsc.br (A.A.F. Zielinski).

<https://doi.org/10.1016/j.ifsset.2025.103926>

Received 8 October 2024; Received in revised form 25 November 2024; Accepted 9 January 2025

Available online 10 January 2025

1466-8564/© 2025 Elsevier Ltd. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Organic solvents such as methanol and ethanol have been used to extract phenolic compounds from natural sources. However, the use of these organic solvents in the food, cosmetic, and pharmaceutical industries is strongly discouraged due to their toxicity and availability of contaminants (Palos-Hernández, Gutiérrez Fernández, Escuadra Burrieza, Pérez-Iglesias, & González-Paramás, 2022). In this way, sustainable processes search the replacing of volatile solvents for biocompatible, greener, and less toxic solvents, for instance, bio-based solvents such as ethyl alcohol, ethyl acetate, ethyl lactate, supercritical carbon dioxide, diethyl carbonate, and deep eutectic solvents (Miyashita, Beppu, Hosokawa, Liu, & Wang, 2020). Thus, based on the presented context, acid whey can be a good bio-based solvent to extract bioactive compounds.

According to a wide scope of evidence, agro-industrial by-products can be sustainable sources of bioactive compounds with high value for the industry, within the concepts of upcycling and circular economy (Benvenuti, Zielinski, & Ferreira, 2022; de Albuquerque et al., 2023; Teixeira et al., 2021). Among promising plant by-products, discarded red cabbage leaves stand out as great sources of anthocyanins (Kowalski et al., 2021). Anthocyanins are phenolic compounds belonging to the class of flavonoids with high academic and industrial interest due to their bioactivities, such as: effects against cardiovascular diseases (Dong et al., 2022); anti-obesity and anti-diabetic properties (Lee et al., 2022); antimicrobial (García et al., 2019); anti-inflammatory (Corrêa et al., 2015); anticancer (Wu et al., 2022); anti-fungal (García et al., 2019) and antioxidants (Filafarro et al., 2022) potentials. Furthermore, such molecules hold natural pigmentation, displaying red, violet, and blue colors (Machado et al., 2022; Molina, Corrêa, Prieto, Pereira, & Barros, 2023; Tena, Martín, & Asuero, 2020). Accordingly, anthocyanin compounds present a huge potential for food, pharmaceutical, and cosmetic fuel applications.

The anthocyanin recovery by green extraction can valorize the red cabbage by-product and substitute the dominant economic model “take, make and dispose of” (Sendri, Singh, Bhatt, Bhatt, & Bhandari, 2022). This approach aligns with the Sustainable Development Goals proposed by the United Nations 2030 agenda (Chen et al., 2020). Besides the solvent employed, acid whey, for a sustainable extraction process, promising alternatives such as ultrasound-assisted technology and high-pressure extraction methods are advantageous as they improve the extraction efficiency while allowing short process time and low solvent consumption (Benvenuti et al., 2022; Teixeira et al., 2021).

Considering all the above, this study aimed to obtain an anthocyanin-rich extract from red cabbage by-product employing acid whey as the solvent and through emerging extraction technologies such as Ultrasound-Assisted Extraction (UAE) and Pressurized Liquid Extraction (PLE) to evaluate the antioxidant, antimicrobial, and anti-inflammatory properties of the obtained extracts as well as their application in a food model.

2. Material and methods

2.1. Materials

Acid whey from cheese processing was donated by a dairy company (Hollandês, Biguaçu, SC, Brazil), and the red cabbage leaves by-product was provided by local producers (Florianópolis, SC, Brazil). Solvents such as 99.5 % ethanol and 98–100 % citric acid were purchased from Êxodo Científica (Sumaré, SP, Brazil). Other chemicals such as DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Folin-Ciocalteu, and standards Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) (97 % purity) and gallic acid (> 97 % purity) were obtained from Sigma Aldrich (Steinheim, Germany). Cyanidin-3-O-glucoside standard (with purity ≥ 90 % as determined by HPLC) was acquired from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid, Trolox, Tris, ascorbic acid, ellipiticine, and dimethyl

sulfoxide (DMSO), thiobarbituric acid, all of analytical grade, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Porcine (*Sus scrofa*) brain tissue and sheep (*Ovis aries*) blood utilized for antioxidant activity assessments were sourced from animals slaughtered under official procedures. Microbiology supplies including Malt extract broth (MEB), blood (sheep blood 7 %), and MacConkey agars were purchased from LiofilChem S.R.L (Roseto d. Abruzzi, TE, Italy). Antibiotics such as ampicillin, imipenem and vancomycin utilized for control assays were sourced from Fisher Scientific (Janssen Pharmaceutical, Belgium), while the antifungal ketoconazole was obtained from Frilabo (Porto, Portugal). For cell culture, Dulbecco's modified Eagle's (DMEM) and Roswell Park Memorial Institute (RPMI 1640) media, Hank's balanced salt solution (HBSS), fetal bovine serum (FBS) were acquired from Hyclone (Logan, UT, USA). LC/MS-grade methanol, ethanol, acetonitrile, and extra pure formic acid were provided by Fisher Scientific (Leicestershire, UK).

2.2. Sample preparation

The acid whey was pasteurized, filtrated, and stored at -18 °C until the assays. Its proximal composition was evaluated in terms of moisture (925.09), fixed mineral residue (ashes) (923.03), total lipid content (920.39), total protein content (954.01), performed according to AOAC methodologies (AOAC, 2012). Total carbohydrates were determined by difference. The pH value determination was performed using a HI 5221 model pH meter (Hanna Instruments, USA).

The red cabbage leaves were selected, washed, sanitized, and dried using an air circulation oven (Lucadema, Model 82/27, SP, Brazil) at 60 °C. The husks were ground in a knife mill (Marconi, Model MA340, SP, Brazil) and sieved to standardize particle size (between 0.30 and 0.85 mm).

2.3. Anthocyanin-rich extract recovery from red cabbage leaf waste

Extraction assays were conducted to compare Pressurized Liquid Extraction (PLE), Ultrasound-Assisted Extraction (UAE), and Heating and Stirring Extraction (HSE) in terms of yield and extract quality. Two solvents were used for each extraction method: acid whey (AW) (pH 4.5) and ethanol acidified (EA) (pH 1.5), composed by ethanol: citric acid solution (0.1 mol L^{-1}) in a ratio of 70:30 (v:v) commonly used for anthocyanin recovery. The extraction times were determined experimentally based on kinetics: PLE (15 min), UAE (15 min), and HSE (15 min). The other extraction parameters were established based on the study of Teixeira et al. (2021), with some modifications. PLE was carried out in continuous mode, with a pressure of 100 bar, and 45 g of glass spheres inside the extraction column, forming a fixed bed (5 g of sample, at 80 °C, with acid whey or ethanol acidified with citric acid as solvents, and a flow rate of 3 mL/min). For both HSE and UAE, a solid-liquid ratio of 1:20 (w:v) was used at 80 °C. The HSE was performed in a thermostatic bath (Microquímica, MQBTC99-20, Brazil), while the UAE was performed using an ultrasonic probe (Eco-Sonics, Ultronique Q3.0/37 A, Brazil) with 40 % power. After extraction, the UAE and HSE samples were centrifuged at 412 g for 20 min (Quimis, Q222T, Brazil).

2.4. Total monomeric anthocyanin (TMA) and polymeric color (PC)

The Total monomeric anthocyanin (TMA) and the polymeric color (PC) of the extracts were quantified according to Mónica Giusti & Wrolstad, (2005) adapted for a microplate reader (Multileader Infinite M200 TECAN, ZH, Switzerland). The TMA content was determined by the pH differential method using potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). TMA concentrations were calculated using (1), (2): The total monomeric anthocyanins was calculated and expressed in mg equivalent of cyanidin-3-glycoside according to Eq. (1).

$$TMA = (Abs * MW * FD * 10^3) / \epsilon * 1 \quad (1)$$

$$Abs = (Abs_{520nm} - Abs_{700nm})_{pH1.0} - (Abs_{520nm} - Abs_{700nm})_{pH4.5} \quad (2)$$

Where: MW = molecular weight: 449.2 g.mol⁻¹ for cyanidin-3-glycoside; FD = established dilution factor: 15; 1 = size of the path that the light travels (thickness of the cuvette) in cm; 10³ = conversion factor from g to mg; ϵ = molar extinction coefficient: 2690 L.mol⁻¹.cm⁻¹ for cyanidin-3-glycoside.

