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Polyphenols from *Plinia jaboticaba* peels: cellular antioxidant activity, anti-obesity effects, microencapsulation, controlled release, and functional beverage development

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ABSTRACT

Polyphenols from jabuticaba berry (*Plinia jaboticaba*) peels were recovered via ultrasound-assisted extraction, with sample-to-solvent ratio and extraction time optimised using response surface methodology. Optimal conditions were established at a sample-to-solvent ratio of 1:40 (*w/v*) and a reaction time of 6.75 min. The resulting freeze-dried jabuticaba peel extract (JPE) exhibited a total phenolic content of 9403 ± 364 mg/100 g, with flavonoids contributing 5196 ± 518 mg/100 g. Major phenolic constituents included ellagic acid (3766 ± 98 mg/100 g), procyanidin B2 (1828 ± 27 mg/100 g), vescalagin (555 ± 21 mg/100 g), gallic acid (307 ± 4 mg/100 g), (−)-epicatechin (243 ± 31 mg/100 g), and cyanidin-3-*O*-glucoside (196 ± 2 mg/100 g). JPE exhibited chemical antioxidant capacity across multiple assays, consistent with cellular antioxidant activity in H_2O_2-challenged human-derived hepatocellular carcinoma (HepG2) cells. In vitro assays also revealed anti-cholesterolemic (IC_{50} = 40.91 μg/mL), antidiabetic (IC_{50} = 33.52 μg/mL), and anti-obesity (IC_{50} = 30.50 μg/mL) potential. Anthocyanins in JPE exhibited high structural reversibility (90.52 ± 0.40%) during pH cycling from pH 2 to 10. The ionotropic gelation technique effectively entrapped the phenolic fraction within an alginate-carboxymethylcellulose matrix, achieving an encapsulation efficiency of 75.23 ± 1.67%. Antioxidant release from microcapsules was greater under simulated gastric than intestinal conditions. Incorporation of JPE into a model beverage enhanced both chemical and cellular antioxidant activity, with pasteurisation preserving flavonoid and anthocyanin contents. The JPE-enriched beverage achieved an acceptability index of 71%, with no significant differences ($p > 0.05$) across gender or age groups. Collectively, JPE represents a promising functional ingredient for sustainable food applications aimed at mitigating oxidative stress.

1. Introduction

In response to the global push for sustainable food systems and circular economy principles, increasing attention is being paid to food processing by-products as valuable sources of health-promoting compounds. The food industry produces substantial quantities of agricultural and processing residues, many of which are rich in bioactive compounds but are often discarded. These underutilised side streams offer considerable potential for valorisation through the development of functional ingredients and nutritionally enhanced food products (Boruah & Ray, 2024; Yadav et al., 2024). Jabuticaba (*Plinia jaboticaba* (Vell.) Kausel, also known as *Myrciaria jaboticaba*) is a berry-like fruit native to Brazil that is consumed fresh or used to produce juices, jellies, wines, and fermented beverages.

Jabuticaba peel, constituting approximately 40% of the fruit's weight, is often discarded during processing (Ferreira et al., 2020; Marsiglia et al., 2021). Jabuticaba peels (JP) are rich in polyphenols, particularly anthocyanins (cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside) and other flavonoids (quercetin, rutin, catechin, epicatechin), which have been linked to antioxidant,

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anti-inflammatory, and anticancer activities, making them ideal candidates for incorporation into functional foods (Nascimento et al., 2024; Plaza et al., 2016; Vuolo et al., 2019). Additionally, JP has already been studied for its anticholesterolemic effect in mice (Alves Castilho et al., 2021) and its in vitro protective effects on the formation of advanced glycation end products, which are associated with diabetes complications, cardiovascular disease, and chronic inflammation (Borges et al., 2022). However, in some cases, non-food-grade solvent systems have been used, such as acetone-water (70:30 *v*/v), distilled water, acidified ethanol (85% ethanol +1.5 mol/L HCl), and 50% methanol, for jabuticaba peel extraction (de Oliveira et al., 2018). While effective in recovering polyphenols, these solvents are not fully compatible with food applications, limiting their translational potential in functional ingredient development.

Another aspect of attention when recovering polyphenols from JP is the extraction methodology. Emerging extraction approaches—including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurised liquid extraction (PLE), and optimised solid–liquid extraction—offer promising alternatives by reducing solvent consumption, shortening processing times, and improving extraction yields (Friedrichsen et al., 2025). Yet, critical challenges remain in integrating JP phenolic-rich extracts into real food systems. Key uncertainties include the stability and controlled release of JP polyphenols during processing, storage, and consumption, as well as their potential impact on sensory attributes. Addressing these knowledge gaps through systematic research is crucial for advancing beyond laboratory-scale extraction to the development of robust, consumer-acceptable functional foods enriched with JP polyphenols. Such efforts will not only strengthen the scientific basis for their application but also accelerate innovation in sustainable food systems. Microencapsulation represents one technology to improve JP polyphenol stability and bioavailability (Naumovski et al., 2020). Vibrating nozzle technology has emerged as a potential alternative for encapsulating polyphenols from diverse sources (Quiroz-Eraso et al., 2024). In this technique, edible wall materials, such as sodium alginate, are used to entrap polyphenols within beads, thereby enhancing their bioaccessibility and bioactivity. By improving JP polyphenols bioavailability and functionality using microencapsulation, it not only supports the valorisation of JP by-products but also provides a logical extension of previous findings demonstrating the rich polyphenolic composition and bioactivity of jabuticaba-derived ingredients.

The valorisation of JP and seeds as functional ingredients aligns with the principles of sustainable development and the circular economy. Our previous studies on jabuticaba by-products demonstrated that jabuticaba seeds and leaves are rich sources of ellagitannins and anthocyanins, and exhibit antimicrobial, antioxidant, antihyperglycemic, antihypertensive, and cytotoxic activities in vitro (Fidelis et al., 2020). When incorporated into yoghurt, jabuticaba seed extract demonstrated prebiotic potential by improving gut microbiota composition and abundance (Fidelis et al., 2021). Similarly, Coelho et al. (2024) microencapsulated jabuticaba peel extract and incorporated it into dairy drinks, and the authors found that the drinks reduced glucose absorption. Other studies have explored the incorporation of JP flour or extracts into foods, such as cookies (Zago et al., 2015), mortadella (Baldin et al., 2018), and isotonic drinks (Ferreira et al., 2020; Porfírio et al., 2019). However, despite the advances, the development and analysis of a functional beverage specifically designed to preserve bioactivity after processing and to evaluate cellular-level antioxidant effects remains limited. In this context, the present study introduces an integrated and innovative approach by combining optimised extraction, vibrating-nozzle microencapsulation, gastrointestinal release assessment, and HepG2-based cellular antioxidant evaluation within a beverage matrix, thereby expanding current knowledge beyond formulation-focused or chemical analyses alone.

2. Materials and methods

2.1. Reagents and cell line

Folin-Ciocalteu 2 N phenol reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichlorofluorescein diacetate (DCFDA), (+)-catechin, 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), ascorbic acid, gallic acid, hydrogen peroxide (H_2O_2), neocuproin (2,9-dimethyl-1,10-phenanthroline), ethylenediaminetetraacetic acid tripotassium salt dihydrate (EDTA), dithiothreitol (DTT), ferrozine, copper (II) chloride dihydrate, 3,5-dinitrosalicylic acid (DNS), acetonitrile, phosphoric acid (85% purity in water), 1,10-phenanthroline, recombinant human 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), nicotinamide adenine dinucleotide phosphate (NADPH), *p*-nitrophenyl butyrate (pNPB), Orlistat, porcine pancreatic amylase, and iron (II) sulphate heptahydrate were obtained from Sigma-Aldrich-Merck (Darmstadt, Germany). Food-grade carboxymethylcellulose sodium salt and sodium alginate were obtained from Ingreland (Shanghai, China) and Guangzhoushi Yuanmeng Trading Co., Ltd. (Guangzhou, China), respectively. Food-grade calcium chloride dihydrate was purchased from Intra-Laboratories (Plymouth, United Kingdom). Acetic acid was obtained from J. T. Baker (Mallinckrodt Baker Inc., Utrecht, the Netherlands). Aluminium chloride hexahydrate, sodium hydroxide, and sodium carbonate were purchased from Panreac Quimica S.A. (Barcelona, Spain). HPLC standards (>98% purity) of delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, procyanidin B2, (−)-epicatechin, ferulic acid, ellagic acid, and hesperidin were purchased from Extrasynthese (Genai, France). Vescalagin and castalagin were obtained from Phytolab GmbH & Co. KG (Vestenbergsgreuth, Germany). The following ingredients were used in the functional beverage: Blueberry flavour drops (MyProtein, Warrington, Cheshire, United Kingdom), xanthan gum (Special Ingredients, Chesterfield, Derbyshire, England), and natural elderberry red colouring (Creative Flavours, Limerick, Ireland). The human hepatocellular carcinoma cell line HepG2 (ATCC® HB-8065™), derived from a 15-year-old Caucasian male with liver cancer, was obtained from the American Type Culture Collection (Manassas, Virginia, USA).

2.2. Jabuticaba peels and extraction procedure

2.2.1. Preparation of jabuticaba peel sample powder

Ripe jabuticaba fruits [*Plinia jaboticaba* (Vell.) O.Berg] cv. Sabará (10 kg) were harvested at a ripe stage in Araucaria, Paraná, Brazil (geographical coordinates: 25°2924.7″S 49°2637.8″W), Brazil, in December 2018. Fruits were sanitised with 100 mg/L sodium hypochlorite for 15 min. The pulp and seeds were manually extracted, and the peels were dried at 40 °C for 48 h. Dried peels were ground to standardise the particle size (42 Tyler mesh). This project is registered in the Brazilian National System of Management of Genetic Heritage (SisGen) (register# AA51DBC).

2.2.2. Jabuticaba peel powder extraction: Preliminary tests

To assess the effect of the ethanol concentration on the polyphenol extraction (e.g., total phenolic content, total anthocyanin content, and instrumental colour – Section 2.3) an initial screening was conducted using a sample-to-solvent ratio of 1:30 *w*/*v*, with varying ethanol concentrations (20%, 40%, 60%, 80%, and 100%) and 100% water for comparison purposes. An ultrasonic liquid processor (model CL-334, serial no. 2021070112, Thermo Fisher Scientific, Karlsruhe, Germany) with a power output of 500 W and a frequency of 20 kHz was used. Extractions were carried out for 5 min in duplicate in a 50 mL centrifuge tube covered with aluminium foil to protect the sample from light, and under an ice bath to prevent degradation from temperature increases. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were conducted to identify significant differences ($p < 0.05$) between extraction conditions and to determine the optimal ethanol

concentration. All statistical calculations were performed using TIBCO Statistica v.13 (TIBCO Statistica Inc., Palo Alto, CA, USA).

2.2.3. Response surface design: Optimising the UAE extraction conditions

The ethanol concentration that maximised total phenolic content during extraction was then selected for optimisation using a design of experiments. For this purpose, the effects of sample-to-solvent ratio (1:20, 1:30, and 1:40 *w/v*) and time (5, 7.5, and 10 min) were studied using a 3^2-factorial design (levels −1, 0 and + 1) where the central point (solid-to-solvent ratio of 1:30 w/v, 7.5 min) was conducted three times to estimate the error (**Table 1**), totalling 12 extraction conditions. Extractions were performed twice in random order to minimise carry-over effects, and the extracts were then pooled for further analysis. The extracts were centrifuged at 1200 ×*g* for 5 min, then vacuum-filtered through qualitative filter paper. The extracts were analysed within 24 h post-extraction and stored at −18 °C.

2.3. Bioactive compounds, colour, and antioxidant capacity

The total phenolic content (TPC) was determined by UV–Vis spectrophotometry using the Folin-Ciocalteu method (Margraf et al., 2015). An analytical curve was prepared using gallic acid, and the results were expressed in mg of gallic acid equivalent per gram (mg GAE/g). The total monomeric anthocyanin content (TAC) was assessed by the pH differential method based on the structural modifications of anthocyanins at pH 1 and pH 4.5. The results were expressed as mg of cyanidin-3-*O*-glucoside equivalent per g (mg C3G/g). The total flavonoid content (TFC) of the extracts was determined in quadruplicate by the aluminium chloride colourimetric method described by Zhishen et al. (1999), with (+)-catechin as the standard. The results were expressed as milligrams of (+)-catechin equivalent per 100 g (mg CTE/100 g).

