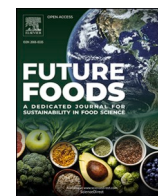


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Sustainable, functional food design: Characterizing and utilizing passion fruit by-product extract in anthocyanin-enriched delivery systems

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ABSTRACT

This study evaluated various conditions, including ethanol concentration (60 to 80 %) and ultrasonic power (300 to 500 W), to determine the optimal ultrasound-assisted extraction (UAE) parameters for anthocyanins from purple passion fruit (*Passiflora edulis* f. *edulis*) peel. The results identified that UAE at 500 W for 10 min using 80 % ethanol was the most effective condition, yielding 6.47 mg GAE/g of total phenolic content and 0.28 mg CYE/g of total anthocyanins. The extract demonstrated a significant 62 % inhibition of reactive oxygen species generation in human red blood cells and exhibited cupric-ion-reducing antioxidant capacity (27.45 mg AAE/g). In a gummy model, adding the optimal extract followed by *in vitro* digestion simulation increased the bioaccessibility of anthocyanins by 112 % and the total phenolic content by 163 %. The extract also enhanced the ferric-reducing antioxidant power (FRAP) with a bioaccessibility of 128 %, while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity decreased after digestion (bioaccessibility of 84 %). However, gummies made with sucrose and artificial red pigment achieved a higher acceptability index (76 %) than those with passion fruit peel extract (66 %). In conclusion, we identified the optimal extraction conditions for producing a food-grade and sustainable extract rich in anthocyanins. This extract can be utilized as a natural coloring and antioxidant in functional food applications.

1. Introduction

Fruit waste and by-products from fruit processing are a growing challenge in today's society, with their prolonged disposal contributing to global environmental concerns. Developing innovative methods to transform these by-products into value-added products offers a practical solution to waste while fostering sustainable economic growth within a bioeconomy framework. In tropical and subtropical regions of South America, especially in Brazil, passion fruit (*Passiflora edulis* Sims.) stands out as a highly profitable crop. However, its peels comprise about 50 % of the fruit's total mass and are commonly discarded as waste during processing. For instance, in 2019, approximately 95 % of the passion fruit harvest was directed to juice and pulp production, generating nearly 390,000 tons of passion fruit peels (PFP). Notably, PFP is rich in bioactive compounds, such as phenolic acids and flavonoids, including anthocyanins and cyanidin-3-glucoside (C3G). These compounds exhibit functional and pharmaceutical properties, making them valuable as potential functional ingredients for applications in food and

pharmaceutical industries, offering significant economic benefits (Mominur Rahman et al., 2021).

Anthocyanins are water-soluble polyphenolic pigments in the flavonoid class, typically in glycosylated forms. They are the key contributors to the purple, blue, and red coloration of fruits, vegetables, and flowers (Mohammadi et al., 2024c). These compounds are susceptible to variations in pH, light, and temperature. Known for their numerous health-promoting properties, sustained dietary consumption of anthocyanins exhibits antibacterial, anti-inflammatory, anticancer, anti-aging, and neuroprotective effects *in vivo* (Mohammadi et al., 2024b, c). They are widely utilized as natural colorants in the food industry, offering a sustainable alternative to synthetic dyes in food products and nutritional supplements (Mohammadi et al., 2024c). This approach also supports food producers in offering "clean label" foods and ingredients.

Numerous studies have explored using PFP in developing various food ingredients and products. For instance, flour extracted from PFP was utilized to make bakery products (Reis et al., 2020), pasta

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(Fogagnoli and Seravalli, 2014), and biscuits (Weng et al., 2021). Other studies focused on extracting soluble pectin (Lin et al., 2020) from PFP or incorporating them into fermented yogurts (do Espírito Santo et al., 2012).