For the percentage of polymeric color (% PC), the extracts diluted in potassium chloride buffer (pH 1.0) were bleached with potassium metabisulfite (0.9 M). The color density of the control fraction (DC), polymeric color of the bleached fraction (PC) and the percentage of polymeric color were calculated through eqs. (3), (4) and (5) respectively. Where: DF is the dilution factor used.

$$DC = [(Abs_{420nm} - Abs_{700nm}) + (Abs_{520nm} - Abs_{700nm})] * DF \quad (3)$$

$$PC = [(Abs_{420nm} - Abs_{700nm}) + (Abs_{520nm} - Abs_{700nm})] * DF \quad (4)$$

$$\%polymeric\ color = (PC/DC) * 100 \quad (5)$$

2.5. Anthocyanin profile by LC-MS

The phenolic profile of red cabbage leaf extracts was determined by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The lyophilized extracts were redissolved at a concentration of 5 mg/mL with ethanol:water mixture (40:60, v/v). Individual anthocyanins were determined according to the methodology described by Gonçalves et al. (2017). Detection was performed on DAD (520 nm) and a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source, whereas the non-anthocyanins compounds were separated and identified as described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016) with detection performed in a DAD (280 and 370 nm). Thus, for the quantification of anthocyanins, a standard of cyanidin-3-glycoside was used ($y = 134,578x - 3,000,000$, $R^2 = 0.9986$, detection limit = 9.94 μ g/mL and quantification limit = 30.13 μ g/mL). However, for identified phenolic compounds for which a commercial standard was not available, quantification was performed using the calibration curve of the most similar available standard.

2.6. In vitro antioxidant activity

To assess the antioxidant activity of the red cabbage leaf waste extracts, five different assays were employed: two cell-based and three chemical-based assays.

2.6.1. OxHLIA and TBARS assays

For the OxHLIA assay, erythrocytes were obtained as described by Lockowandt et al. (2019) and the results were expressed as extract concentration (μ g/mL) needed to protect 50% of the erythrocyte population from oxidative hemolysis (IC₅₀) for a Δt of 60 min. The inhibition of the generation of thiobarbituric acid reactive substances (TBARS) was estimated as reported by Corrêa et al. (2015). For both assays, the extracts were dissolved in PBS and tested in concentrations ranging from 600 to 2.3 g/mL.

2.6.2. DPPH, ABTS and FRAP assays

The free radical scavenging activity of the extracts were determined using DPPH was performed according to Brand-Williams, Cuvelier, and Berset (1995) and ABTS radicals scavenging, according to Re et al. (1999) and also by Ferric ion Reducing Antioxidant Power (FRAP) according to Benzie and Strain (1996) methods, with adaptations for microplate reader (Infinite M200, TECAN) using Eq. (6). The results were expressed in micromoles of Trolox equivalents per 100 g of dry

waste (μ mol TE/g dw), which were calculated from the standard curves (DPPH = 0,14x - 6,83; $R^2 = 0,99$, ABTS = 0,11x + 4,91; $R^2 = 0,99$) and FRAP = 0,0009x_{absorbance} + 0,0083; $R^2 = 0,99$).

$$\text{Antioxidant activity\%inhibition} = 1 - (Abs\ sample / Abs\ White) * 100 \quad (6)$$

2.6.3. NO-production inhibition

NO-production inhibition was determined according to the method described by (Corrêa et al., 2015). The ability of the studied extract to inhibit the production of the pro-inflammatory mediator nitric oxide (NO) in lipopolysaccharide (LPS) stimulated macrophages (RAW 246.7) was evaluated. Dexamethasone (50 μ M) was used as a positive control and the cells in the presence and in the absence of LPS were used as negative controls. The obtained results were expressed as the sample concentration that inhibited 50 % of nitric oxide production (IC₅₀ values).

2.7. Antiproliferative activity

The evaluation of the antiproliferative activity of the extracts was performed according to the method previously described by Corrêa et al. (2017). The extracts rich in anthocyanins were dissolved in water at a final concentration of 8 mg/mL and further diluted in water (8–0.125 mg/mL), being the final concentrations tested comprised between 400 and 6.25 g/mL. Four human tumoral cell lines were tested, the gastric (AGS), colorectal (CaCo-2), and breast adenocarcinomas (MCF-7). The non-tumor cell line from the African green monkey kidney (VERO) was also tested. The obtained results were expressed as the extract concentration responsible for inhibiting 50 % of cell proliferation (GI₅₀ values, g/mL). The commercial Ellipticine was used as the positive control, and cells without samples as the negative control.

2.8. Antibacterial and antifungal potential

The evaluation of antibacterial and antifungal activity was determined using the protocol described by Corrêa et al. (2015). To evaluate the antibacterial activity, five Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*) and three Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes* and *Methicillin-resistant Staphylococcus aureus* - MRSA) were used. The antibiotics ampicillin, imipenem and vancomycin were used as positive controls.

The antifungal activity of the extracts was tested against *Aspergillus fumigatus* and *Aspergillus brasiliensis* using the protocol described by Corrêa et al. (2015). Ketoconazole was used as a positive control. The results were expressed as Minimum Inhibitory Concentrations (MIC, mg/mL) and Minimum Bactericidal or Fungicidal concentrations (MBC and MFC, respectively, mg/mL).

2.9. Application of PLE anthocyanin-rich extract in a food model

The lyophilized anthocyanin-rich extract obtained by PLE using acid whey as the solvent was added to a marshmallow model formulation. The base formulation contained sugar (173 g), impalpable sugar (43 g), corn starch (26.6 g), corn glucose (26.6 g), colorless unflavored gelatin (10.6 g), and water (44 mL). With the base ready, two samples were prepared: (A) control sample, without the addition of colorant, and (B) sample with the addition of anthocyanin-rich extract (4 g).

The ingredients were incorporated and subjected to aeration at maximum mixed in a planetary mixer (Deluxe SX84, Arno, Brazil). The lyophilized extracts were dissolved in a water and solubilized at room temperature, then added to the formulation. After aeration, the marshmallow mass was transferred to a container, remaining at rest for 12 h at room temperature. After this period, the dough was cut into cubes of approximately 3 cm in height and 2 cm in width.

2.9.1. Instrumental color analysis

The photos of the samples of the developed marshmallow confection were taken using a photographic camera (Nikon D5300, resolution 4496 × 3000 pixels) placed in a booth equipped with white light (D65 lighting). The photos were treated with ImageJ software (Health National Institute, Bethesda, MD, USA) and converted to the CieLab system. Six color readings were performed (parameters L*, a*, and b*) for each triplicate of the developed samples. The L*, a* and b* values of the samples were measured and the chromaticity (C*) and the hue angle (h°) were calculated using eqs. (7) and (8).

$$h^{\circ} = \arctan(b^*/a^*) \quad (7)$$

$$C^* = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2} \quad (8)$$

2.9.2. Analysis of bioactive compounds

The bioactive compounds were recovered from marshmallow samples, using a solution of ethanol: citric acid 30:70 (v/v) 0.1 mol L⁻¹ at 60 °C for 30 min in a thermostatic bath (Microquímica, MQBTC99-20, Brazil). Sequentially, TMA and antioxidant capacity by FRAP and ABTS methods were determined for marshmallow-type confectionery, according to the procedures presented are Section 2.4 and 2.6.1.

2.9.3. Texture profile

The texture properties of the samples were determined using a texture analyzer (TAHD plus Texture Analyzer, Stable Micro Systems). For the analysis, a P/5 probe (5 mm in diameter) was used, and

compression was applied at 30 % of the height of the samples. The samples were conditioned by keeping them at room temperature before measurement and the texture profile was analyzed.

2.10. Statistical analysis

Results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's test (*p* < 0.05) were used to compare means using Statistica v. 13.5 (TIBCO Software Inc., Palo Alto, CA, USA).