The extracts' colour attributes were analysed by UV-VIS spectrophotometry at 420 nm (yellow pigments), 520 nm (red pigments), and 620 nm (blue pigments), where the sum of these absorbance values represents the colour intensity of each extract. The percentage of each pigment was calculated based on the colour intensity.

The Fe^{2+} chelating ability was evaluated using the ferrozine-$FeSO_4$ method as described by Santos et al. (2017). Na-EDTA was used to generate the analytical curve, and results were expressed as mg EDTAE/100 g. The ferric-reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain (1996). Ascorbic acid was used as the standard, and results were expressed as mg AAE/100 g of sample. The hydroxyl radical scavenging activity (HRSA) of the extracts was measured using the method of Mukhopadhyay et al. (2016), which employed H_2O_2 and 1,10-phenanthroline. Gallic acid was used to plot the analytical curve, and results were expressed as mg GAE/100 g of sample. All analyses were run in quadruplicate.

2.4. Response surface modelling and optimisation

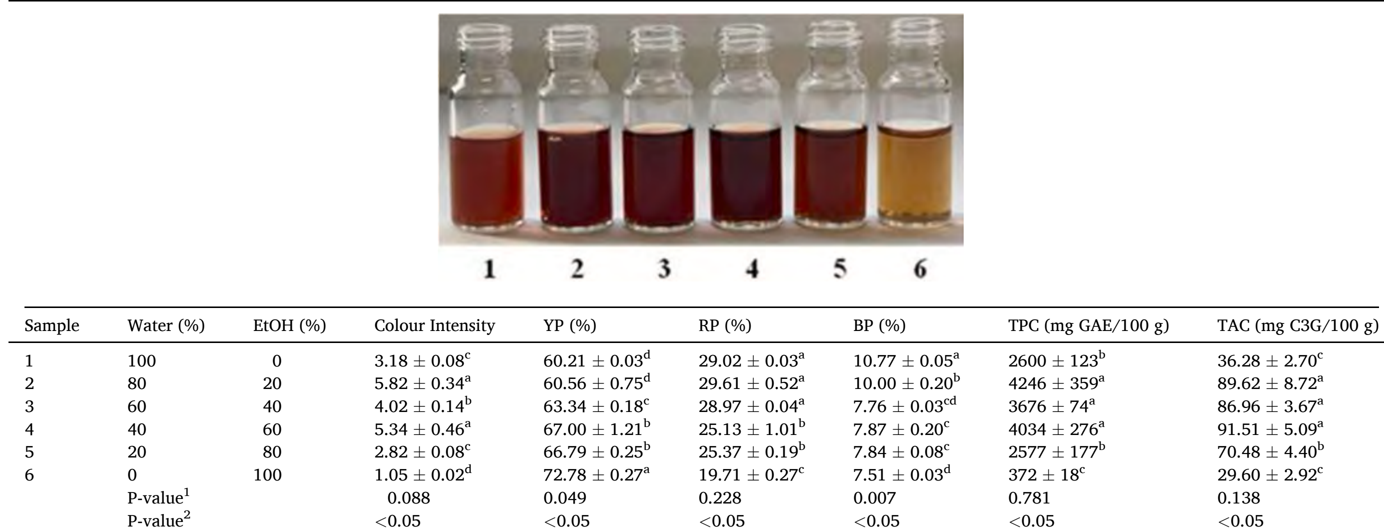
The results were utilised to generate multiple regression models via Response Surface Modelling (RSM) using a second-order polynomial equation (Eq. 1). This allowed for the assessment of the effects of sample-to-solvent ratio (x_i) and extraction time (x_j) on the colour attributes, TPC, TAC, and antioxidant capacity of the extracts. RSM models were proposed based on statistically significant regression coefficients ($p < 0.10$), and the determination coefficient (R^2) and the adjusted R^2 were calculated to assess model fit. R^2 values higher than 0.70 were considered suitable.

$$\text{Response} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} {x_1}^2 + \beta_{22} {x_2}^2 + \beta_{12} x_1 x_2 + \varepsilon \quad (1)$$

The optimal UAE conditions were determined using the desirability function approach, as described by Derringer and Suich (1980). TAC and HRSA were selected as key quality indicators of the extracts to maximise both responses. A multi-response optimisation was performed by assigning individual desirability functions to TAC (+1; maximal value) and HRSA (+1; maximal value), which were then combined into an overall desirability index. The optimisation process employed 100 iterations, each evaluated at exact grid points within the experimental design space, to identify the conditions that yielded the highest TAC and HRSA.

The optimal jabuticaba peel extract (JPE) was obtained using the UAE conditions, following the same experimental procedure described in Section 2.2. For this purpose, 600 mL of extract was produced in 12 batches of 50 mL. Liquid extracts were pooled, filtered through qualitative paper, and centrifuged for 5 min at 1000 ×*g* to remove solid impurities. The ethanol was removed from the extract under vacuum, and water was added back to compensate for the ethanol loss. This extract

Table 1
Effect of varying ethanol concentrations on pigment recovery, total phenolics, and anthocyanin content in jabuticaba peel extracts.



Sample	Water (%)	EtOH (%)	Colour Intensity	YP (%)	RP (%)	BP (%)	TPC (mg GAE/100 g)	TAC (mg C3G/100 g)
1	100	0	3.18 ± 0.08[c]	60.21 ± 0.03[d]	29.02 ± 0.03[a]	10.77 ± 0.05[a]	2600 ± 123[b]	36.28 ± 2.70[c]
2	80	20	5.82 ± 0.34[a]	60.56 ± 0.75[d]	29.61 ± 0.52[a]	10.00 ± 0.20[b]	4246 ± 359[a]	89.62 ± 8.72[a]
3	60	40	4.02 ± 0.14[b]	63.34 ± 0.18[c]	28.97 ± 0.04[a]	7.76 ± 0.03[cd]	3676 ± 74[a]	86.96 ± 3.67[a]
4	40	60	5.34 ± 0.46[a]	67.00 ± 1.21[b]	25.13 ± 1.01[b]	7.87 ± 0.20[c]	4034 ± 276[a]	91.51 ± 5.09[a]
5	20	80	2.82 ± 0.08[c]	66.79 ± 0.25[b]	25.37 ± 0.19[b]	7.84 ± 0.08[c]	2577 ± 177[b]	70.48 ± 4.40[b]
6	0	100	1.05 ± 0.02[d]	72.78 ± 0.27[a]	19.71 ± 0.27[c]	7.51 ± 0.03[d]	372 ± 18[c]	29.60 ± 2.92[c]
	P-value[1]		0.088	0.049	0.228	0.007	0.781	0.138
	P-value[2]		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Note: Values represent mean ± SD (n = 4). [1]Probability values obtained by the Brown-Forsythe test of homoscedasticity; [2]Probability values obtained by either one-way ANOVA (homoscedastic data) or Welch-ANOVA (heteroscedastic data); YP = yellow pigments; RP = red pigments; BP = blue pigments; TPC = total phenolic content; GAE = gallic acid equivalent; TAC = total anthocyanin content; C3G = cyanidin-3-*O*-glucoside equivalent. Different superscript letters within the same column indicate statistically significant differences (p < 0.05).

was analysed for bioactive compounds and antioxidant capacity (Section 2.3). The predicted values from each RSM equation and the actual values were used to generate ±95% prediction intervals.

2.5. Optimal JPE extract: phenolic compounds by HPLC-DAD/TOF-MS

Anthocyanins in the optimal JPE were analysed by high-performance liquid chromatography (HPLC) with a diode-array detector (DAD) using an Agilent Technologies (Agilent 1200 Infinity Series) system under the same chromatographic conditions as those employed by Mohammadi et al. (2024). Four anthocyanins were screened in the analysis: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside, and delphinidin-3-*O*-rutinoside - of which only cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside were identified and quantified in the sample (Fig. 1).

The analysis of gallic acid, vescalagin, castalagin, procyanidin B2,

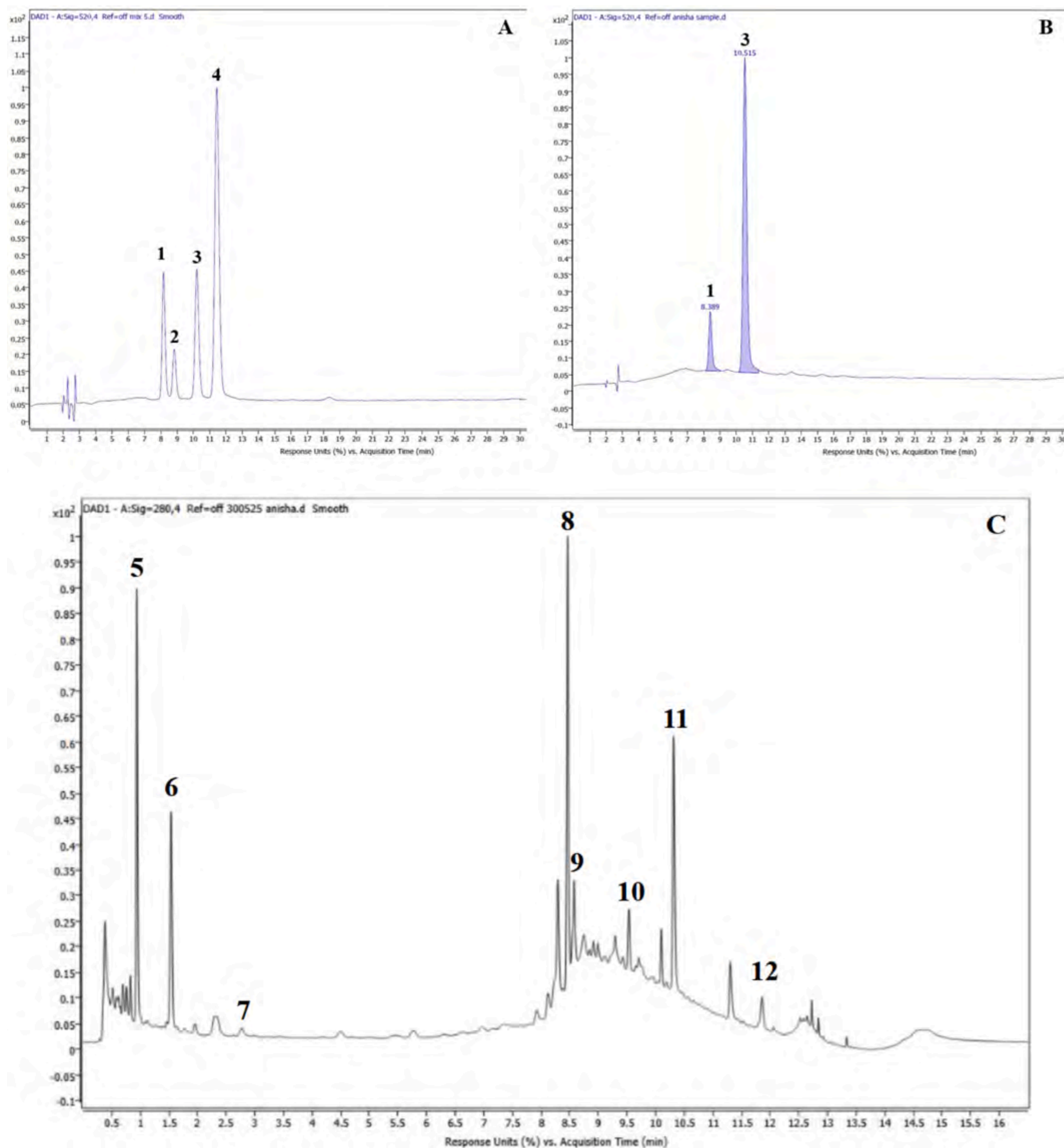


Fig. 1. HPLC-DAD at 520 nm of anthocyanin standards (**A**), namely **1** – delphinidin-3-*O*-glucoside, **2** – delphinidin-*O*-rutinoside, **3** – cyanidin-3-*O*-glucoside, and **4** – cyanidin-3-*O*-rutinoside; (**B**) identification of the two major anthocyanins in jabuticaba peel extract. (**C**) HPLC-DAD profile of jabuticaba peel extract phenolics at 280 nm, where **5** – gallic acid, **6** – vescalagin, **7** – castalagin, **8** – procyanidin B2, **9** – (−)-epicatechin, **10** – ferulic acid, **11** – ellagic acid, **12** – hesperidin.