Various methods for extracting anthocyanins from PFP have been reported, including conventional solid–liquid extraction with solvents such as ethanol, citric acid–water and water (Ghada et al., 2020; Liu et al., 2018). Among green extraction techniques, ultrasound-assisted extraction (UAE) stands out as an efficient method. It enhances heat and mass transfer through cavitation, disrupting plant cells and improving yield. Key factors affecting extraction include temperature, solvent-to-material ratio, pH, and time (Herrera-Ramirez et al., 2020). However, previous studies have not thoroughly explored the green extraction of anthocyanins from PFP using response surface methodology (RSM) in functional products like gummy formulations, nor have they examined anthocyanin bioaccessibility and antioxidant capacity before and after digestion. This approach could offer a comprehensive understanding of anthocyanin bioaccessibility while promoting the replacement of artificial colors with natural alternatives, aligning with circular economy principles to deliver safe, clean-label products that meet consumer demand.

This study analyzed the physicochemical properties of PFP extracts, including their composition, color pigments, anthocyanin content, and erythrocyte cellular antioxidant activity, which has not been previously reported. It also explored the fortification of gummies with PFP extracts as natural colorants and antioxidant sources, assessing their chemical composition and bioaccessibility of anthocyanins before and after digestion. The novelty of this study lies in its comprehensive evaluation of both the bioactive properties of PFP extracts and their application in functional gummies.

2. Materials and methods

2.1. Chemical reagents

Ethanol, 2,9-dimethyl-1,10-phenanthroline (neocuproin), Folin-Ciocalteu 2 N phenol reagent, 3,4,5-trihydroxybenzoic acid monohydrate (gallic acid), ammonium acetate, ascorbic acid, copper (II) chloride dihydrate, hydrochloric acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), α -amylase from porcine pancreas (14 U/mg, A3176-500KU), pepsin from porcine gastric mucosa (≥ 500 U/mg 77,160–100 G), bile salts (B8756–100 G), pancreatin from porcine pancreas (P1750–100 G), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), phosphate, dichlorodihydrofluorescein diacetate (DCFH-DA), iron chloride hexahydrate, magnesium chloride, ammonium carbonate, potassium chloride, and sodium acetate were obtained from Merck KGaA (Darmstadt, Germany), while 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) was purchased from Honeywell (Rolle, Switzerland). The other reagents were of analytical grade. HPLC standard (>98 % purity) of C3G was purchased from Extrasynthese (Genay, France). Beef gelatine powder (Dr. Oetker, Dublin, Ireland), xylitol and inulin extracted from chicory root (fructooligosaccharides content of 89 %), Bulk Sports Supplements Limited, Essex, United Kingdom), and blueberry flavoring drops (My Protein, Manchester, United Kingdom).

2.2. Purple passion fruit by-product and extraction of anthocyanins

Purple passion fruits (*Passiflora edulis* f. *edulis*), sourced initially from Columbia, were purchased from a supermarket in Limerick, Ireland. A total of 1 kg of purple passion fruits were processed by removing seeds; the peels were separated and dried in the laboratory oven at 60°C for 48 h. The dried PFP were ground into powder and stored in a dry place at room temperature. From 1 kg of passion fruit, 884 g were by-products, and approximately 116 g were pulp, and after drying, 194.4 g of passion fruit by-products were ground until a 60 Tyler mesh powder was obtained.

The 2² factorial design of the experiment was used in this study to find the optimal extraction conditions, where the ethanol concentration (60 % and 80 %) and ultrasonic power (300 W and 500 W) were used as the independent variables and tested at two levels. One central point (ethanol concentration of 70 %, and ultrasonic power of 400 W) was used to estimate the pure error and increase the degrees of freedom in the design. The choice of ethanol concentrations (60, 70, and 80 %) is based on the research conducted by (Ghada et al., 2020) and (de Santana et al., 2017). Triplicate extractions (100 mL) of 10 min were carried out using ultrasound-assisted extraction (UAE) (Fisherbrand™ Model 505 Sonic Dismembrator, Germany) using a solvent-to-material ratio of 20 mL:1 g. The beaker was placed in an ice bath to prevent temperature increases during the process. After the extraction, the extracts were centrifuged (4000 g for 5 min), and the supernatants were filtered using vacuum filtration and collected for further analysis.