3. Results and discussion

3.1. Proximate composition of the acid whey

The proximate composition of the acid whey presented the following values: 94.77 ± 0.003 % of moisture, 0.0032 ± 0.0002 g. L⁻¹ of fixed mineral residue (Ash), 0.0020 ± 0.0004 g.L⁻¹ of total fat (Soxhlet) and 0.0056 ± 0.0005 g. L⁻¹ of protein (N × 6.25). Menchik et al. (2019) reported a similar composition for cottage cheese in terms of moisture (93.8 %) and proteins (0.00505 g. L⁻¹). However, they found a higher ash content (0.33 mg/g) and a lower lipid level (0.01 %). The variation in the proximate composition is mainly related to factors such as the origin of the milk, the type of processing, and the heat treatment applied (Rocha-Mendoza et al., 2021).

Table 1

Chemical composition and antioxidant activity of the anthocyanin-rich extracts obtained from red cabbage leaf residue.

Extraction Methods	Solvent Type		Chemical-based methods				Cell-based methods		
	AE	AW	TMA mg C3G/g de	% polymeric color	ABTS μmol ET/100g	DPPH μmol ET/100g	FRAP μmol ET/100g	OxHLIA IC ₅₀ ; μg/mL *	TBARS IC ₅₀ ; μg/mL
HSE			7.12±0.30 ^c	49.90±0.40 ^b	8586±50 ^f	9060±20 ^f	7709±80 ^d	223±13 ^b	153±9 ^d
			6.60±0.10 ^f	51.80±0.40 ^a	10612±40 ^d	9197±90 ^c	7666±90 ^c	477±13 ^a	204±9 ^b
UAE			7.58±0.40 ^b	45.00±0.40 ^d	10057±70 ^c	13827±10 ^d	7204±60 ^f	18.71±0.1 ^c	161±9 ^c
			7.29±0.10 ^d	48.30±0.10 ^c	11625±50 ^c	13931±50 ^c	9080±60 ^b	115±7 ^c	250±4 ^a
PLE			8.08±0.10 ^a	40.00±0.90 ^f	12865±16 ^b	14639±90 ^a	9274±40 ^a	8±1 ^e	116±0.3 ^f
			7.51±0.10 ^c	43.60±0.90 ^c	14006±60 ^a	13963±60 ^b	8284±90 ^c	9±1 ^f	120±6 ^c
Trolox	-	-	-	-	-	-	-	21.80±0.20 ^d	5.8±0.6 ^e

Values expressed as mean ± standard deviation (ANOVA followed by Tukey test, equal letters mean no significant difference (*p* < 0.05). AE: extract obtained with acidified ethanol, AW: extract obtained with acid whey, HSE: Heating and Stirring Extraction, UAE: ultrasound-assisted extraction, PLE: pressurized liquid extraction, ET: Trolox equivalent, TMA: total monomeric anthocyanins, C3G: cyanidin-3-glycoside dry extract, ABTS: 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl radical, FRAP: Ferric-Reducing Antioxidant Power), OxHLIA: Oxidative hemolysis inhibition assay, TBARS: thiobarbituric acid reactive substances, Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid and ET: Trolox equivalent. *Δt = 60 min.

3.2. Influence of the solvent and extraction method in anthocyanin recovery

The results obtained using acid whey as a solvent for anthocyanin extraction were comparable to those achieved with the conventional solvent, acidified ethanol (Table 1). The total monomeric anthocyanin (TMA) recovered with acid whey was 7.31 % lower than that obtained with acidified ethanol in the Heating and Stirring Extraction (HSE) method, 3.83 % lower in the Ultrasonic Assisted Extraction (UAE) method, and 7.06 % lower in the Pressurized Liquid Extraction (PLE)

method. These results suggest that acid whey, a byproduct of the dairy industry, holds significant potential as a sustainable alternative solvent for anthocyanin extraction.

Polar solvents, such as water and ethanol, are commonly used for the extraction of bioactive compounds, including anthocyanins, due to their polar affinity with these compounds, which improves their solubility in the medium (Kruszewski & Boselli, 2024). The acidification of the extraction medium is crucial for breaking down cell membranes in plant tissues, facilitating the release of anthocyanins. Furthermore, an acidic environment stabilizes the flavilium cation, preserving the color of the

Table 2

Identification and quantification by LC-MS of the anthocyanin-rich extracts obtained from red cabbage leaf residue.

a) Tentative Identification of anthocyanin compounds present in the extracts						b) Quantification of anthocyanin compounds present in the extracts. The values are expressed in mg/g dry extract.					
Peak	RT (min)	λ_{max} (nm)	$[M + H]^+$ (m/z)	MS^2 (m/z)	Tentative identification	HSE acidified ethanol	HSE Acid Whey	UAE acidified ethanol	UAE Acid Whey	PLE acidified ethanol	PLE Acid whey
1	20.43	511	773	611 (51),449 (32),287 (100)	Cyanidin-3-O-sophoroside-5-O-glucoside	0.36 ± 0.01	0.31 ± 0.01	0.37 ± 0.01	0.34 ± 0.01	0.53 ± 0.01	0.44 ± 0.01
2	21.78	512	611	449 (25),287 (100)	Cyanidin-3-O-glucoside-5-O-glucoside	0.63 ± 0.01	0.44 ± 0.01	0.65 ± 0.01	0.45 ± 0.01	0.75 ± 0.01	0.58 ± 0.01
3	24.04	523	979	817 (12),449 (31),287 (100)	Cyanidin-3-O-(sinapoyl)-sophoroside-5-O-glucoside	0.33 ± 0.01	0.31 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.51 ± 0.01	0.43 ± 0.01
4	25.09	527	1141	979 (18),449 (13),287 (100)	Cyanidin-3-O-(sinapoyl)-triglucoside-5-O-glucoside	0.53 ± 0.01	0.39 ± 0.01	0.55 ± 0.01	0.41 ± 0.01	0.61 ± 0.01	0.55 ± 0.02
5	29.32	522	1081	919 (21),449 (61),287 (100)	Cyanidin-3-O-(caffeoyl)(p-coumaroyl)-diglucoside-5-O-glucoside	0.45 ± 0.01	0.36 ± 0.01	0.47 ± 0.01	0.38 ± 0.01	0.61 ± 0.01	0.52 ± 0.01
6	29.74	523	1111	949 (11),449 (32),287 (100)	Cyanidin-3-O-(feruloyl)-triglucosides-5-O-glucoside	0.51 ± 0.01	0.39 ± 0.01	0.55 ± 0.01	0.41 ± 0.01	0.66 ± 0.01	0.54 ± 0.01
7	31.93	533	1141	979 (21),449 (15),287 (100)	Cyanidin-3-O-(sinapoyl)-triglucoside-5-O-glucoside	0.45 ± 0.01	0.034 ± 0.01	0.44 ± 0.01	0.38 ± 0.01	0.57 ± 0.01	0.49 ± 0.01
8	32.96	534	1317	1155 (16),449 (22),287 (100)	Cyanidin-3-O-(feruloyl)(sinapoyl)-triglucoside-5-O-glucoside	0.58 ± 0.01	0.41 ± 0.01	0.53 ± 0.01	0.44 ± 0.01	0.63 ± 0.01	0.56 ± 0.01
9	36.89	522	949	757 (32),449 (6),287 (100)	Cyanidin-3-O-(feruloyl)-sophoroside-5-O-glucoside	1.90 ± 0.03	0.98 ± 0.02	1.88 ± 0.03	1.07 ± 0.01	1.59 ± 0.04	1.21 ± 0.04
10	39.01	534	1125	963 (10),449 (54),287 (100)	Cyanidin-3-O-(feruloyl)(feruloyl)-diglucoside-5-O-glucoside	0.42 ± 0.01	0.32 ± 0.01	0.41 ± 0.01	0.35 ± 0.01	0.54 ± 0.01	0.46 ± 0.01
11	39.39	534	1155	993 (16),449 (9),287 (100)	Cyanidin-3-O-(feruloyl)(sinapoyl)-diglucoside-5-O-glucoside	0.52 ± 0.01	0.36 ± 0.01	0.52 ± 0.01	0.40 ± 0.01	0.59 ± 0.01	0.52 ± 0.01
12	39.77	534	1185	1023 (8),449 (26),287 (100)	Cyanidin-3-O-(sinapoyl)(sinapoyl)-diglucoside-5-O-glucoside	0.96 ± 0.02	0.52 ± 0.01	0.94 ± 0.02	0.58 ± 0.01	0.78 ± 0.01	0.69 ± 0.01
Total anthocyanins						7.63 ± 0.08^e	5.12 ± 0.05^f	7.69 ± 0.03^b	5.52 ± 0.10^d	8.38 ± 0.09^a	7.01 ± 0.09^c

values expressed as mean ± standard deviation (ANOVA followed by the Tukey test, equal letters mean no significant difference ($p < 0.05$), RT: retention time, λ_{max} : maximum absorbance, MS^2 : ion mass fragments, EA: extract obtained with acidified ethanol, AW: extract obtained with acid whey, HSE: Heating and Stirring Extraction, UAE ultrasound-assisted extraction and PLE: pressurized liquid extraction.