epicatechin, ferulic acid, ellagic acid, and hesperidin in the optimal JPE was performed by HPLC coupled with time-of-flight mass spectrometry (LC/MS 6230 TOF), using a system from Agilent Technologies equipped with a DAD. The separation was carried out on a Poroshell 120 EC-C18 column (3 × 50 mm, 2.7 μm, Agilent Technologies), maintained at 40 °C, with a flow rate of 0.8 mL/min. The mobile phase consisted of solvent A (water acidified with 0.1% formic acid) and solvent B (acetonitrile), using a gradient elution program as follows: 95% A/5% B at 0.00 min (held until 3.60 min); 90% A/10% B at 5.60 and 6.30 min; 85% A/15% B at 7.00 min; 75% A/25% B at 10.80 min; 30% A/70% B at 11.70 and 12.20 min; returning to 95% A/5% B at 13.10 min and maintained until 16.10 min. The injection volume was 1 μL, and detection of vescalagin, castalagin, and ellagic acid was at 254 nm, and gallic acid, procyanidin B2, (−)-epicatechin, ferulic acid, and hesperidin were identified at 280 nm. The compounds were identified and quantified by comparing their retention times, analytical curves, and UV spectral characteristics with those of external standards.

2.6. Reversibility of anthocyanins in the optimal jabuticaba peel extract

The pH stability of the optimal JPE was evaluated by sequentially adjusting the sample from pH 2 to 4, 6, 8.7, and 10 using a 2 mol/L NaOH solution, with aliquots collected at each pH (Sari et al., 2012). After reaching pH 10, the extract was readjusted to pH 2 with 1 mol/L HCl to assess the reversibility of the anthocyanins. Colour attributes (e.g., redness, yellowness, and blueness) and antioxidant capacity (Fe^{2+} chelating ability and HRSA) were measured as described in Section 2.3 to monitor changes in antioxidant potential across the pH gradient.

2.7. Effects of the optimal jabuticaba peel extract on HepG2 cell viability and cellular antioxidant activity

The effects of the optimal JPE and functional beverage on HepG2 cell viability were determined by the MTT assay. HepG2 cells were seeded into 96-well plates at a density of 1×10^4, and after adherence, treatments with JPE and beverages were performed at various concentrations (5, 20, 50, 100, 200, and 400 μg/mL) for 24 h. Subsequently, 10% MTT (5 mg/mL) was added, and after 4 h of the MTT reaction, the culture medium was removed, and 200 μL of DMSO was added. The samples were analysed in a spectrophotometer at 570 nm. Two independent experiments were performed, each with three technical replicates, and the results are presented as the mean ± standard deviation.

The effects of JPE and beverages on the scavenging of intracellular ROS in HepG2 cells (1 × 104 cells/well) were investigated using the DCFDA method. Briefly, HepG2 cells were treated with samples at concentrations of 5, 20, 50, 100, 200, and 400 μg/mL, for 24 h. After treatment, a DCFDA solution (5 μmol/L) was added to each well, and the reaction proceeded at 37 °C for 30 min in the dark. Afterwards, a 1 mmol/L H_2O_2 solution was added to the wells and incubated for 20 min. The negative control comprised cell culture media without H_2O_2. The fluorescence intensity was measured at $\lambda_{excitation}$ = 450 nm and $\lambda_{excitation}$ = 520 nm. The results were expressed as a percentage of the fluorescence intensity relative to the positive control. Two independent experiments were performed, each with three technical replicates, and the results are presented as the mean ± standard deviation.

2.8. In vitro cholesterol-lowering potential, anti-obesity and antidiabetic activity of the optimal jabuticaba peel extract

The potential cholesterol-lowering activity of JPE against HMGCR was evaluated using a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent HMGCR activity assay, as described by Iqbal et al. (2014). Recombinant human HMGCR (0.1 U/mL) was prepared in phosphate-buffered saline (PBS; 100 mM, pH 7.4) supplemented with 1 mM EDTA and 2 mM dithiothreitol (DTT). JPE was initially dissolved in dimethyl sulfoxide (DMSO) at 100 mg/mL and subsequently diluted in PBS to achieve final test concentrations of 25, 50, 75, 100, and 125 μg/mL, maintaining DMSO at ≤1%. In a 96-well microplate, 50 μL of enzyme solution was preincubated with 50 μL of either JPE or a control (PBS as the negative control; 1 μM simvastatin as the positive control) at 37 °C for 15 min. The reaction was initiated by adding 100 μL of substrate solution containing 0.15 mM HMG-CoA and 0.2 mM NADPH in PBS. The NADPH consumption was monitored by measuring the decrease in absorbance at 340 nm every 30 s for 10 min on a microplate reader. The initial rate (ΔA_{340nm}/min) was calculated from the linear portion of the absorbance-time curve. All experiments were performed three times in triplicate. The enzymatic activity inhibition was calculated according to Eq. 2:

$$\text{HMGCR in}\textit{hibition}\ (\%) = 100\,x\left(\frac{V_{control} - V_{sample}}{V_{control}}\right) \quad (2)$$

where $V_{control}$ and V_{sample} represent the reaction velocities in the absence and presence of the enzyme inhibitor, respectively. The concentration at which JPE or the control exerts half of its maximal inhibitory effect (IC_{50}) was calculated using regression analysis and expressed as μg/mL.

The anti-obesity potential was assessed using 0.1 mg/mL porcine pancreatic lipase (PPL; 10 U/mL) prepared in PBS (50 mM phosphate, pH 8) (Nayebhashemi et al., 2023). A 96-well microplate containing JPE or Orlistat as the positive control (25, 50, 75, 100, and 125 μg/mL, 20 μL) was combined with 20 μL of a 10 mM pNPB solution and 60 μL of lipase. The microplate was incubated for 10 min at 37 °C, and the absorbance was measured at 405 nm. Lipase inhibition was determined by the reduction in PPL activity in the presence of JPE. The assay was performed in triplicate across three independent experiments. The percentage inhibition was calculated using Eq. 3:

$$\textit{Lipase inhibition}\ (\%) = 100\,x\left(1 - \frac{B - B_c}{A - A_c}\right) \quad (3)$$

where A = enzymatic activity without inhibitor, A_c = Negative control without inhibitor, B = enzymatic activity with inhibitor, B_c = Negative control with inhibitor. The IC_{50} was calculated using regression analysis and expressed as μg/mL.

The in vitro antidiabetic potential of JPE was assessed using the α-amylase inhibition assay, with acarbose as the positive control (Oluwagunwa et al., 2021). JPE or acarbose (25, 50, 75, 100, and 125 μg/mL; 100 μL) was mixed with 100 μL of a 0.006 M NaCl solution to maintain ionic strength and enzyme stability, 200 μL of PBS (pH 6.9), and 100 μL of porcine pancreatic α-amylase (0.05 mg/mL). The reaction was initiated by adding 500 μL of a 1% (*w/v*) starch solution, followed by incubation at 37 °C for 10 min. The enzymatic reaction was terminated by adding 500 μL of 3,5-dinitrosalicylic acid (DNS) reagent and heating at 100 °C for 5 min. After cooling to room temperature, the reaction mixtures were diluted to a final volume of 5 mL with distilled water, and absorbance was measured at 540 nm. The percentage of α-amylase inhibition was calculated using Eq. 4:

$$\alpha - \text{Amylase inhibition}\ (\%) = 100\,x\left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \quad (4)$$

where $A_{control}$ is the absorbance of the reaction mixture without inhibitor (i.e., enzyme and substrate) without JPE/acarbose, and A_{sample} is the absorbance of the reaction mixture containing the JPE. The IC_{50} was calculated using regression analysis and expressed as μg/mL.

2.9. Microencapsulation of the optimal jabuticaba peel extract and kinetic release under gastric and intestinal fluids

JP polyphenol-enriched microbeads were produced via an ionotropic gelation-based encapsulation technique using an alginate solution, under the optimal conditions established by our research group (Farrell et al., 2024). A homogeneous solution of freeze-dried JPE (2%), sodium

alginate (1%), and carboxymethyl cellulose (CMC) (1%) in distilled water was prepared using a homogeniser (IKA Ultra-Turrax, Staufer, Germany) under continuous shaking at 10,000 rpm for 5 min. Then, the solution was homogenised under magnetic stirring for 45 min to hydrate and eliminate bubbles. This inlet solution was extruded using a micro-encapsulator B-390 (Büchi Labortechnik AG, Postfach, Switzerland) under preset conditions of frequency (1000 Hz), electrode potential (250 V), and pressure (396 mbar). For complete cross-linking, the resulting microbeads were stirred in a calcium chloride solution (5%) for 30 min. An aliquot of the initial stock solution and the hardening solution were collected after the gelation period for further analysis. Subsequently, the microbeads were filtered, and the filtrate was collected. The microbeads were then washed with water, air-dried for 24 h, and stored at 4 °C until use. Encapsulation efficiency (EE), a measure of the extent to which the intended polyphenols are incorporated within the microbeads, was calculated using Eq. 5.

$$EE\ (\%) = (A_a/A_b)^{*}100 \tag{5}$$

where A_a is the total phenolic content after encapsulation, and A_b is the total phenolic content before encapsulation, both expressed as mg GAE/100 g.

Dried microcapsules (100 mg) were dispersed in 10 mL of simulated gastric fluid [SGF at pH 3.0, composition in a litre: 17.25 mL of 0.5 mol/L KCl, 2.25 mL of a 0.5 mol/L KH_2PO_4, 31.25 mL of 1 mol/L $NaHCO_3$, 29.5 mL of 2 mol/L NaCl, 1 mL of 0.15 mol/L $MgCl_2.6H_2O$, and 1.25 mL of 0.5 mol/L $(NH_4)_2CO_3$] and incubated at 37 °C under orbital stirring (180 rpm) for 4 h. During the gastric phase, 700 μL aliquots were collected every 2 h and immediately replaced with an equal volume of fresh SGF to maintain constant volume. After the 4 h incubation, the mixture was centrifuged at 3000 $\times g$ for 5 min, and the precipitated microcapsules were re-suspended in 10 mL of simulated intestinal fluid (SIF, pH 7.0; composition in one litre: 17 mL of 0.5 mol/L KCl, 2 mL of a 0.5 mol/L KH_2PO_4, 106.25 mL of 1 mol/L $NaHCO_3$, 24 mL of 2 mol/L NaCl, and 2.75 mL of 0.15 mol/L $MgCl_2.6H_2O$). The intestinal phase was maintained at 37 °C with stirring (180 rpm) for 6 h. During this stage, 700 μL aliquots were withdrawn every 2 h, and the same volume (700 μL) of fresh SIF was added back immediately to maintain constant volume. Collected aliquots from both gastric (t = 0, 2, and 4 h) and intestinal (t = 0, 2, 4, and 6 h) phases were clarified by centrifugation and analysed for TPC, TFC, FRAP, and CUPRAC as described in Section 2.3.

2.10. Application of optimal jabuticaba peel extract on functional beverage development

Ethanol-free JPE obtained after 30 min of magnetic stirring was used to formulate a functional beverage for sensory analysis. The approach outlined by Saleem et al. (2025) was adapted with certain modifications, and the beverage was prepared in a food-grade laboratory. JPE (50%), spring water (50%), natural blueberry flavour (0.5%), and xanthan gum (0.15%) were blended to produce a functional beverage. A control beverage (blank) was prepared by replacing JPE with water, and natural elderberry red colour (0.045%) was added to mimic JPE's colour. Both beverages were subjected to pasteurisation (81 °C/1 min) (Waghmare, 2024) using a stainless-steel pasteurizer and stored at 4 °C in glass jars. For analytical purposes, the JPE-enriched beverage was formulated using optimal JPE obtained through UAE after ethanol elimination. A corresponding control beverage (blank), prepared without JPE. Both followed the above-mentioned formulation, excluding xanthan gum.