2.3. Physicochemical characterization of the extracts

The color pigments in the PFP extract were analyzed in triplicate using the methods outlined by Mohammadi (2024). The absorbances (A) were measured using a spectrophotometer at wavelengths of 420 nm (yellow pigments), 520 (red pigments) nm, and 620 nm (blue pigments). The color intensity (IC) was then calculated to determine the percentage of yellow, red, and blue pigments using Eqs. (1)–4:

$$\text{Color intensity (IC)} = A_{420} + A_{520} + A_{620} \quad (1)$$

$$\text{Yellow pigments (\%)} = \left(\frac{A_{420}}{\text{IC}} \right) \times 100 \quad (2)$$

$$\text{Red pigments (\%)} = \left(\frac{A_{520}}{\text{IC}} \right) \times 100 \quad (3)$$

$$\text{Blue pigments (\%)} = \left(\frac{A_{620}}{\text{IC}} \right) \times 100 \quad (4)$$

The total anthocyanin contents (TAC) of the PFP extracts were determined in quadruplicate using the pH differential method at pH 1 and pH 4.5 (Mohammadi et al., 2024c). Absorbances were read at 520 and 700 nm, and C3G's molecular weight (449 g/mol) and a molar absorption coefficient (26,900 M⁻¹ cm⁻¹) were used in the calculations. Results were expressed as mg cyanidin-3-glucoside equivalent per g of dried PFP (mg CYE/g).

Total phenolic contents (TPC) were determined in quadruplicate using the Folin-Ciocalteu assay (Mohammadi et al., 2024c). The absorbance was measured at 725 nm after a 60-minute reaction, and the results were expressed as mg gallic acid equivalent per g of dried PFP (mg GAE/g).

The cupric-ion-reducing antioxidant capacity (CUPRAC) assay was performed in quadruplicate to estimate the extracts' antioxidant capacity (Mohammadi et al., 2024a). The absorbance was measured at 450 nm after a 30-minute reaction, and the results were expressed in mg of ascorbic acid equivalents per g of dried PFP (mg AAE/g).

2.4. Identification and characterization of the optimal extraction conditions

2.4.1. Response surface methodology coupled to inferential analysis

Response Surface Methodology (RSM) was used to model the effects of ethanol concentration and ultrasonic power on the content of bioactive compounds, color, and antioxidant activity. The quadratic model used for the analysis is expressed in Equation 5:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (5)$$

Where Y is the predicted response (e.g., TAC, TPC, CUPRAC, color attributes), X_i and X_j are the independent variables (e.g., ultrasonic power

and ethanol concentration), β_0 is the intercept, β_i , β_{ii} , and β_{ij} are the coefficients for the linear, quadratic, and interaction terms, respectively.

The significance of the model and its parameters were checked using the ANOVA procedure. Regression coefficients with p -values lower than 0.05 were regarded as significant, and the coefficient of determination (R^2) was calculated using TIBCO Statistica v. 13.1 software (TIBCO Statistica Inc., Palo Alto, CA, USA). In addition to the RSM modeling, one-way ANOVA followed by Tukey's test ($p < 0.05$) was used to assess differences between extraction conditions and highlight the optimal extract using a statistical approach. Graphical representations were generated using GraphPad Prism v. 8.4.3 (GraphPad Software Inc., San Diego, CA, USA). The optimal liquid extract (600 mL) was produced according to the procedures detailed in Section 2.2. For the procedures described in Section 2.4.2 (HPLC analysis), Section 2.4.3 (cellular antioxidant activity), and Section 2.4 (gummy production), ethanol was evaporated under vacuum at 40 °C and subsequently replaced with distilled water to maintain the original mass-to-solvent ratio.