anthocyanins and preventing of color change (Patras, 2019). In this study, the acidified ethanol solvent had a pH of 1.5, while the acid whey solvent had a pH of 4.5.

The results from Azman, Nor, Charalampopoulos, and Chatzifragkou (2022) highlight the importance of pH in optimizing extraction efficiency. A concentration of Cyanidin-3-O-glucoside (C3G) at pH 3, after 2 h of extraction, yielded 10.9 mg/100 g of black currant pomace extract, which was significantly lower than the concentration obtained at pH 1.5, which was 114 mg/100 g, emphasizing the benefits of a more acidic extraction environment.

Although the pH of acid whey, a common byproduct in the dairy industry, typically ranges from pH 4.0–4.5 (Menchik et al., 2019), slightly above the ideal pH range for anthocyanin extraction, it can still be effective for recovering bioactive compounds.

Regarding degradation methods, both UAE and PLE provided higher yields compared to the conventional HSE method. In UAE, the yields were 7.58 mg C3G/g with acidified ethanol and 7.29 mg C3G/g with acid whey. In UAE, ultrasonic waves to induce the cavitation, which aids in breaking down plant tissues and releasing the desired compounds (Kumar Awasthi et al., 2022). The PLE method, which applies high pressure and temperature, yielded 8.08 mg C3G/g with acidified ethanol and 7.51 mg C3G/g with acid whey, benefiting from operational conditions that reduce solvent viscosity and surface tension, facilitating solvent penetration and mass transfer into the plant tissue (Zielinski et al., 2021).

PLE also showed good results in terms of anthocyanin stability, as indicated by the polymeric color index, which reflects the degree of anthocyanin degradation. The polymeric color values were lower for the extracts obtained by PLE (40.0 % AE and 43.6 % AW) compared to those obtained by HSE (49.9 % AE and 51.8 % AW) and UAE (45.0 % AE and 48.3 % AW). These results suggest that PLE minimizes anthocyanin degradation by providing conditions that prevent oxidation and the formation of brown pigments, such as melanodins (Tena et al., 2020). Oxidation reactions, which are more likely to occur in open-system extraction methods like HSE and UAE, likely contributed to the increase in polymeric color observed in these extracts.

Therefore, the combination of acid whey and PLE technology appears promising for the extraction of anthocyanins with greater stability and less degradation, offering a sustainable and efficient alternative to traditional extraction methods.

3.3. Anthocyanins identification

Anthocyanins from red cabbage leaf residues were tentatively identified by LC-MS analyses, using retention time (RT, min), maximum absorbance (λ_{\max} , nm), and ion mass fragments (MS^2 , m/z) (Table 2). Twelve anthocyanins were detected with a common ion mass fragment at 287 m/z corresponding to a cyanidin aglycone (Mansour, Moustafa, & Abdelkhalik, 2021; Wiczowski, Szawara-Nowak, & Topolska, 2013). Moreover, five acylated and five diacylated anthocyanins were detected.

Peaks 1 ($[M + H]^+$ at 773 m/z) and **2** ($[M + H]^+$ at 611 m/z), released three and two ion mass fragments at MS^2 , respectively, revealing the successive loss of glucoside moieties (162 mu). Some studies with red cabbage have tentatively identified these compounds as cyanidin-3-O-sophoroside-5-O-glucoside and cyanidin-3-O-glucoside-5-O-glucoside, respectively (Charron, Clevidence, Britz, & Novotny, 2007; Mansour et al., 2021; Wiczowski et al., 2013). **Peaks 3, 4, 6, 7** and **9** showed an acylation in the spectrometric analyses. **Peak 3** ($[M + H]^+$ at 979 m/z) released three ion fragments at 817 m/z ($[M + H]-162$), 449 ($[M + H]-162-162-206$) and 287 m/z , after a loss of glucoside moiety. According to the literature, the ion mass fragment 206 u can correspondent with a sinapic acid. Therefore, this compound has been tentatively identified as cyanidin-3-O-(sinapoyl)-sophoroside-5-O-glucoside (Mansour et al., 2021).

Peaks 4 and **7**, which showed the same pseudomolecular ion $[M + H]^+$ at 1141 m/z , revealed fragmentation of four glucoside units and a

sinapic acid unit, these compounds were tentatively identified as cyanidin-3-O-(sinapoyl)-triglucoside-5-O-glucoside (Wiczowski et al., 2013). **Peak 6** ($[M + H]^+$ at 1111 m/z). Revealed a loss of a glucoside unit at 949 m/z ($[M + H]-162$)⁺, followed by the loss of other two glucoside units and a ferulic acid residue (176 mu) at 449 m/z ($[M + H]-162-162-162-176$)⁺, and finally, the detachment of one more glucoside unit revealed the presence of a cyanidin (287 m/z), therefore this peak was tentatively identified as cyanidin-3-O-(feruloyl)-triglucoside-5-O-glucoside (Mansour et al., 2021). **Peak 9** ($[M + H]^+$ at 949 m/z), had a fragmentation behavior at tandem mass spectrometry similar to **peak 6**, except for having in its composition one less glucoside unit was detected. In the literature, this compound was identified as cyanidin-3-O-(feruloyl)-sophoroside-5-O-glucoside (Mansour et al., 2021).

Peaks 5, 8, 10, 11, and **12** were tentatively identified as diacylated anthocyanins. **Peak 5** ($[M + H]^+$ at 1081 m/z), released fragment ions at 919 ($[M + H]-162$), at 449 m/z ($[M + H]-162-162-146$), and at 287 m/z ($[M + H]-162-162-162-146-162$), according to other authors, this compound can be content a caffeic and a *p*-coumaric acids residues (162 and 147 mu , respectively), and has been identified as cyanidin-3-O-(caffeoyl)(*p*-coumaroyl)-diglucoside-5-O-glucoside (Mansour et al., 2021). **Peak 8** ($[M + H]^+$ at 1317 m/z) showed three fragments, at 1155 m/z ($[M + H]-162$), at 449 m/z ($[M + H]-162-162-176-206$), and at 287 m/z . This compound has been described as diacylated anthocyanin with ferulic acid (176 mu) and sinapic acid (206 mu) and has been identified as cyanidin-3-O-(feruloyl)(sinapoyl)-triglucoside-5-O-glucoside. **Peak 10** ($[M + H]^+$ at 1125 m/z), depicting three MS^2 fragment ions, at 963 m/z ($[M + H]-162$), at 449 m/z ($[M + H]-162-176-176$), and at 287 m/z ($[M + H]-162-176-176-162$), the spectrometry mass analysis suggesting that this compound undergo double acylation with ferulic acid, being tentatively identified as cyanidin-3-O-(feruloyl)(feruloyl)-diglucoside-5-O-glucoside (Mansour et al., 2021; Wiczowski et al., 2013).

Peak 11 ($[M + H]$ at 1155 m/z), presented a fragmentation behavior similar to the one of peak 8; however, this compound only released three glucoside units, therefore it was tentatively identified as cyanidin-3-O-(feruloyl)(sinapoyl)-diglucoside-5-O-glucoside (Mansour et al., 2021; Wiczowski et al., 2013).