2.10.1. Effect of temperature on antioxidant stability and other physicochemical properties

Each of the two analytical beverages (JPE and blank) was further divided into two portions: one portion was pasteurised (81 °C for 1 min), while the other remained unpasteurised (e.g., blank). This enabled assessment of the effects of thermal treatment on antioxidant stability and other physicochemical properties (Section 2.3). Pasteurised samples were cooled to room temperature using an ice bath, and all the samples were stored at 4 °C before analysis.

2.10.2. Sensory analysis

A sensory evaluation of the JPE-enriched functional beverage and the blank was conducted in individual sensory booths. Ethics approval was obtained from the University of Limerick - Science and Engineering Research Ethics Committee (protocol number 2025_04_13_S&E), and participants were informed about the sensory procedure/samples before giving written and verbal consent. A total of 53 untrained participants (20 males and 33 females) aged 18–50 years participated in the study. Participants were instructed to evaluate each sample (coded with a three-digit number) independently using a 9-point hedonic scale, assessing four sensory attributes: colour, taste, texture (mouthfeel), and overall impression (1 = dislike extremely to 9 = like extremely). Additionally, consumer purchase intent was assessed using a 6-point willingness-to-pay scale. Options ranged from “I would not buy it” to “I would pay €3.00”. The acceptability Index (AI) was also calculated using Eq. 6.

$$AI\ (\%) = (A/B)^{*}100 \tag{6}$$

where, A is the average score, and B is the maximum score given by the candidates.

2.11. Statistical analyses

Differences among groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Comparisons between two samples during in vitro digestion were performed using a paired Student's t-test. Enzyme inhibition by JPE was compared with that of reference positive controls (Section 2.8) using an unpaired Student's t-test. Statistical analysis and graphical representation were performed using GraphPad Prism software version 8.0 (GraphPad Software, Inc., San Diego, CA, USA) and Microsoft Excel version 2011. A p-value of <0.05 was considered statistically significant.

3. Results and discussion

3.1. Effects of ethanol concentrations on colour, antioxidant capacity, and polyphenols

In this study, ethanol–water mixtures were evaluated for their ability to extract bioactive compounds from jabuticaba peel, as assessed by TPC, TAC, pigment composition, and colour intensity. Solvent systems containing 20–60% ethanol showed the highest extraction efficiency (**Table 1**), with 20% ethanol yielding the highest TPC (4246 ± 359 mg GAE/100 g), TAC (89.62 ± 8.72 mg C3G/100 g), red pigment content (29.61 ± 0.52%), and colour intensity (5.82 ± 0.34 a.u.). These results indicate that 20% ethanol provides an optimal balance between bioactive recovery and desirable chromatic properties. These findings differ from those of Rodrigues et al. (2015), who reported higher phenolic and anthocyanin yields using 46% acidified ethanol combined with UAE, emphasizing yield maximisation through cavitation and low pH. In contrast, the present study demonstrates that a milder, non-acidified solvent can achieve comparable bioactive recovery, likely by favouring the extraction of more polar phenolics. Similarly, Tarone et al. (2021) reported markedly lower phenolic yields using 50% ethanol under UAE, reinforcing the effectiveness of 20% ethanol in this work. Although higher ethanol concentrations have been shown to enhance anthocyanin solubilisation (Nunes Mattos et al., 2022; Paludo et al., 2019), they may reduce antioxidant recovery due to limited extraction of hydrophilic compounds. Overall, these comparisons highlight the strong influence of solvent composition, matrix characteristics, and extraction conditions on bioactive yield and functionality.

Bioactive compounds such as polyphenols generally exhibit solubility that depends strongly on solvent polarity, following the like-dissolves-like principle. JP polyphenols comprise compounds with varying polarity, including relatively polar phenolic acids and glycosylated anthocyanins, as well as less polar flavan-3-ols and procyanidins. Accordingly, the use of ethanol–water mixtures enables effective tuning of solvent polarity, allowing the simultaneous solubilisation of both hydrophilic and moderately hydrophobic phenolics. While water promotes the extraction of highly polar, hydrogen-bonding compounds, ethanol enhances the solubility of less polar aromatic structures and reduces solvent viscosity, thereby improving mass transfer and anthocyanin extraction efficiency (Borges et al., 2020; Brglez Mojzer et al., 2016; Plaskova & Mlcek, 2023). In addition, ethanol is also easily removed using scalable processes, making it well suited for food applications (Lee et al., 2024).

Pearson correlation analysis was conducted to explore the relationships among colour intensity, pigment composition (yellow, red, and blue pigments), TPC, and TAC. The results are summarised in **Table S1.** Colour intensity showed a strong positive correlation with both TPC ($r = 0.942$, $p < 0.001$) and TAC ($r = 0.837$, $p < 0.001$), indicating that higher phenolic and anthocyanin contents significantly contribute to the colour strength of the extract. RP was positively correlated with TPC ($r = 0.717$, $p < 0.001$), emphasising the importance of red pigments in phenolic-rich extracts. Moreover, a strong positive correlation was observed between TPC and TAC ($r = 0.850$, $p < 0.001$), confirming that phenolic-rich samples also contained higher levels of anthocyanins.

3.2. Effects of sample-to-solvent ratio and time on chemical composition and antioxidant capacity of jabuticaba peel extracts

According to Table 2, decreasing the sample-to-solvent ratio (i.e., increasing solvent content) generally increased the extraction yield of bioactive compounds. Higher solvent availability (e.g., 1:40 *w/v* solid-to-solvent ratio) promoted greater recovery of TPC, TFC, TAC, and antioxidant capacity, whereas lower solvent contents (e.g., 1:20 g/mL) resulted in reduced yields. This trend indicates that increased solvent volume enhances solubilisation and mass transfer of phenolic compounds, while insufficient solvent limits extraction efficiency.

TPC ranged from 1639 ± 176 to 2513 ± 370 mg GAE/100 g, with the highest average value obtained at a 1:30 *w/v* solid-to-solvent ratio for 7.5 min. Similar trends have been reported for ultrasound-assisted extraction of jabuticaba peel, in which lower solvent volumes reduced polyphenol yield (Bueno et al., 2024). This effect is attributed to improved solubilisation and diffusion of phenolics with increased solvent availability, as also observed in microwave-assisted extraction of macadamia skin, where solvent volume was the main determinant of yield (Dailey & Vuong, 2016). TFC followed a similar pattern, reaching a maximum of 984 ± 32 mg CTE/100 g for the extract obtained with a 1:40 *w/v* solid-to-solvent ratio for 7.5 min.

TAC also peaked at the 1:40 w/v solid-to-solvent ratio, with the highest concentration (62.95 ± 5.03 mg C3G/100 g) obtained at 5 min. Longer extraction times or lower solvent volumes led to lower anthocyanin recovery, likely due to their susceptibility to oxidation or degradation. Extending extraction beyond 7.5 min did not improve TAC yields. Comparable trends were reported by Fernández-Barbero et al. (2019), who identified solvent volume as a major driver of polyphenol and anthocyanin extraction efficiency from jabuticaba, supporting the role of enhanced mass transfer at higher solvent ratios.

Antioxidant activity, measured by HRSA and FeCA, was greatest at the 1:40 w/v solid-to-solvent ratio and 7.5 min (698 ± 12 mg GAE/100 g and 273 ± 30 mg EDTAE/100 g, respectively, Table 2). These results are consistent with the elevated TPC, as phenolics are known contributors to radical scavenging and metal-chelating activity. Lower solvent ratios or prolonged extraction times consistently reduced antioxidant performance, reinforcing the need for sufficient solvent availability and controlled exposure to prevent degradation. Similar influences of extraction parameters on antioxidant activity in jabuticaba peel were reported by Fidelis et al. (2020). In contrast, colour intensity and red pigment content showed different responses to extraction conditions. The highest colour intensity (3.94 ± 0.08 a.u.) and red pigment content (31.50 ± 0.02%) were observed at lower solid-to-solvent ratios (1:20–1:30 *w/v*) and shorter extraction times (5 min). Increasing the

Table 2
Effect of sample-to-solvent ratio and extraction time on total phenolic content, total flavonoids, total anthocyanin content, hydroxyl radical scavenging capacity, and Fe^{2+} chelating ability of jabuticaba peel extracts.

Sample	Solid-to-solvent ratio (*w/v* - g: mL)	Time (min)	TPC (mg GAE/100 g)	TFC (mg CTE/100 g)	TAC (mg C3G/100 g)	HRSA (mg GAE/100 g)	FeCA (mg EDTAE/100 g)	Colour intensity (a.u.)	Red pigments (%)
1	20	5	2084 ± 155^{ab}	614 ± 11^{d}	35.86 ± 2.84^{de}	444 ± 6^{c}	88 ± 10^{c}	3.94 ± 0.08^{a}	31.28 ± 0.13^{b}
2	20	7.5	1990 ± 49^{ab}	551 ± 4^{e}	29.31 ± 3.31^{e}	401 ± 15^{d}	123 ± 7^{c}	3.45 ± 0.09^{cd}	30.34 ± 0.07^{e}
3	20	10	1639 ± 176^{b}	595 ± 18^{d}	33.61 ± 4.19^{e}	433 ± 10^{cd}	86 ± 7^{c}	3.80 ± 0.12^{ab}	30.28 ± 0.05^{e}
4	30	5	2222 ± 276^{ab}	829 ± 14^{bc}	47.93 ± 5.42^{bc}	583 ± 4^{b}	196 ± 14^{b}	3.19 ± 0.04^{ef}	31.50 ± 0.02^{a}
5	30	7.5	2234 ± 298^{ab}	834 ± 20^{bc}	47.30 ± 3.72^{bcd}	583 ± 20^{b}	177 ± 10^{b}	3.22 ± 0.03^{ef}	30.87 ± 0.04^{c}
6	30	10	2263 ± 254^{ab}	861 ± 19^{b}	41.82 ± 5.92^{cde}	593 ± 10^{b}	79 ± 7^{c}	3.55 ± 0.13^{bc}	30.57 ± 0.04^{d}
7	40	5	2090 ± 65^{ab}	943 ± 33^{a}	62.95 ± 5.03^{a}	681 ± 18^{a}	184 ± 26^{b}	2.80 ± 0.01^{g}	31.41 ± 0.03^{ab}
8	40	7.5	2476 ± 411^{a}	984 ± 32^{a}	62.37 ± 6.50^{a}	698 ± 12^{a}	273 ± 30^{a}	2.98 ± 0.07^{fg}	30.89 ± 0.06^{c}
9	40	10	2376 ± 222^{a}	935 ± 10^{a}	56.44 ± 4.29^{ab}	686 ± 18^{a}	189 ± 17^{b}	2.82 ± 0.08^{g}	30.62 ± 0.00^{d}
10	30	7.5	2513 ± 370^{a}	823 ± 8^{bc}	51.39 ± 6.18^{abc}	584 ± 11^{b}	86 ± 39^{c}	3.33 ± 0.06^{de}	30.97 ± 0.02^{c}
11	30	7.5	2263 ± 309^{ab}	823 ± 14^{bc}	53.85 ± 2.81^{abc}	580 ± 8^{b}	86 ± 9^{c}	3.42 ± 0.07^{cde}	30.99 ± 0.02^{c}
12	30	7.5	2097 ± 248^{ab}	802 ± 11^{c}	52.59 ± 5.66^{abc}	591 ± 17^{b}	107 ± 16^{c}	3.32 ± 0.11^{de}	30.93 ± 0.03^{c}
P-value[1]			0.396	0.525	0.754	0.499	0.562	0.402	<0.001*
P-value[2]			0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Values represent mean ± SD (n = 4). [1]Probability values obtained by the Brown-Forsythe test of homoscedasticity; [2]Probability values obtained by either one-way ANOVA (homoscedastic data) or Welch-ANOVA (*heteroscedastic data); TPC = total phenolic content; GAE = gallic acid equivalent; TFC = total flavonoid content; CTE = catechin equivalent; TAC = total anthocyanin content; C3G = cyanidin-3-*O*-glucoside equivalent; HRSA = hydroxyl radical scavenging ability; FeCA = Fe^{2+} chelating ability; EDTAE = EDTA equivalent. Values sharing different superscript letters in a column are significantly different at the 0.05 level ($p < 0.05$).

solvent volume, particularly to a 1:40 w/v solid-to-solvent ratio, reduced colour intensity despite higher anthocyanin levels. This suggests that colour expression depends not only on anthocyanin concentration but also on pigment stability and composition (Khoo et al., 2017; Miranda et al., 2021). Prolonged extraction may promote pigment transformation or degradation, reducing perceived redness. Similar findings were reported by Miranda et al. (2021), who showed that milder extraction conditions better preserved red hues in jabuticaba peel extracts.