2.4.2. Anthocyanin composition of the optimal extract

The chromatographic analysis was conducted for the optimal PFP extract (80 % ethanol, 500 W ultrasonic power). Dealcidolized PFP extract (400 μ L) was mixed with ultrapure water acidified with 1 % phosphoric acid (1600 μ L) and filtered using a 0.22 μ m cellulose acetate filter. The anthocyanin profile of the PFP extract was then analyzed using an HPLC Agilent 1200 Infinity Series chromatograph (Agilent Technologies, Santa Clara, CA, USA) with a Telos LU C18 column (ID 5 μ m, 150 $\times$ 4.6 mm). The injection volume was 30 μ L, and the column was maintained at 35 °C. The mobile phase, consisting of solvent A (ultrapure water acidified with 1 % phosphoric acid) and solvent B (acetonitrile) was run at a flow rate of 0.8 mL/min. The gradient condition was 92 % A at 0 min, 88 % A at 3 min, 86 % A at 25 min, 84 % A at 28 min, and 92 % A at 30 min). Four anthocyanins were used as standards: cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside, and delphinidin-3-rutinoside, and analytical curves were created using different concentrations (linearity: 5 to 49 mg/L, $R^2 > 0.99$). The anthocyanins were monitored using the diode array detector (DAD) at 520 nm, and the injections were performed in triplicate (Mohammadi et al., 2024c).

2.4.3. Erythrocyte cellular antioxidant capacity

The AAPH-induced intracellular ROS assay followed the procedures outlined by Cruz et al. (2024). Red blood cells (RBCs) were collected from a healthy male volunteer (40 years old, body mass index = 24.8 kg/m²). Dealcidolized PFP extract was prepared at 20 $\times$ and 40 $\times$ dilutions in PBS solution to check for dose-dependency behavior. Standard solutions of quercetin (25, 50, and 100 mg/L) were included alongside positive and negative controls. RBCs (100 μ L) were added to each solution and incubated at 37 °C, 150 rpm, for 20 min. Subsequently, 200 μ L of AAPH solution was added to all tubes except negative controls, which received 100 μ L of PBS. After gentle mixing, tubes were incubated at 37 °C for 2 h. Following incubation, the mixtures were centrifuged at 1200 $\times$ g at 4 °C for 10 min, and the supernatant was discarded. This washing process was repeated twice, with 400 μ L of PBS added each time, and the final 140 μ L of the washed RBC suspension was transferred to a 96-well microplate. Fluorometric measurements were performed in quadruplicate with excitation and emission wavelengths of 485 nm and 520 nm, respectively. ROS generation (%) was calculated using Eq. (6).

$$\text{ROS generation (\%)} = \left(\frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{positive control}} - A_{\text{blank}}} \right) \times 100 \quad (6)$$

The protocol was approved by the Science and Engineering Research Ethics Committee, University of Limerick (approval number 2023_02_01_S&E).

2.5. Application of purple passion fruit by-product extract on a functional food model

2.5.1. Gummy formulation

The gummy formulations followed Mohammadi et al. (2024). For PFP-enriched gummies, 1.5 g of xylitol and 2.5 g of inulin were dissolved in 17.5 g of water and heated to 40 °C. Then, 11.5 g of honey was added and dissolved, followed by the gradual addition of 5 g of gelatin, with the mixture heated to 60 °C. Once the texture was homogenous, the mixture was cooled to 40 °C, and 11.5 g of PFP extract and 0.5 g of flavoring agent were added. For the control gummies, 4 g of sucrose and 11.5 g of honey were dissolved in 28.75 g of water and heated to 40 °C. Next, 5 g of gelatin was added, and the mixture was heated to 60 °C until fully dissolved. After cooling to 40 °C, 0.5 g of flavoring agent and 0.25 g of color agent were incorporated. Both mixtures were poured into molds, cooled for 5 min, frozen for 10 min, and stored in airtight containers in the fridge.