Finally, **peak 12** ($[M + H]$ at 1185 m/z), showed three MS^2 ion fragments at 1023 m/z ($[M + H]-162$), at 449 m/z ($[M + H]-162-206-206$), and at 287 m/z ($[M + H]-162-162-206-206-162$), revealing a cyanidin aglycone after a loss of three glucoside units and two sinapic acid residue. This compound has been tentatively identified as cyanidin-3-O-(sinapoyl)(sinapoyl)-diglucoside-5-O-glucoside (Mansour et al., 2021; Wiczowski et al., 2013).

3.4. Antioxidant potential of anthocyanin-rich extract

The *in vitro* antioxidant activity was assessed using the ABTS, DPPH, and FRAP methods, yielding results ranging from 8586 to 14,006 $\mu\text{mol ET}/100\text{ g}$, 9060 to 14,639 $\mu\text{mol ET}/100\text{ g}$ and 7204 to 9274 $\mu\text{mol ET}/100\text{ g}$ respectively (Table 1). The utilization of acid whey as an extraction solvent produced an extract with antioxidant activity comparable to acidified ethanol (AE) and values reported in the literature. Patras (2019) identified antioxidant activity ranging from 6321 to 14,337 mol TE/L using the ABTS method in extracts of red cabbage leaf residues. In red cabbage leaves, Yiğit, Turabi Yolaçaner, Hamzaloğlu, and Gökmen (2022) obtained antioxidant activity values ranging from 141.13 to 369.37 mmol TE/kg using the DPPH method. These results underscore the efficacy of acid whey as a biological solvent for the extraction of antioxidant compounds.

In the analysis of antioxidant activity through the cellular methods OxHLIA and TBARS, extracts derived from pressurized whey extraction also stood out (Table 1). According to Garcia et al. (2019), the classification of plant extracts can be based on IC_{50} values, indicating high activity ($IC_{50} < 50\ \mu\text{g}/\text{mL}$), moderate activity ($50 < IC_{50} < 100\ \mu\text{g}/\text{mL}$), weak activity ($100 < IC_{50} < 200\ \mu\text{g}/\text{mL}$), or inactivity ($IC_{50} > 200\ \mu\text{g}/\text{mL}$).

mL). Remarkably, PLE extracts exhibited the highest antioxidant capacity in the TBARS method, with IC₅₀ values ranging between 116 and 120 µg/mL, indicating a weak activity. The HSE and UAE extracts, with values of 153–204 µg/mL and 161–250 µg/mL, respectively, were classified as weakly active or inactive in this method. For the OxHLIA method, the UAE extracts showed moderately active activity, with IC₅₀ values ranging from 19 to 115 µg/mL, while the HSE extracts were inactive in this method. However, PLE extracts demonstrated high activity in reducing hemolysis, with values between 8 and 9 µg/mL, statistically superior (*p* < 0.05) to the positive control (Trolox 21.8 g/mL), indicating excellent antioxidant potential.

3.5. Antibacterial and antifungal activity

The values referring to the minimum inhibitory concentration (MIC) of red cabbage leaf extract against five Gram-negative and three Gram-positive bacteria are presented in (Table 3). All extracts tested showed inhibitory capacity against Gram-positive and Gram-negative bacteria, but none have presented bactericide effects, with MFC values higher than 10 mg/mL. Extracts obtained by acidified ethanol and acid whey were more active against Gram-positive bacteria than Gram-negative bacteria. A similar trend was observed by Garcia et al. (2019) when exploring the antibacterial activity of an anthocyanin extract obtained from Juçara fruit residues (*Euterpe edulis Martius*).

As described by Corrêa et al. (2017) natural extracts can be classified according to their minimum inhibitory concentration (MIC) into strong inhibitors (MIC below 0.5 mg/mL), moderate inhibitors (MIC between 0.6 and 1.5 mg/mL) and weak inhibitors (MIC above 1.6 mg/mL). Accordingly, although red cabbage extracts showed microbial inhibition, they can be considered weak bacterial growth inhibitors.

Table 3
Evaluation of the antibacterial activity of anthocyanin extracts from cabbage leaves.

Extract Type	Extraction Methods						Control Positive					
	HSE		UAE		PLE		Ampicillin 10 mg/mL		Imipenem 1 mg/mL		Vancomycin 1 mg/mL	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria												
<i>Escherichia coli</i>	10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
<i>Klebsiella pneumoniae</i>	10	>10	>10	>10	>10	>10	10	10	<0.0078	<0.0078	n.t.	n.t.
<i>Morganella morganii</i>	10	>10	10	>10	10	>10	>10	>10	<0.0078	<0.0078	n.t.	n.t.
<i>Proteus mirabilis</i>	10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
<i>Pseudomonas aeruginosa</i>	10	>10	10	>10	10	>10	>10	>10	0.5	1	n.t.	n.t.
Gram-positive bacteria												
<i>Enterococcus faecalis</i>	5	>10	5	>10	5	>10	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
<i>Listeria monocytogenes</i>	10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	2.5	>10	2.5	>10	5	>10	<0.15	<0.15	n.t.	n.t.	0.25	0.5
EA												
HSE		UAE		PLE		Ampicillin 10 mg/mL		Imipenem 1 mg/mL		Vancomycin 1 mg/mL		
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	
Gram-negative bacteria												
<i>Escherichia coli</i>	10	>10	5	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
<i>Klebsiella pneumoniae</i>	10	>10	10	>10	10	>10	10	10	<0.0078	<0.0078	n.t.	n.t.
<i>Morganella morganii</i>	10	>10	10	>10	5	>10	>10	>10	<0.0078	<0.0078	n.t.	n.t.
<i>Proteus mirabilis</i>	10	>10	10	>10	10	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
<i>Pseudomonas aeruginosa</i>	10	>10	10	>10	10	>10	>10	>10	0.5	1	n.t.	n.t.
Gram-positive bacteria												
<i>Enterococcus faecalis</i>	10	>10	10	>10	10	>10	<0.15	<0.15	n.t.	n.t.	<0.0078	<0.0078
<i>Listeria monocytogenes</i>	10	>10	5	>10	5	>10	<0.15	<0.15	<0.0078	<0.0078	n.t.	n.t.
MRSA	2.5	>10	2.5	>10	5	>10	<0.15	<0.15	n.t.	n.t.	0.25	0.5

AW: extract obtained with acid whey, AE: extract obtained with acidified ethanol, HSE: heating and stirring extraction, UAE: extraction assisted by ultrasound and PLE: extraction with pressurized liquid, MRSA: Methicillin-resistant *Staphylococcus aureus*, MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration, nt: not-tested.

The same potential was observed for antifungal activity (Table 4), as all extracts presented weak inhibitory profiles. Mansour et al. (2021) when investigating the antimicrobial activity of anthocyanins and flavanols from red cabbage, reported moderate effects against two types of cultures *Aspergillus niger* and *Candida albicans*, and no effect against *Pseudomonas aeruginosa*. However, it is worth mentioning that all microorganisms used in this study are clinically isolated multidrug-resistant strains that have a resistance profile much superior to ATCC-licensed standard strains.

Table 4
Evaluation of the antifungal potential of anthocyanin extracts from red cabbage leaves.

Extraction methods	Types of extract	<i>Aspergillus brasiliensis</i> (MIC/MFC)	<i>Aspergillus fumigatus</i> (MIC/MFC)
HSE	AE	10/>10	>10/>10
	AW	10/>10	10/>10
UAE	AE	10/>10	>10/>10
	AW	10/>10	10/>10
PLE	AE	>10/10	10/>10
	AW	5/>10	10/>10
Ketoconazole	–	0.06/0.125	0.5/1

AE: extract obtained with acidified ethanol, AW: extract obtained with acid whey, HSE: heating and stirring extraction, UAE: extraction assisted by ultrasound and PLE: extraction with pressurized liquid. MIC: minimum inhibitory concentration; MFC: minimum fungicidal concentration.