Pearson correlation analysis (**Table S2**) revealed significant associations ($p < 0.05$) among bioactive and colour parameters. TFC was strongly correlated with HRSA ($r = 0.977$), indicating a major contribution of flavonoids to antioxidant activity. TAC correlated positively with both TFC ($r = 0.867$) and HRSA ($r = 0.852$), confirming the role of anthocyanins in antioxidant performance. In contrast, colour intensity was negatively correlated with HRSA ($r = -0.815$) and TFC ($r = -0.782$), likely reflecting pigment instability under conditions favouring higher phenolic extraction. Red pigment percentage showed moderate correlations ($p < 0.05$) with TAC ($r = 0.440$) and TFC ($r = 0.401$), suggesting a compositional influence of anthocyanins and flavonoids on visual attributes.

3.3. Predictive modelling and optimisation of UAE conditions

The effects of extraction time and sample-to-solvent ratio on the bioactive and pigment properties of JPEs were assessed using RSM, with results summarised in Table 3 and visualised in contour plots (**Fig. S1**). The models incorporated linear, quadratic, and interaction effects of the two variables. Regression analysis confirmed that both factors significantly influenced recovery of bioactive compounds (e.g., TFC, TPC, and TAC) and pigment characteristics ($p < 0.05$), with strong model performance for most responses. Significant interaction effects indicated that optimisation depended on the combined influence of extraction time and solvent ratio rather than on individual parameters alone.

Using the desirability function of optimisation, a sample-to-solvent ratio of 1:40 g/mL and an extraction time of 6.75 min were found to be optimal, yielding a high desirability value (*d*-value) of 0.915 (**Fig. S2**). Collectively, these predictive models and surface plots provide strong statistical justification for selecting a 1:40 w/v sample-to-solvent ratio and a 7.5-min extraction time as the optimal condition for bioactivity, while highlighting milder conditions (1:20–1:30, 5 min) as preferable for enhanced colour quality.

Our results showed that for TFC, the model prediction (±95% prediction interval: 929–1005 mg CTE/100 g) aligned well with the experimental value (977 ± 3 mg CTE/100 g), indicating model accuracy. The comparison of model-predicted values and experimental results for TAC and HRSA in jabuticaba peel extracts showed an acceptable TAC value (±95% prediction interval: 50.70–73.21 mg C3G/100 g; experimental value 51.68 ± 1.67 mg C3G/100 g). On the other hand, the experimental HRSA value (1274 ± 65 mg GAE/100 g) was significantly higher than the ±95% prediction interval (670–728 mg GAE/100 g). Similarly, FeCA shows good agreement between predicted (±95% prediction interval: 192–336 mg EDTAE/100 g) and the experimental value (259 ± 27 mg EDTAE/100 g). RP% also demonstrates acceptable accuracy, with a predicted mean value of 30.99% (±95% prediction interval: 30.86–31.12%) compared to the experimental value of 32.05 ± 0.01%. The colour intensity also showed a strong predictive ability, with the predicted value (±95% prediction interval: 2.75–3.19 a.u.) closely aligned with the experimental value (3.33 ± 0.08 a.u.). Overall, while the model performs well for some parameters, it shows limited predictive reliability for HRSA.

3.4. Characterisation of the optimal jabuticaba peel extract

3.4.1. Polyphenol composition and antioxidant potential

The liquid optimal jabuticaba peel extract (JPE) exhibited a pronounced chromatic profile, with a red colour intensity of 32.05 ± 0.01%, indicative of a high concentration of anthocyanins (51.68 ± 1.67 mg C3G/100 g) (**Table S3**). Quantitative analysis revealed a high TPC (3482 ± 284 mg GAE/100 g) and TFC (977 ± 3 mg CTE/100 g), positioning JPE among the most phenol-rich matrices derived from native Brazilian fruits, such as butia, araca, red and purple pitanga, and blackberry (Denardin et al., 2015). This phenolic abundance is a critical determinant of the extract's antioxidant efficacy, given the well-established redox and metal chelating properties of polyphenols. The liquid extract demonstrated a high single-electron transfer (SET) ability, as confirmed by CUPRAC (34,822 ± 1393 mg AAE/100 g) and FRAP (8178 ± 54 mg AAE/100 g) assays and hydrogen-atom transfer (HAT), as confirmed by the HRSA assay (1275 ± 65 mg GAE/100 g).

The phenolic composition of freeze-dried JPE is presented in Table 4. Total phenolic, flavonoid, and anthocyanin contents measured spectrophotometrically were 9403 ± 364 mg GAE/100 g, 5196 ± 518 mg CTE/100 g, and 236 ± 8 mg C3G/100 g, respectively. Ellagic acid was the predominant compound (3766 ± 98 mg/100 g), accounting for more than half of the quantified phenolics. Procyanidin B2 (1828 ± 27 mg/100 g) was the second most abundant, followed by vescalagin (555 ± 21 mg/100 g), gallic acid (307 ± 4 mg/100 g), (−)-epicatechin (243 ± 31 mg/100 g), castalagin (124 ± 7 mg/100 g), hesperidin (84 ± 1 mg/100 g), and ferulic acid (50 ± <1 mg/100 g). The total concentration of phenolics quantified by HPLC was 7198 mg/100 g.

Similar values have been described in the literature, although differences are evident depending on the species and the extraction conditions applied. de Andrade Neves et al. (2021) analysed several jabuticaba species and found TPC values ranging from 600 to 1149 mg GAE/100 g. Despite the lower concentrations, their results consistently highlighted cyanidin-3-*O*-glucoside as the main compound, followed by delphinidin-3-*O*-glucoside and ellagic acid derivatives, confirming the predominance of anthocyanins and ellagitannins in jabuticaba peel. These comparisons demonstrate the importance of extraction optimisation to achieve higher yields of phenolic and bioactive compounds.

The predominance of ellagic acid in freeze-dried JPE is consistent with previous reports identifying ellagitannins and their derivatives as the major phenolics in jabuticaba peel (Abe et al., 2012; Alezandro et al., 2013). Ellagic acid and ellagitannins are well recognised for their antioxidant, anti-inflammatory, and chemopreventive activities in vivo (Quatrin et al., 2018). Vescalagin and castalagin, characteristic compounds of jabuticaba peel and seeds, are also noted for their chemical stability and associated antioxidant and anti-inflammatory effects (de Andrade Neves et al., 2021; Fidelis et al., 2021). In addition, procyanidin B2, a dimeric proanthocyanidin commonly found in Myrtaceae fruits, contributes to radical scavenging and metal-chelating activity (Sakano et al., 2005).

Delphinidin-3-*O*-glucoside (45 ± <1 mg/100 g) and cyanidin-3-*O*-glucoside (196 ± 2 mg/100 g) were the only anthocyanins detected in JPE. These results agree with previous reports on jabuticaba. de Andrade Neves et al. (2021) reported that cyanidin-3-*O*-glucoside accounted for 80.59% of the total anthocyanins (85.27 mg/100 g of 105.78 mg/100 g), a proportion comparable to that observed in this study (81.26%), although with a higher overall anthocyanin content (240.59 mg/100 g). Similarly, Leite-Legatti et al. (2012) identified the same compounds in jabuticaba peel, with markedly higher concentrations (634.75 mg/100 g for delphinidin-3-*O*-glucoside and 1963.57 mg/100 g for cyanidin-3-*O*-glucoside).

The chemical antioxidant potential of freeze-dried JPE was confirmed through multiple assays that combine SET, HAT, and metal chelation mechanisms of action: HRSA (6866 ± 60 mg GAE/100 g), FeCA (938 ± 117 mg EDTAE/100 g), CUPRAC (90,653 ± 578 mg AAE/100 g), and FRAP (24,183 ± 472 mg AAE/100 g). Earlier studies on food-grade JPE have shown that it exhibits antioxidant potential through multiple mechanisms of action, including its use as a natural food preservative (Dalponte Dallabona et al., 2020; Heck et al., 2020).

Table 3
Multiple regression coefficients derived from response surface modelling of experimental data, illustrating the effects of extraction time and sample-to-solvent ratio on the chemical composition, instrumental colour parameters, and antioxidant capacity of jabuticaba peel extract.

Factors	Regression Coefficient	Standard Error	*t*- value	*P*- value	−95% Confidence limit	+95% Confidence limit
			Total phenolic content			
Mean/Interc.	1204	775	1.553	0.130	−373	2781
(1)Sample-to-solvent ratio(L)	86.050	48.408	1.778	0.085	−12.437	185
Sample-to-solvent ratio(Q)	−1.649	0.784	−2.104	0.043	−3.243	−0.055
Extraction time(Q)	−15.820	5.536	−2.858	0.007	−27.082	−4.558
1 L by 2Q	0.493	0.178	2.771	0.009	0.131	0.856
R^2	0.426					
R^2_{Adj}	0.364					
			Total flavonoids			
Mean/Interc.	811	235	3.459	0.002	334.153	1288.359
(1)Sample-to-solvent ratio(L)	24.553	14.336	1.713	0.096	−4.613	53.720
Sample-to-solvent ratio(Q)	−0.664	0.260	−2.549	0.016	−1.193	−0.134
(2)Extraction time(L)	−253	42.348	−5.969	<0.001	−338.942	−166.627
Extraction time(Q)	12.492	2.101	5.947	<0.001	8.218	16.765
1 L by 2 L	4.660	1.875	2.486	0.018	0.846	8.474
1Q by 2 L	0.082	0.044	1.873	0.070	−0.007	0.172
1Q by 2Q	−0.011	0.002	−5.124	<0.001	−0.015	−0.006
R^2	0.988					
R^2_{Adj}	0.986					
			Total anthocyanin content			
Mean/Interc.	592.681	241.884	2.450	0.020	101	1085
(1)Sample-to-solvent ratio(L)	−38.218	16.787	−2.277	0.029	−72.371	−4.066
Sample-to-solvent ratio(Q)	0.614	0.280	2.194	0.035	0.045	1.183
(2)Extraction time(L)	−180.061	66.876	−2.692	0.011	−316	−44.001
Extraction time(Q)	12.625	4.437	2.845	0.008	3.597	21.653
1 L by 2 L	12.209	4.638	2.632	0.013	2.773	21.645
1 L by 2Q	−0.860	0.308	−2.791	0.009	−1.487	−0.233
1Q by 2 L	−0.189	0.077	−2.449	0.020	−0.347	−0.032
1Q by 2Q	0.013	0.005	2.598	0.014	0.003	0.024
R^2	0.850					
R^2_{Adj}	0.813					
			Hydroxyl radical scavenging activity			
Mean/Interc.	633	38.644	16.377	<0.001	554	711
(2)Extraction time(L)	−168.970	14.101	−11.983	<0.001	−198	−140
Extraction time(Q)	8.832	0.943	9.367	<0.001	6.914	10.751
1 L by 2 L	6.392	0.390	16.371	<0.001	5.598	7.186
1 L by 2Q	−0.251	0.021	−11.725	<0.001	−0.295	−0.208
1Q by 2 L	−0.043	0.006	−7.456	<0.001	−0.055	−0.031
R^2	0.983					
R^2_{Adj}	0.981					
			Fe^{2+} chelating ability			
Mean/Interc.	−6548	1551	−4.222	<0.001	−9704	−3392
(1)Sample-to-solvent ratio(L)	484	108	4.497	<0.001	265.066	703
Sample-to-solvent ratio(Q)	−8.328	1.794	−4.643	<0.001	−11.978	−4.679
(2)Extraction time(L)	1725	429	4.021	<0.001	852	2597
Extraction time(Q)	−102	28.455	−3.602	0.001	−160	−44.590
1 L by 2 L	−126	29.742	−4.235	<0.001	−186	−65.436
1 L by 2Q	7.456	1.977	3.772	0.001	3.435	11.477
1Q by 2 L	2.201	0.496	4.436	<0.001	1.192	3.210
1Q by 2Q	−0.131	0.033	−3.972	<0.001	−0.198	−0.064
R^2	0.790					
R^2_{Adj}	0.738					
			Colour intensity			
Mean/Interc.	17.993	1.624	11.079	<0.001	14.689	21.297
(1)Sample-to-solvent ratio(L)	−0.657	0.089	−7.352	<0.001	−0.839	−0.475
Sample-to-solvent ratio(Q)	0.006	0.001	4.410	<0.001	0.003	0.009
(2)Extraction time(L)	−3.144	0.357	−8.802	<0.001	−3.871	−2.417
Extraction time(Q)	0.159	0.021	7.486	<0.001	0.116	0.202
1 L by 2 L	0.126	0.015	8.435	<0.001	0.096	0.156
1 L by 2Q	−0.005	0.001	−6.834	<0.001	−0.006	−0.003
1Q by 2 L	−0.001	<0.001	−5.023	<0.001	−0.001	−0.001
R^2	0.938					
R^2_{Adj}	0.925					