2.5.2. Chemical composition, antioxidant capacity, and in vitro static digestion simulation

The digestion method followed the INFOGEST protocol proposed by Brodkorb et al. (2019) and Minekus et al. (2014). 5 g of each gummy (PFP-enriched and control) were solubilized with 5 g of purified water and heated to 40 °C until it melted. The melted gummies (5 g) were mixed with 4 mL of salivary fluid, 475 μ L ultrapure water, and 25 μ L of CaCl₂, followed by 500 μ L of amylase. The mixture was incubated at 37 °C, 100 rpm for 2 min, then cooled in an ice bath to stop the enzymatic activity before transitioning to the gastric phase. Gastric fluid (6.5 μ L) and 5 μ L of CaCl₂ were added, followed by pH adjustment to 3 using HCl. After adjusting the pH, 500 μ L of pepsin solution was added, and the mixture was incubated at 37 °C, 100 rpm for 2 h. The enzymatic activity was stopped using an ice bath before moving to the intestinal phase. Intestinal fluid (6.5 μ L) and 40 μ L of CaCl₂ were added, and pH was adjusted to 7 using NaOH. After pH adjustment, 2.5 mL of bile solution and 5 mL of pancreatic solution were added. The tubes were incubated at 37 °C, 100 rpm for 2 h. The enzymatic activity was then stopped by cooling in an ice bath. TCA was added to prevent protein precipitation before centrifugation at 3000 g for 1 h (Brodkorb et al., 2019; Minekus et al., 2014). Samples were frozen for further analysis. TAC and TPC were determined as described in Section 2.3.

The FRAP assay was performed by mixing each sample (20 μ L) with 180 μ L of FRAP reagent, and incubating for 20 min. Absorbance was measured at 593 nm, and results were expressed as mg AAE/30 g gummy (Mohammadi et al., 2024d). For the DPPH assay, each sample (40 μ L) was mixed with 260 μ L of 0.1 mmol/L methanolic DPPH solution, incubated in the dark at 25 °C for 30 min, and absorbance was measured at 517 nm. Results were expressed as mg AAE/30 g gummy (Mohammadi et al., 2024a). Bioaccessibility (%) of the released compounds was calculated using Eq. (7) (Yu et al., 2021).

$$\text{Bioaccessibility (\%)} = \frac{\text{CI}}{\text{CA}} \times 100 \quad (7)$$

where CI is the final contents (after digestion), and CA is the initial contents (before digestion).

2.5.3. Sensory analysis of gummies

The sensory evaluation was conducted at the Science and Engineering building, University of Limerick, in Ireland, with ethical approval (2024_07_03_S&E). Fifty-one participants evaluated two gummy samples (PFP-enriched and control) using a 9-point hedonic scale for color, taste, texture, and overall impression, where 1 = dislike extremely and 9 = like extremely. Each sample's acceptability index (%) was calculated using Eq. (8).

$$\text{Acceptability index (\%)} = \left(\frac{\text{Average of overall impression}}{\text{The highest score of overall impression}} \right) \times 100 \quad (8)$$

2.6. Statistical analysis

Data are expressed as the mean $\pm$ standard deviation. Significant differences between groups ($p < 0.05$) were determined using an unpaired Student's *t*-test for two-group comparisons, and one-way analysis of variance (ANOVA) with Tukey's post-hoc test for comparisons involving three or more groups. Correlation analysis was performed using Pearson's correlation coefficient. All statistical analyses were performed with TIBCO Statistica v.13 (TIBCO Statistica Inc., Palo Alto, CA, USA), and graphs were generated using OriginPro 2021 (version 9.8.0.200) from OriginLab Corporation, USA.

3. Results and discussion

3.1. Extraction optimization of passion fruit peel extracts: chemical composition, antioxidant capacity, and chromatic attributes