3.6. NO-production inhibition and antiproliferative activities

The antiproliferative effects of extracts from red cabbage leaf residue, evaluated for the inhibition of the four cell lines (AGS, CaCo-2, MCF-7, and VERO) proliferation are presented in (Table 5). The results were expressed as the extracts concentrations responsible for inhibit in 50 % cell proliferation (GI_{50}).

None of the samples studied exhibit the capacity to inhibit the proliferation of the tested cell lines ($GI_{50} > 400 \mu\text{g/mL}$). Likewise, the anthocyanin-rich extracts also demonstrated no ability to inhibit the production of NO in LPS-stimulated RAW 246.7 cells.

Therefore, the anthocyanin-rich extracts obtained using acid whey or acidified ethanol as solvents also did not reveal toxicity, which is an extremely positive result in terms of their potential application in food matrices. However, complementary *in vivo* tests are still needed to prove the absence of toxicity and to determine safe doses.

3.7. Application of anthocyanin-rich extract in marshmallow confectionery

The anthocyanin-rich extract obtained by the PLE method, using acid whey as the solvent, was incorporated into marshmallows to assess both coloration capability and antioxidant potential in a model food system (Fig. 1). This choice aligns with a sustainable approach, maintaining the upcycling application cycle through the use of extracts from cabbage leaf residues recovered by a bio-based solvent reused from cheese processing.

Color is one of the main quality parameters of food products, which impresses the acceptance of food by consumers. The marshmallows added to extract (B) exhibited a positive a^* parameter, within the red color spectrum, and a negative b^* parameter, corresponding to the blue spectrum and reduced luminosity (L^*) (Table 6). According to (Ghareaghajlou, Hallaj-Nezhadi, & Ghasempour, 2021) anthocyanins form four different structures which are in equilibrium with each other in aqueous solutions in a wide range of colors: at pH 1–3, the flavylium cation (red) is predominant; at pH 3–4 and 4–5 there are mixtures of 4 forms: carbinol pseudo base, flavylium cation, chalcone, quinonoid base in different proportions (violet to blue); at pH 6–7, a mixture of 3 forms: quinoidal base, carbinol pseudo base and chalcone (colorless forms); and; at pH > 7, anthocyanins are degraded (Patras, 2019). In this way, the color variation of the solution is a consequence of one or more anthocyanin configurations obtained by changes in the pH of the medium. The pH of the developed product was around (~5.5) which explains the expression of colors indicating shade in the purple color aspect. The marshmallow showed high chromaticity (C^*), which indicates the greater intensity of the color of the products. In addition, according to (Mojica, Berhow, & Gonzalez de Mejia, 2017) the increase in chromaticity may be related to a higher concentration of anthocyanins.

Sample B presents TMA of 1.31 mg C3G/g with antioxidant values of 511 and 771 $\mu\text{mol ET}/100 \text{ g}$, for ABTS and FRAP, respectively. In this way, it can be deduced that anthocyanin extract properties were

Table 5
Antiproliferative and anti-inflammatory potentials of extracts.

	AE			AW			Positive Controls	
	HSE	UAE	PLE	HSE	UAE	PLE	Ellipticin ($\mu\text{g/mL}$)	Dexamethasone ($\mu\text{g/mL}$)
AGS (GI_{50} , $\mu\text{g/mL}$)	>400	>400	>400	>400	>400	>400	1.23 \pm 0.03	–
CaCo-2 (GI_{50} , $\mu\text{g/mL}$)	>400	>400	>400	>400	>400	>400	1.21 \pm 0.02	–
MCF-7 (GI_{50} , $\mu\text{g/mL}$)	>400	>400	>400	>400	>400	>400	1.02 \pm 0.02	–
VERO (GI_{50} , $\mu\text{g/mL}$)	>400	>400	>400	>400	>400	>400	1.41 \pm 0.06	–
RAW 246.7 (IC_{50} , $\mu\text{g/mL}$)	>400	>400	>400	>400	>400	>400	–	6.3 \pm 0.4

AE: extract obtained with acidified ethanol, AW: extract obtained with acid whey, HSE: heating and stirring extraction, UAE: extraction assisted by ultrasound and PLE: extraction with pressurized liquid, AGS: gastric adenocarcinoma, CaCo2: colon adenocarcinoma, MCF-7: adenocarcinoma, in addition to the non-tumor line VERO (African green monkey kidney cells) and RAW 246.7: mouse macrophage-like cell line.

preserved in the food model evaluated. The red cabbage extract obtained in this study showed a predominance of acylated and diacylated groups as previously reported. According to (Zhao et al., 2017) the acyl structures present greater stability through intramolecular copigmentation phenomena, reducing the probability of a nucleophilic attack of water and preventing the hydrolysis of the red flavylium cation to the colorless carbinol base, allowing the formation of colorless carbinol, blue and purple quinoid bases.

In addition to coloring, texture is also an important factor in determining the quality of confectionery product products such as gummies, jellies, and marshmallows (De Roos, 2003). In the industrial scope, texture control is necessary, since the hardening of the product is seen as a technological problem due to the vitreous state or the formation of gelatin gel caused by the crystallization of sugar, being an undesirable factor and one of the main causes. Deterioration in confectionery products (Tan & Lim, 2008).

The marshmallows showed significant improvements in their texture with the addition of anthocyanin-rich extracts from purple cabbage. Compared to samples without the extracts, the samples with the extracts had lower values of hardness, chewiness, and cohesion ($p < 0.05$). Cohesion values ranged from 49.60 % to 75.50 %, hardness values ranged from 2.12 to 9.43 N, and chewiness values ranged from 1.56 to 5.70 N.

These results are consistent with previous studies showing that the addition of bioactive compounds can improve the texture properties of foods. In similar studies, the addition of microencapsulated bioactive compounds from eggplant peel to confectionery coatings resulted in a reduction in firmness (Horincar et al., 2020). While the addition of *Ficus carica* L. and *Prunus spinosa* L. extracts as anthocyanin-based food colorants in icing and “beijinho” candies led to an increase in softness and chewiness of the products (Backes et al., 2020). Furthermore, the anthocyanin-rich extracts also improved the elasticity of the marshmallows, with all sample elasticity values being above 70 %. This indicates that the addition of the extracts not only improved the texture of the marshmallows but also contributed to the formation of a stable foam with the proper texture.

4. Conclusion

The use of acid whey as a biological solvent satisfactory recovered anthocyanin from red cabbage leaf residues. Among the evaluated extraction methods, pressurized liquid extraction (PLE) resulted in better anthocyanin recovery with higher *in vitro* antioxidant activity determined by chemical and cell-based cells and lower polymeric color formation, which is related to anthocyanin degradation. In addition, the anthocyanin-rich extracts obtained by ethanol or acid whey showed as weak fungistatic and bacteriostatic activity. The extracts did not present anti-proliferative and anti-inflammatory activity against the evaluated tumor lines (AGS, CaCo2, MCF-7, and VERO). However, the extracts did not show cytotoxicity, indicating their safe application in food without harmful risks.

Anthocyanin extracts obtained by PLE with acid whey also



Fig. 1. Use of powdered anthocyanins in marshmallow.

A: control sample (no natural colorant) and B: sample with AWE powder extract (acid whey solvent extraction).

Table 6

Chemical and physical characterization of Marshmallows.