(*continued on next page*)

Table 3 (*continued*)

Factors	Regression Coefficient	Standard Error	*t*- value	*P*- value	−95% Confidence limit	+95% Confidence limit
			Red pigments			
Mean/Interc.	51	3	18	0	45	56
(1)Sample-to-solvent ratio(L)	−1	0.191	−5.33	<0.001	−1.410	−0.631
Sample-to-solvent ratio(Q)	0.015	0.003	4.63	<0.001	0.008	0.021
(2)Extraction time(L)	−6.007	0.763	−7.88	<0.001	−7.558	−4.455
Extraction time(Q)	0.385	0.051	7.61	<0.001	0.282	0.488
1 L by 2 L	0.332	0.053	6.28	<0.001	0.225	0.440
1 L by 2Q	−0.022	0.004	−6.30	<0.001	−0.029	−0.015
1Q by 2 L	−0.005	0.001	−5.52	<0.001	−0.007	−0.003
1Q by 2Q	<0.001	<0.001	5.59	<0.001	<0.001	<0.001
R^2	0.984					
R^2_{Adj}	0.980					

Table 4
Phenolic composition and antioxidant capacity of the optimal freeze-dried jabuticaba peel extract (JPE).

Phenolic composition – UV/Vis spectrophotometry	
Total phenolic content (mg GAE/100 g)	9403 ± 364
Total flavonoid content (mg CTE/100 g)	5196 ± 518
Total anthocyanin content (mg C3G/100 g)	236 ± 8
Anthocyanins	
Delphinidin-3-*O*-glucoside (mg/100 g)	45 ± <1
Cyanidin-3-*O*-glucoside (mg/100 g)	196 ± 2
Phenolic acids and derivatives	
Gallic acid (mg/100 g)	307 ± 4
Ferulic acid (mg/100 g)	50 ± <1
Ellagic acid (mg/100 g)	3766 ± 98
Flavan-3-ols and proanthocyanidins	
(−)-Epicatechin (mg/100 g)	243 ± 31
Procyanidin B2 (mg/100 g)	1828 ± 27
Ellagitannins	
Vescalagin (mg/100 g)	555 ± 21
Castalagin (mg/100 g)	124 ± 7
Non-anthocyanin flavonoids	
Hesperidin (mg/100 g)	84 ± 1
Total phenolic content identified by HPLC (mg/100 g)	7198
Antioxidant capacity	
HRSA (mg GAE/100 g)	6866 ± 60
FeCA (mg EDTAE/100 g)	938 ± 117
CUPRAC (mg AAE/100 g)	90,653 ± 578
FRAP (mg AAE/100 g)	24,183 ± 472
In vitro *porcine pancreatic lipase inhibition*	
IC_{50} (μg/mL) - JPE	30.50 ± 0.74^b
IC_{50} (μg /mL) - Orlistat	49.80 ± 3.55^a
In vitro *recombinant human 3-hydroxy-3-methylglutaryl-CoA inhibition*	
IC_{50} (μg/mL) - JPE	40.91 ± 0.59^a
IC_{50} (μg/mL) - Simvastatin	32.96 ± 2.30^b
In vitro *α-amylase inhibition*	
IC_{50} (μg/mL) - JPE	33.52 ± 1.57^b
IC_{50} (μg/mL) - Acarbose	50.50 ± 0.63^a

Note: TPC = Total phenolic content; GAE = Gallic acid equivalent; TFC = Total flavonoid content; CTE = Catechin equivalent; TAC = Total anthocyanin; C3G = cyanidin-3-*O*-glucoside equivalent; HRSA = Hydroxyl radical scavenging ability; FeCA = Fe^{2+} chelating ability; EDTAE = EDTA equivalent; CUPRAC = Cupric ion reducing antioxidant capacity; AAE = Ascorbic acid equivalent; FRAP = Ferric reducing antioxidant power. Different uppercase letters indicate statistically significant differences between JPE and standards.

3.4.2. *Effects of pH on anthocyanin reversibility*

Anthocyanin colour expression is reversible and strongly pH dependent. As shown in Fig. 2, red pigment content peaked at pH 2, corresponding to the highest visual redness and HRSA. Jabuticaba peel anthocyanins displayed high structural reversibility (90.52 ± 0.40%) after pH cycling from 2 to 10 and back to 2, indicating minimal irreversible degradation under alkaline conditions. This reversibility was higher than that reported for black rice anthocyanins (e.g., cyanidin-3-*O*-glucoside), which retained only ~50% of absorbance at 520 nm following similar pH treatment (Pedro et al., 2016).

Shifting the pH from 2 to 10 resulted in a pronounced decrease in red pigmentation and an increase in yellow tones, reflecting the multistate pH behaviour of JPE anthocyanins. Under alkaline conditions, the flavylium cation converts to the colourless hemiketal and subsequently to the yellow *cis*- - and *trans*-chalcone forms, which are less stable (Singh et al., 2018). The extent and reversibility of these transformations depend on anthocyanin structure, particularly glycosylation and acylation patterns, which govern interconversion kinetics between molecular species (Basílio et al., 2016). At pH 10, both redness and antioxidant activity were reduced; however, re-acidification to pH 2 (pH 2R) partially restored colour intensity and fully recovered HRSA (Fig. 2). This suggests that although visual pigment recovery is incomplete, the redox-active features responsible for antioxidant function remain largely preserved and favoured under acidic conditions. In contrast, FeCA showed a distinct pH response, increasing markedly from acidic to neutral–alkaline conditions and peaking between pH 6 and 10 (>800 mg EDTAE/100 g). This increase likely reflects enhanced deprotonation of hydroxyl and carboxyl groups, facilitating metal coordination. Upon re-acidification, FeCA declined sharply, recovering only partially (~200 mg EDTAE/100 g), consistent with reports of irreversible phenolic oxidation or structural alteration at high pH (Janeiro & Brett, 2007).

3.4.3. *Anti-obesity, anti-cholesterolemic, and antidiabetic potential of JPE*

Table 4 presents the bioactivity profile of JPE, highlighting its anti-diabetic, anti-obesity, and anti-cholesterolemic potential based on in vitro assays. Rich in phenolic compounds—including ellagic acid, vescalagin/castalagin, procyanidin B2, and (−)-epicatechin—JPE exhibited superior inhibitory activity against porcine pancreatic lipase (IC_{50} = 30.50 μg/mL) compared to Orlistat (IC_{50} = 49.80 μg/mL), a clinically approved lipase inhibitor commonly used to support weight loss and prevent weight regain in adults (Subramaniyan & Hanim, 2025). JPE also showed anti-cholesterolemic effects (IC_{50} = 40.91 μg/mL) by inhibiting recombinant human 3-hydroxy-3-methylglutaryl-CoA, a key enzyme in the cholesterol biosynthesis pathway (Wang et al., 2023), but its inhibition was lower than that of simvastatin (IC_{50} = 32.96 μg/mL). JPE also inhibited α-amylase activity (IC_{50} = 33.52 μg/mL) more efficiently than acarbose (IC_{50} = 50.50 μg/mL), an oral medication used to manage postprandial hyperglycemia (Singh et al., 2025). It is important to note that our findings provide only preliminary biochemical evidence and should not be interpreted as proof of physiological effects.

In a human adipogenic stem cell (*h*ASCs) model, Okla et al. (2015)

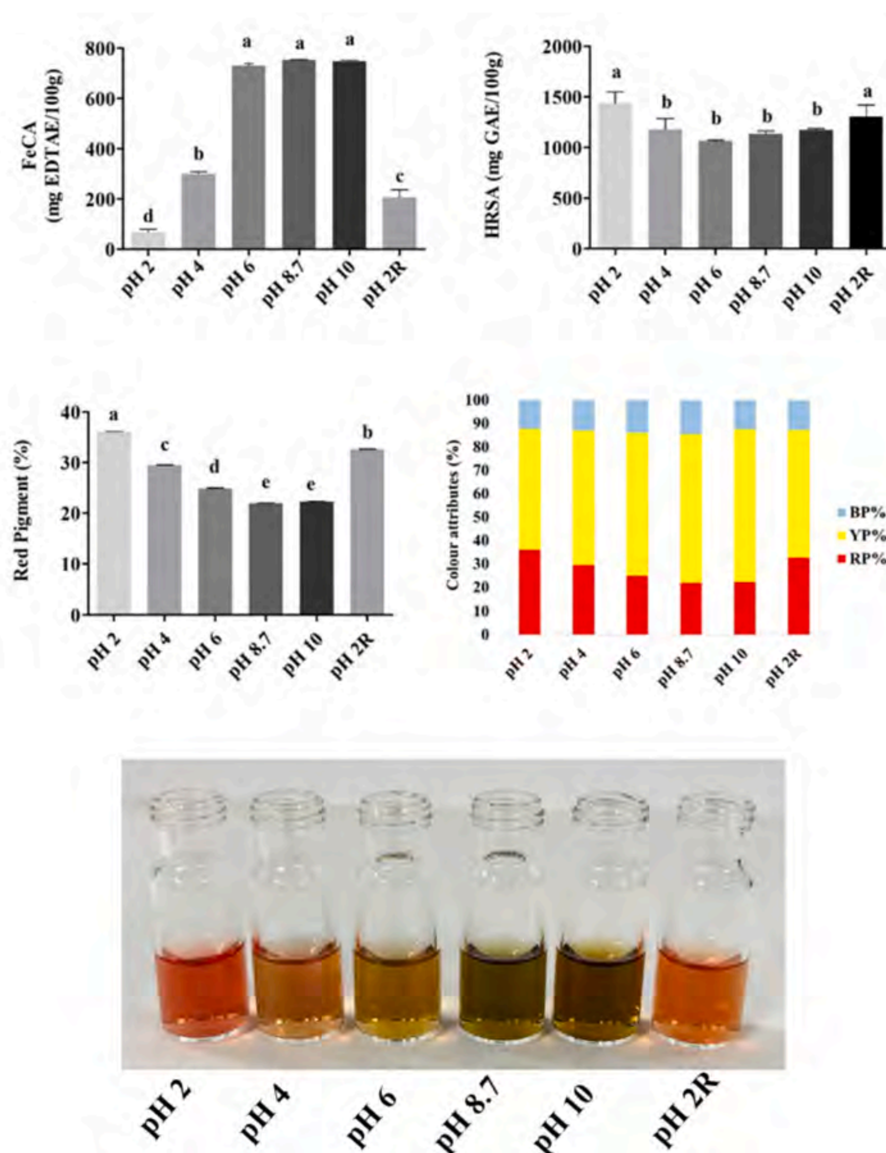


Fig. 2. Effect of pH shift (2, 4, 6, 8.7 and 10) on colour attributes (e.g., red pigments, RP; yellow pigments, YP; blue pigments, BP) and antioxidant capacity (HRSA, FeCA) of optimal jabuticaba peel extract using reverse spectrophotometric titration. Observed colour changes (BP, RP, YP) reflect the reversible structural transformations of anthocyanins under varying pH. Different lowercase superscript letters indicate significant differences between pH values ($p < 0.05$). Data are represented as mean ± SD ($n = 4$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demonstrated that ellagic acid from muscadine grape powder significantly modulated adipogenesis and suppressed de novo fatty acid and triglyceride synthesis in mature adipocytes. Borges et al. (2022) reported that jabuticaba peel extract, prepared with 75% (*v*/v) ethanol acidified to pH 2.0 with HCl, inhibited porcine pancreatic lipase (IC_{50} = 111.51 mg/mL) and α-amylase (IC_{50} = 1.88 mg/mL). Similarly, Alves Castilho et al. (2021) used a 70% hydroalcoholic extract of jabuticaba peel and observed lipase inhibition (IC_{50} = 143.9 μg/mL), although cyanidin-3-*O*-glucoside was not implicated in this activity. Oral administration of this extract (250 and 500 mg/kg) also reduced starch absorption in mice, supporting the in vitro findings.