Analysis	Marshmallow samples	
	A	B
pH	5.7 ± 0.1	5.5 ± 0.1
TAM (mg C3G/g)	–	1.31 ± 0.03
% polymeric color	–	55.69 ± 0.50
Antioxidant activity		
ABTS (μmol ET/100 g)	–	511 ± 0.40
FRAP (μmol ET/100 g)	–	771 ± 0.02
Textura		
Cohesiveness (%)	75.50 ± 0.01 ^a	49.60 ± 0.02 ^b
Chewability (N)	5.70 ± 1.02 ^a	1.56 ± 6.72 ^b
hardness (N)	9.43 ± 1.07 ^a	2.12 ± 1.90 ^c
Elasticity (%)	74.28 ± 0.97 ^b	83.15 ± 1.08 ^a
Color		
L*	80.02 ± 0.20 ^a	60.61 ± 0.10 ^b
a*	2.07 ± 0.02 ^b	19.45 ± 0.10 ^a
b*	6.62 ± 0.03 ^a	–21.85 ± 0.07 ^b
°h	72.09 ± 0.30 ^a	48.32 ± 0.30 ^b
C*	6.93 ± 0.02 ^b	29.25 ± 0.40 ^a

Values expressed as mean ± standard deviation (t-test, equal letters mean no significant difference ($p < 0.05$)). AMT: total monomeric anthocyanins, C3G: cyanidin-3-glycoside, EAG: gallic acid equivalent, ET: Trolox equivalent, A: control sample (no dye), B: sample with AWE extract powder (solvent extraction acid whey), L*: brightness, a*: green/ red, b*: blue/yellow color component, C*: chromaticity and h°: matrix angle.

demonstrated potential as natural colorants for marshmallows, providing not only a desirable purple hue but also significant improvements in texture parameters. In summary, our results highlight the prominent potential of acid whey as a bio-based and green alternative solvent.

CRedit authorship contribution statement

Jaime Oliveira: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laís Benvenuti:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Bianca R. Albuquerque:** Formal analysis. **Tiane C. Finimundy:** Formal analysis. **Filipa Mandim:** Formal analysis. **Tânia C.S.P. Pires:** Formal analysis. **Carla Pereira:** Formal analysis. **Rúbia Carvalho Gomes Corrêa:** Writing – review & editing, Supervision. **Lillian Barros:** Writing – review & editing, Conceptualization. **Acácio Antonio Ferreira Zielinski:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Acknowledgment

The authors are also grateful to CAPES (OLIVEIRA, J. scholarship) and CNPq (Grant number 304020/2022-2), and to FCT/MCTES (PID-DAC): CIMO, UIDB/00690/2020 (DOI: [10.54499/UIDB/00690/2020](https://doi.org/10.54499/UIDB/00690/2020)) and UIDP/00690/2020 (DOI: [10.54499/UIDP/00690/2020](https://doi.org/10.54499/UIDP/00690/2020)); and Sus-TEC, LA/P/0007/2020 (DOI: [10.54499/LA/P/0007/2020](https://doi.org/10.54499/LA/P/0007/2020)); also to National funding by FCT, P.I., through the individual scientific employment program-contract for L. Barros and C. Pereira contracts. R. C. G. Corrêa is a research grant recipient of Cesumar Institute of Science, Technology, and Innovation (ICETI).

Data availability

Data will be made available on request.

References

- de Albuquerque, B. R., Corrêa, R. C. G., de Lima Sampaio, S., & Barros, L. (2023). Bioactive compounds from food and its by-products: Current applications and future perspectives. *Food Waste Conversion*, 3–41. https://doi.org/10.1007/978-1-0716-3303-8_1
- AOAC. (2012). *AOAC - official methods of analysis from the Association of Official Analytical Chemists International Arlington*. AOAC International.
- Azman, E. M., Nor, N. D. M., Charalampopoulos, D., & Chatzifragkou, A. (2022). Effect of acidified water on phenolic profile and antioxidant activity of dried blackcurrant (*Ribes nigrum* L.) pomace extracts. *LWT*, 154, Article 112733. <https://doi.org/10.1016/j.lwt.2021.112733>
- Backes, E., Leichtweis, M. G., Pereira, C., Carochi, M., Barreira, J. C. M., Kamal Genena, A., ... Ferreira, I. C. F. R. (2020). *Ficus carica* L. and *Prunus spinosa* L. extracts as new anthocyanin-based food colorants: A thorough study in confectionery products. *Food Chemistry*, 333. <https://doi.org/10.1016/j.foodchem.2020.127457>
- Benvenuti, L., Zielinski, A. A. F., & Ferreira, S. R. S. (2022). Pressurized aqueous solutions of deep eutectic solvent (DES): A green emergent extraction of anthocyanins from a Brazilian berry processing by-product. *Food Chemistry: X*, 13. <https://doi.org/10.1016/j.fochx.2022.100236>
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70–76.
- Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.F.: An underexploited and highly disseminated species. *Industrial Crops and Products*, 89, 45–51. <https://doi.org/10.1016/j.indcrop.2016.04.065>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30.
- Charron, C. S., Clevidence, B. A., Britz, S. J., & Novotny, J. A. (2007). Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage (*Brassica oleracea* L. Var. capitata). *Journal of Agricultural and Food Chemistry*, 55(13), 5354–5362. <https://doi.org/10.1021/jf0710736>
- Chen, T. L., Kim, H., Pan, S. Y., Tseng, P. C., Lin, Y. P., & Chiang, P. C. (2020). Implementation of green chemistry principles in circular economy system towards sustainable development goals: Challenges and perspectives. *Science of the Total Environment*, 716. <https://doi.org/10.1016/j.scitotenv.2020.136998>
- Corrêa, R. C. G., De Souza, A. H. P., Calhelha, R. C., Barros, L., Glamoclija, J., Sokovic, M., ... Ferreira, I. C. F. R. (2015). Bioactive formulations prepared from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom *Pleurotus ostreatus* Singer. *Food & Function*, 6(7), 2155–2164. <https://doi.org/10.1039/c5fo00465a>
- Corrêa, R. C. G., Haminiuk, C. W. I., Barros, L., Dias, M. I., Calhelha, R. C., Kato, C. G., ... Ferreira, I. C. F. R. (2017). Stability and biological activity of Merlot (*Vitis vinifera*) grape pomace phytochemicals after simulated in vitro gastrointestinal digestion and

- colonic fermentation. *Journal of Functional Foods*, 36, 410–417. <https://doi.org/10.1016/j.jff.2017.07.030>
- De Roos, K. B. (2003). Effect of texture and microstructure on flavour retention and release. *International Dairy Journal*, 13(8), 593–605. [https://doi.org/10.1016/S0958-6946\(03\)00108-0](https://doi.org/10.1016/S0958-6946(03)00108-0)
- Dong, Y., Wu, X., Han, L., Bian, J., He, C., El-Omar, E., Gong, L., & Wang, M. (2022). The potential roles of dietary anthocyanins in inhibiting vascular endothelial cell senescence and preventing cardiovascular diseases. *Nutrients*, 14(14). <https://doi.org/10.3390/nu14142836>
- Filafarro, M., Codeluppi, A., Brighenti, V., Cimurri, F., González-Paramás, A. M., Santos-Buelga, C., ... Vitale, G. (2022). Disclosing the antioxidant and neuroprotective activity of an anthocyanin-rich extract from sweet cherry (*Prunus avium* L.) using in vitro and in vivo models. *Antioxidants*, 11(2). <https://doi.org/10.3390/antiox11020211>
- García, J. A. A., Corrêa, R. C. G., Barros, L., Pereira, C., Abreu, R. M. V., Alves, M. J., ... Ferreira, I. C. F. R. (2019). Chemical composition and biological activities of Juçara (*Euterpe edulis* Martius) fruit by-products, a promising underexploited source of high-added value compounds. *Journal of Functional Foods*, 55, 325–332. <https://doi.org/10.1016/j.jff.2019.02.037>
- Ghareaghajlou, N., Hallaj-Nezhadi, S., & Ghasempour, Z. (2021). Red cabbage anthocyanins: Stability, extraction, biological activities and applications in food systems. *Food Chemistry* (Vol. 365). <https://doi.org/10.1016/j.foodchem.2021.130482>. Elsevier Ltd.
- Gonçalves, G. A., Soares, A. A., Correa, R. C. G., Barros, L., Haminiuk, C. W. I., Peralta, R. M., ... Bracht, A. (2017). Merlot grape pomace hydroalcoholic extract improves the oxidative and inflammatory states of rats with adjuvant-induced arthritis. *Journal of Functional Foods*, 33, 408–418. <https://doi.org/10.1016/j.jff.2017.04.009>
- Horincar, G., Enachi, E., Barbu, V., Andronoiu, D. G., Răpeanu, G., Stănciuc, N., & Aprodă, I. (2020). Value-added pastry cream enriched with microencapsulated bioactive compounds from eggplant (*Solanum melongena* L.) peel. *Antioxidants*, 9(4). <https://doi.org/10.3390/antiox9040351>
- Kowalski, A., Agati, G., Grzegorzewska, M., Kosson, R., Kusznierevicz, B., Chmiel, T., ... Kaniszewski, S. (2021). Valorization of waste cabbage leaves by postharvest photochemical treatments monitored with a non-destructive fluorescence-based sensor. *Journal of Photochemistry and Photobiology B: Biology*, 222. <https://doi.org/10.1016/j.jphotobiol.2021.112263>
- Kruszewski, B., & Boselli, E. (2024). Blackcurrant pomace as a rich source of anthocyanins: Ultrasound-assisted extraction under different parameters. *Applied Sciences*, 14(2), 821. <https://doi.org/10.3390/app14020821>
- Kumar Awasthi, M., Paul, A., Kumar, V., Sar, T., Kumar, D., Sarsaiya, S., ... Taherzadeh, M. J. (2022). Recent trends and developments on integrated biochemical conversion process for valorization of dairy waste to value added bioproducts: A review. *Bioresource Technology*, 344. <https://doi.org/10.1016/j.biortech.2021.126193>
- Lee, H. J., Lee, D. Y., Chun, Y. S., Kim, J. K., Lee, J. O., Ku, S. K., & Shim, S. M. (2022). Effects of blue honeysuckle containing anthocyanin on anti-diabetic hypoglycemia and hyperlipidemia in Ob/Ob mice. *Journal of Functional Foods*, 89. <https://doi.org/10.1016/j.jff.2022.104959>
- Lockowandt, L., Pinela, J., Roriz, C. L., Pereira, C., Abreu, R. M. V., Calheta, R. C., ... Ferreira, I. C. F. R. (2019). Chemical features and bioactivities of cornflower (*Centaurea cyanus* L.) capitula: The blue flowers and the unexplored non-edible part. *Industrial Crops and Products*, 128, 496–503. <https://doi.org/10.1016/j.indcrop.2018.11.059>
- Machado, M. H., da Almeida, A. R., de Maciel, M. V. O. B., Vitorino, V. B., Bazzo, G. C., da Rosa, C. G., ... Barreto, P. L. M. (2022). Microencapsulation by spray drying of red cabbage anthocyanin-rich extract for the production of a natural food colorant. *Biocatalysis and Agricultural Biotechnology*, 39. <https://doi.org/10.1016/j.cbab.2022.102287>
- Mansour, K. A., Moustafa, S. F., & Abdelkhalik, S. M. (2021). High-resolution UPLC-MS profiling of anthocyanins and flavonols of red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* DC.) cultivated in Egypt and evaluation of their biological activity. *Molecules*, 26(24). <https://doi.org/10.3390/molecules26247567>
- Menchik, P., Zuber, T., Zuber, A., & Moraru, C. I. (2019). Composition of coproduct streams from dairy processing: Acid whey and milk permeate. *Journal of Dairy Science*, 102(5), 3978–3984. <https://doi.org/10.3168/jds.2018-15951>
- Miyashita, K., Beppu, F., Hosokawa, M., Liu, X., & Wang, S. (2020). Bioactive significance of fucoxanthin and its effective extraction. *Biocatalysis and Agricultural Biotechnology*, 26. <https://doi.org/10.1016/j.cbab.2020.101639>
- Mojica, L., Berhow, M., & Gonzalez de Mejia, E. (2017). Black bean anthocyanin-rich extracts as food colorants: Physicochemical stability and antidiabetes potential. *Food Chemistry*, 229, 628–639. <https://doi.org/10.1016/j.foodchem.2017.02.124>
- Molina, A. K., Corrêa, R. C. G., Prieto, M. A., Pereira, C., & Barros, L. (2023). Bioactive natural pigments' extraction, isolation, and stability in food applications. *Molecules*, 28(3). <https://doi.org/10.3390/molecules28031200>
- Palos-Hernández, A., Gutiérrez-Fernández, M. Y., Escudera Burrieza, J., Pérez-Iglesias, J. L., & González-Paramás, A. M. (2022). Obtaining green extracts rich in phenolic compounds from underexploited food by-products using natural deep eutectic solvents. Opportunities and challenges. *Sustainable Chemistry and Pharmacy*, 29. <https://doi.org/10.1016/j.scp.2022.100773>
- Patras, A. (2019). Stability and colour evaluation of red cabbage waste hydroethanolic extract in presence of different food additives or ingredients. *Food Chemistry*, 275, 539–548. <https://doi.org/10.1016/j.foodchem.2018.09.100>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237.
- Reguengo, L. M., Salgaço, M. K., Sivieri, K., & Maróstica Júnior, M. R. (2022). Agro-industrial by-products: Valuable sources of bioactive compounds. *Food Research International*, 152. <https://doi.org/10.1016/j.foodres.2021.110871>
- Rocha-Mendoza, D., Kosmerl, E., Krentz, A., Zhang, L., Badiger, S., Miyagusuku-Cruzado, G., Mayta-Apaza, A., Giusti, M., Jiménez-Flores, R., & García-Cano, I. (2021). Invited review: Acid whey trends and health benefits. *Journal of Dairy Science*, 104(2), 1262–1275. <https://doi.org/10.3168/jds.2020-19038>
- Sendri, N., Singh, S., Bhatt, V., Bhatt, P., & Bhandari, P. (2022). Valorization of red cabbage pomace for stabilization of anthocyanins in *Rhododendron arboreum*. *Industrial Crops and Products*, 187. <https://doi.org/10.1016/j.indcrop.2022.115371>
- Tan, J. M., & Lim, M. H. (2008). Effects of gelatine type and concentration on the shelf-life stability and quality of marshmallows. *International Journal of Food Science and Technology*, 43(9), 1699–1704. <https://doi.org/10.1111/j.1365-2621.2008.01756.x>
- Teixeira, R. F., Benvenuti, L., Burin, V. M., Gomes, T. M., Ferreira, R. S., & Zielinski, A. A. F. (2021). An eco-friendly pressure liquid extraction method to recover anthocyanins from broken black bean hulls. *Innovative Food Science and Emerging Technologies*, 67. <https://doi.org/10.1016/j.ifset.2020.102587>
- Tena, N., Martín, J., & Asuero, A. G. (2020). State of the art of anthocyanins: Antioxidant activity, sources, bioavailability, and therapeutic effect in human health. *Antioxidants*, 9(5). <https://doi.org/10.3390/antiox9050451>
- Wiczowski, W., Szawara-Nowak, D., & Topolska, J. (2013). Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. *Food Research International*, 51(1), 303–309. <https://doi.org/10.1016/j.foodres.2012.12.015>
- Wu, C. F., Wu, C. Y., Lin, C. F., Liu, Y. W., Lin, T. C., Liao, H. J., & Chang, G. R. (2022). The anticancer effects of cyanidin 3-O-glucoside combined with 5-fluorouracil on lung large-cell carcinoma in nude mice. *Biomedicine and Pharmacotherapy*, 151. <https://doi.org/10.1016/j.biopha.2022.113128>
- Yiğit, Ü., Turabi Yolaçaner, E., Hamzaloğlu, A., & Gökmen, V. (2022). Optimization of microwave-assisted extraction of anthocyanins in red cabbage by response surface methodology. *Journal of Food Processing and Preservation*, 46(1). <https://doi.org/10.1111/jfpp.16120>
- Zhao, C. L., Yu, Y. Q., Chen, Z. J., Wen, G. S., Wei, F. G., Zheng, Q., ... Xiao, X. L. (2017). Stability-increasing effects of anthocyanin glycosyl acylation. *Food Chemistry*, 214, 119–128. <https://doi.org/10.1016/j.foodchem.2016.07.073>
- Zielinski, A. A. F., del Sanchez-Camargo, A. P., Benvenuti, L., Ferro, D. M., Dias, J. L., & Ferreira, S. R. S. (2021). High-pressure fluid technologies: Recent approaches to the production of natural pigments for food and pharmaceutical applications. *Trends in Food Science and Technology*, 118(November), 850–869. <https://doi.org/10.1016/j.tifs.2021.11.008>