3.5. Microencapsulation of the optimal jabuticaba peel extract

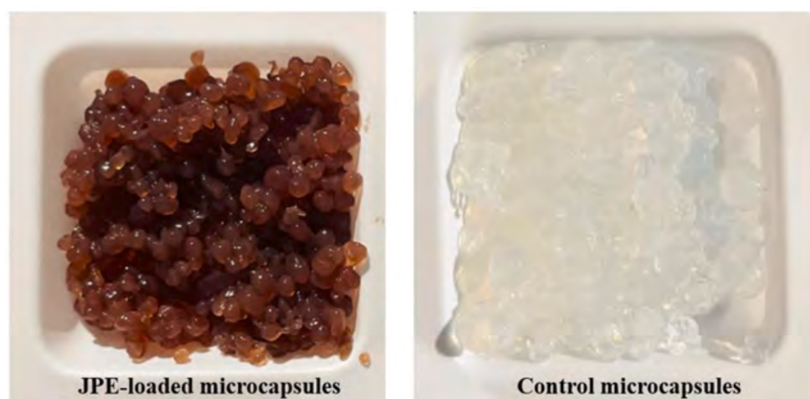
3.5.1. Encapsulation efficiency

The ionotropic gelation method successfully entrapped the phenolic fraction of the JPE within the alginate matrix, achieving an encapsulation efficiency of 75.23 ± 1.67%. The stock solution had TPC (before encapsulation) of 17,744 ± 761 mg GAE/100 g, which decreased to 4387 ± 111 mg GAE/100 g after the process. This reduction reflects the inevitable loss of phenolics during bead hardening; however, a substantial proportion remained well preserved within the polymeric structure. Comparable outcomes have been reported in the literature: Cabral et al. (2018) obtained encapsulation efficiencies ranging from 52.71 to 89.74% for JPE microparticles produced by spray drying with chitosan as encapsulating polymer. In contrast, Dalponte Dallabona et al. (2020) achieved a much higher efficiency of 98.1% when co-encapsulating jabuticaba peel and propolis extracts in alginate beads, highlighting how extract composition, bead size, and encapsulation conditions can strongly influence retention.

3.5.2. Kinetic release of bioactive compounds under gastric and intestinal fluids

The release kinetics of bioactive compounds from JPE-loaded microcapsules were evaluated in gastric and intestinal fluids to assess changes in antioxidant capacity (**Table 5**). During the gastric phase, antioxidant capacity increased markedly, with CUPRAC rising from 73 ± 12 to 5433 ± 160 mg AAE/100 g over 4 h and FRAP increasing almost

Table 5
Kinetic release of total flavonoids (TFC), total phenolics (TPC), and antioxidant capacity (CUPRAC and FRAP) from the optimal freeze-dried microencapsulated jabuticaba peel extract (JPE) using gastric and intestinal fluids at different time intervals.



Digestion phase and time	CUPRAC (mg AAE/100 g)	FRAP (mg AAE/100 g)	TFC (mg CTE/100 g)	TPC (mg GAE/100 g)
Gastric 0 h	73 ± 12	112 ± 2	178 ± 18	100 ± 8
Gastric 2 h	4264 ± 180	1004 ± 21	444 ± 70	1362 ± 27
Gastric 4 h	5433 ± 160	1046 ± 43	1295 ± 0	1633 ± 19
Intestine 0 h	ND	34 ± 2	81 ± 5	ND
Intestine 2 h	1894 ± 138	70 ± 4	134 ± 18	100 ± 2
Intestine 4 h	928 ± 12	138 ± 4	107 ± 18	152 ± 3
Intestine 6 h	268 ± 10	66 ± 5	104 ± 10	107 ± 11

Note: ND = not detected. Values are expressed as mean ± standard deviation (n = 4). GAE = Gallic acid equivalent; AAE = Ascorbic acid equivalent; CTE = catechin equivalent.

tenfold (112 ± 2 to 1046 ± 43 mg AAE/100 g). Parallel increases in TFC (178 ± 18 to 1295 ± 0 mg CTE/100 g) and TPC (100 ± 8 to 1633 ± 19 mg GAE/100 g) indicate efficient and continuous release of flavonoids and phenolics under acidic conditions, facilitated by matrix swelling and compound solubilisation. In the intestinal phase, release followed a different pattern. At 0 h, CUPRAC and TPC were negligible, indicating delayed compound availability. After 2 h, CUPRAC increased sharply (1894 ± 138 mg AAE/100 g), with measurable FRAP, TFC, and TPC levels, confirming compound release. Prolonged exposure to the intestinal fluid (i.e., up to 6 h) led to gradual declines in most parameters, suggesting partial degradation or transformation under alkaline conditions, with CUPRAC decreasing from 1894 ± 138 to 268 ± 10 mg AAE/100 g between 2 and 6 h, and TPC decreasing from 152 ± 3 to 107 ± 11 mg GAE/100 g between 4 and 6 h. Overall, CUPRAC increased 3.7-fold from the start of the gastric phase to the end of intestinal digestion, while FRAP and TFC decreased by ~40%. The current microencapsulation system favoured gastric release rather than targeted intestinal delivery, representing a limitation of the formulation. The use of acidic gastric fluid promoted early alginate swelling and the diffusion of phenolics, reducing retention and enhancing intestinal release. In addition, the alkaline intestinal environment may have contributed to phenolic degradation, further limiting the antioxidant potential of microcapsules. Comparable behaviour was reported by Barcellos et al. (2025), who observed enhanced phenolic bioaccessibility and antioxidant capacity from maltodextrin-encapsulated jabuticaba juice during digestion. In contrast, Dalponte Dallabona et al. (2020) reported limited gastric release (~40%) from alginate beads at pH 1.2, followed by complete intestinal release. These differences are likely linked to gastric pH conditions, as the pH 3 used in the present study (postprandial) favours alginate swelling and faster phenolic diffusion compared with fasting-state conditions.

3.6. *Functional beverage development and analysis*

3.6.1. *Effects of the optimal jabuticaba peel extract and functional beverage on HepG2 cell viability and cellular antioxidant activity*

In this study, the effects of JPE and beverage formulations (5–400 μg/mL) on HepG2 cell viability and H_2O_2-induced oxidative challenge were evaluated. HepG2 cells, which express phase I and II metabolic enzymes, are widely used to assess the cytotoxicity and safety of foods (Valentin-Severin et al., 2003). Accordingly, MTT and ROS assays were employed to evaluate the safety and antioxidant potential of the extract and beverages. As shown in Fig. 3A, neither the optimal JPE nor the formulated beverages (pasteurised, unpasteurised, or blank) exhibited cytotoxic effects after 24 h of exposure. Based on U.S. National Cancer Institute and Geran criteria, all samples were classified as non-cytotoxic (IC_{50} > 501 μg/mL) under the conditions tested (Goldin et al., 1981). Notably, pasteurisation did not alter cytocompatibility, indicating that thermal processing did not generate harmful degradation products. These findings agree with Albuquerque et al. (2020), who reported low cytotoxicity of JPE toward HepG2 and MCF-7 cells.

Regarding cellular antioxidant activity, both JPE and the beverages significantly reduced intracellular ROS levels across the tested concentration range. JPE reduced oxidative stress by 83–64%, while the unpasteurised and pasteurised beverages achieved reductions of 59–37% and 36–33%, respectively (Fig. 3B). Overall, the beverages, particularly the pasteurised formulation, showed superior protection against oxidative stress compared with the extract alone. This effect is consistent with the high levels of anthocyanins, ellagitannins, gallic acid, ellagic acid, and quercetin reported in jabuticaba peel (Lenquiste et al., 2015; Tarone et al., 2021). Supporting these results, Ferreira et al. (2020) demonstrated that a jabuticaba-based beverage reduced oxidative stress markers in athletes and enhanced antioxidant defenses.

3.6.2. *Influence of pasteurisation on bioactive compounds in a functional beverage*

A statistically significant reduction in red pigment concentration was observed after pasteurisation (from 33.25 to 32.44%; $p < 0.001$)

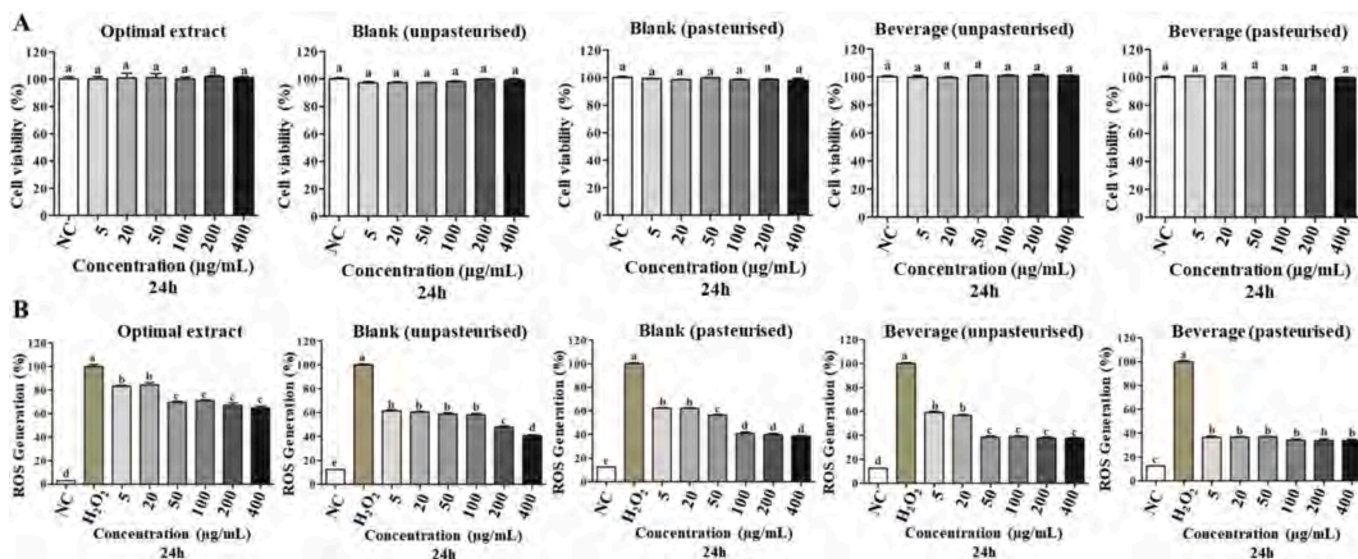
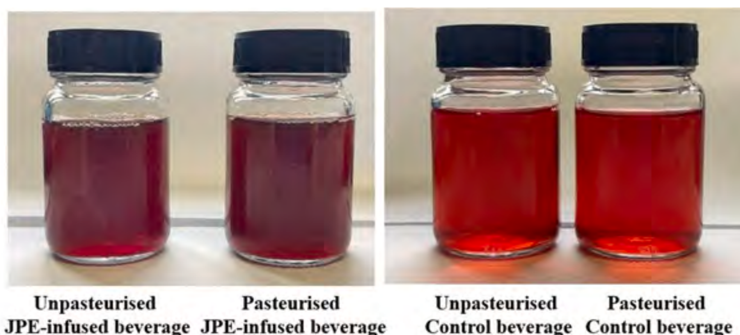


Fig. 3. (A) Effect of jabuticaba peel extract (JPE) obtained at the optimal extraction conditions and JPE-enriched functional beverage on human-derived hepatocellular carcinoma (e.g., HepG2) cells at 5–400 μg/mL concentrations. (B) Cellular antioxidant activity assessed via ROS generation in HepG2 cells treated with JPE and beverage samples (5–400 μg/mL). NC: negative control (culture medium); H_2O_2: hydrogen peroxide (positive control).

Table 6
Effect of pasteurisation on red pigments (RP), total phenolic content (TPC), total flavonoids (TFC), total anthocyanins (TAC), hydroxyl radical scavenging capacity (HRSA), cupric ion reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and Fe^{2+} chelating ability (FeCA) in the functional beverage infused with jabuticaba peel extract.



Treatment	RP (%)	TPC (mg GAE/L)	TFC (mg CTE/L)	TAC (mg C3G/L)	HRSA (mg GAE/L)	CUPRAC (mg AAE/L)	FRAP (mg AAE/L)	FeCA (mg EDTAE/L)
JPE- infused (Unpasteurised)	33.25 ± 0.06^{a}	446 ± 9^{a}	137 ± 3^{a}	8.04 ± 0.40^{a}	102 ± 1^{a}	3693 ± 124^{a}	935 ± 9^{a}	13.53 ± 2.73^{a}
JPE- infused (Pasteurised)	32.44 ± 0.03^{b}	441 ± 6^{a}	139 ± 2^{a}	7.33 ± 1.03^{a}	102 ± 3^{a}	3593 ± 83^{a}	940 ± 18^{a}	18.20 ± 1.19^{a}
Difference (%)	−2.4	−1.1	+1.4	−8.8	0	−2.7	+0.5	+34.5
Blank beverage (Unpasteurised)	66.83 ± 0.56	20.38 ± 3.65	ND	ND	49 ± 2	126 ± 12	83 ± 4	0

Note: TPC = Total phenolic content; GAE = Gallic acid equivalent; TFC = Total flavonoid content; CTE = Catechin equivalent; TAC = Total anthocyanin; C3G = cyanidin-3-*O*-glucoside equivalent; HRSA = Hydroxyl radical scavenging ability; FeCA = Fe^{2+} chelating ability; EDTAE = EDTA equivalent; CUPRAC = Cupric ion reducing antioxidant capacity; AAE = Ascorbic acid equivalent; FRAP = Ferric reducing antioxidant power; RP = red pigments; ND = not determined. Values are expressed as mean ± standard deviation (n = 4). Different superscript letters indicate significant differences ($p < 0.05$) between treatments.

(Table 6), consistent with reports that heat-sensitive anthocyanins are susceptible to thermal degradation (Azofeifa et al., 2015). In contrast, pasteurisation did not significantly affect TPC, TFC, TAC, or antioxidant activities (HRSA, CUPRAC, FRAP, and FeCA), indicating substantial preservation of functional properties. Similar stability was reported by Azofeifa et al. (2015), who found no significant changes in phenolic content or antioxidant capacity of blackberry juice following short-time pasteurisation. Together, these findings support the effectiveness of mild thermal processing in retaining bioactivity while selectively reducing unstable pigments.

More thermally stable phenolics, such as ellagitannins and phenolic acids, are often resilient to pasteurisation (Bolling et al., 2010). Although thermal treatments commonly reduce polyphenols and flavonoids, slight increases have been reported in some matrices due to the release of bound forms or structural modifications that enhance assay reactivity (Mlcek et al., 2016). Consistent with this, TFC showed a non-significant impact after pasteurisation (137 ± 2.55 to 139 ± 1.63 mg CTE/L). While Geraldi et al. (2021) observed increases in phenolics and antioxidant capacity following short, high-temperature treatment of jabuticaba juice, Hu et al. (2020) reported significant losses under

harsher conditions (90 °C for 60 s). These contrasting outcomes highlight the influence of temperature–time intensity on phenolic stability, with milder pasteurisation favouring functional retention.

3.6.3. Sensory evaluation of a functional beverage

Sensory evaluation of the JPE-infused beverage was performed using a 9-point hedonic scale and compared with a blank control (Fig. 4A). Significant differences ($p < 0.05$) were observed for colour and texture: the blank control was rated higher for colour, while the JPE beverage received higher texture scores. No significant differences were found for overall impression or liking of colour and taste ($p > 0.05$). Although purchase intention was slightly higher for the JPE beverage (3.19 ± 1.23) than for the control (2.87 ± 1.36), this difference was not significant. The acceptability index (AI) further supported product acceptance, with the JPE beverage achieving 71% compared with 67% for the blank control. As an AI ≥70% is widely considered indicative of satisfactory consumer acceptance, these results confirm the overall acceptability of the JPE formulation.

Sensory responses were further analysed by age group (Fig. 4B) and gender (Fig. 4C). No significant differences (p > 0.05) were detected among age categories or between genders for any sensory attribute, although participants aged ≥30 years showed slightly higher acceptance. Females showed a marginally higher AI (73%) than males (69%), but both groups demonstrated favourable acceptance. The lack of significant demographic effects suggests the broad appeal of the JPE beverage, consistent with reports that well-formulated plant-based beverages are generally accepted across consumer groups (Wichchukit & O'Mahony, 2015).

4. Conclusions

This study demonstrated that polyphenol extraction from jabuticaba peels, particularly anthocyanins, is most effective using a 20% ethanolic solution. The combination of UAE and RSM enabled optimisation of the extraction process, with optimal conditions identified at a solid-to-solvent ratio of 1:40 *w/v* and a 6.75 min extraction time. The resulting extract was rich in polyphenols, including gallic acid, procyanidin B2, vescalagin, (−)-epicatechin, and cyanidin-3-*O*-glucoside, and exhibited antioxidant, antidiabetic, anti-obesity, and anti-cholesterolemic potential in vitro. Encapsulation in a food-grade matrix allowed targeted

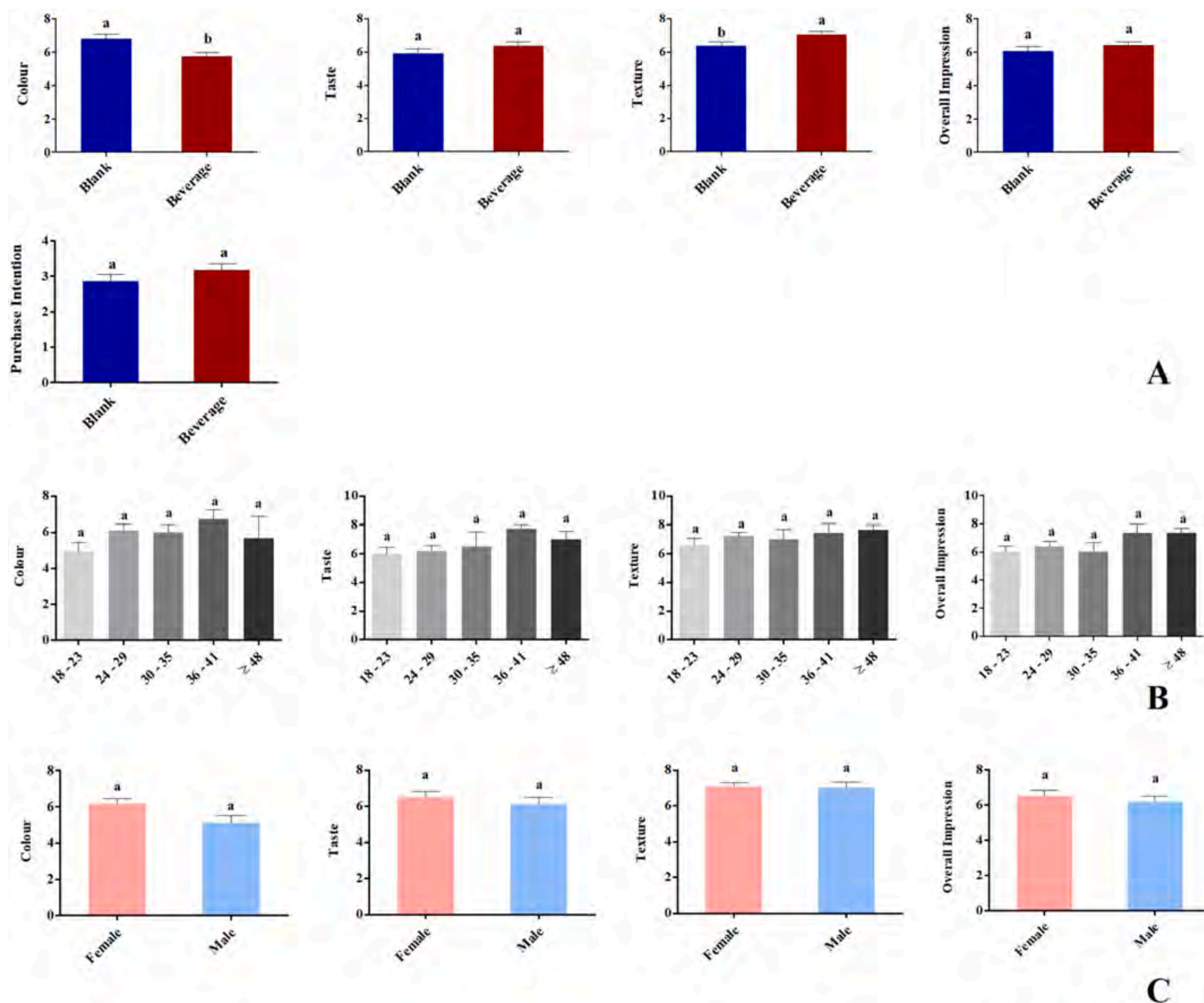


Fig. 4. Sensory evaluation scores of (A) Blank and beverage containing jaboticaba peel extract (B) beverage containing jabuticaba peel extract across age groups (C) JPE-enriched beverage by gender. Mean values are based on ratings from a 9-point hedonic scale. Different letters in the comparison indicate significantly different results ($p < 0.05$).

release under gastric conditions, while incorporation into a beverage model enhanced cellular antioxidant activity without loss of bioactive compounds after pasteurisation. Overall, jabuticaba peel extract shows strong potential as a sustainable functional ingredient for mitigating oxidative stress.

Ethical statement - studies in humans and animals

The sensory evaluation of the beverages was reviewed and approved by the University of Limerick Research Ethics Committee (Science & Engineering) (approval number: 2025_04_13_S&E). Written informed consent was obtained from all participants prior their participation in the study.

Declaration of generative AI and AI-assisted technologies used in the manuscript preparation process

During the preparation of this manuscript, Grammarly Pro was used to assist with corrections to grammar, syntax, and readability. The tool was not used to generate content. The authors subsequently reviewed and edited the text and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Anisha Madathikudiyil Haridas: Writing – original draft, Investigation, Formal analysis, Data curation. **Amanda Lais Alves Almeida Nascimento:** Writing – original draft, Investigation, Formal analysis. **Fahrul Nurkolis:** Writing – original draft, Investigation, Formal analysis. **Carolina Girotto Pressete:** Writing – original draft, Supervision, Investigation, Formal analysis, Data curation. **Daniel Granato:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.119303.

Data availability

Data will be made available on request.

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