Table 1 summarizes the effects of ethanol concentration and ultrasonic power on the chemical composition, antioxidant capacity, and chromatic attributes of the PFP extract. The TPC values ranged from 5.26 ± 0.23 to 7.04 ± 0.49 mg GAE/g, with the highest value observed at 300 W and 60 % ethanol. Ultrasound power at 400 W combined with 70 % ethanol concentration also yielded a relatively high TPC (6.68 ± 0.07 mg GAE/g). In contrast, at 300 W ultrasound power and 80 % ethanol resulted in the lowest TPC, which was significantly different ($p < 0.05$) from the values obtained under the other conditions. In contrast, dos Reis et al. (2018), reported a significantly higher TPC of 1570.80 mg GAE/100 g dry weight, likely due to their exhaustive extraction method (dos Reis et al., 2018). Similarly, da Costa et al. (2023) analyzed immature, mature, and ripe purple PFP with values of 24.09, 58.53, and 112 mg GAE/100 g sample, respectively (da Costa et al., 2023), which are lower than the values obtained in this study. The variations in TPC observed in our study with others may be attributed to the combined influence of extraction parameters, such as ethanol concentration and ultrasound power, which can enhance cell wall disruption and solubilization of phenolics (Bhagya Raj and Dash, 2020).

The TAC of PFP extracts showed limited variability, ranging from 0.20 ± 0.01 to 0.32 ± 0.7 mg CYE/g ($p < 0.05$), with the highest value obtained at 500 W and 80 % ethanol ($p < 0.05$). The increase in TAC at this condition may be linked to improved extraction efficiency of ethanol for anthocyanins, which are typically more soluble in ethanol-rich environments (Celli et al., 2015). The stability of anthocyanins during extraction might also be influenced by ethanol's ability to minimize oxidative degradation (Zou et al., 2011).

The CUPRAC values ranged from 12.68 ± 2.03 to 39.50 ± 1.58 mg AAE/g, with the highest antioxidant capacity observed at 400 W and 70 % ethanol ($p < 0.05$). Higher CUPRAC values observed at moderate

ethanol concentrations (60–70 %) suggest that these conditions effectively extract polar and nonpolar antioxidants. The moderate ethanol concentrations are relevant for the CUPRAC assay, as it measures antioxidant capacity through both Single Electron Transfer (SET) and Hydrogen Atom Transfer (HAT) mechanisms (Apak, 2017). Polar antioxidants primarily act via SET, while nonpolar antioxidants often follow HAT mechanisms. Therefore, the CUPRAC assay is relevant to assessing the antioxidant capacity of mixed-polarity samples.

The chromatic attributes of PFP extract, including color intensity and the proportions of yellow, red, and blue pigments, were significantly influenced by ultrasonic power and ethanol concentration. Color intensity ranged from 1.39 ± 0.04 to 2.01 ± 0.16 a.u., with the highest value at 500 W and 60 % ethanol ($p < 0.05$), highlighting the role of moderate ethanol levels and increased ultrasonic power in enhancing pigment extraction. Yellow pigments, predominantly carotenoids, peaked at 69.12 ± 0.06 % under 300 W and 80 % ethanol, likely due to ethanol's efficiency in solubilizing nonpolar compounds (Linares and Rojas, 2022). Red pigments linked to anthocyanins were highest at 29.68 ± 0.03 % with 300 W and 60 % ethanol, demonstrating that lower ethanol concentrations better stabilize and extract these polar molecules (Castañeda-Ovando et al., 2009). Blue pigments, potentially anthocyanin derivatives, reached their maximum of 11.67 ± 0.26 % under 400 W and 70 % ethanol, aligning with Montero-Calderon et al., who noted the stabilization and enhanced recovery of anthocyanins under similar conditions from orange peel (Montero-Calderon et al., 2019). The optimal extraction conditions for PFP extract were determined using inferential analysis (Table 1) and RSM (Table 2 and Fig. 1). The ideal parameters were 500 W and 80 % ethanol, as it yielded the highest total anthocyanin content (0.32 ± 0.07 mg CYE/g), which was the primary criterion for selection. Only TPC ($R^2=0.875$), total monomeric anthocyanin content ($R^2=0.860$), and color intensity ($R^2=0.872$) were accurately modeled, where most of the data variability could be explained by the RSM models. Based on the results, increased ultrasound power and ethanol concentration raised the TAC ($R^2=0.875$).

The correlation analysis revealed assay-dependent relationships among antioxidant assays and chromatic attributes. TPC was inversely correlated with yellow pigments ($r=-0.675$, $p=0.006$), red pigments ($r=0.700$, $p=0.004$), and CUPRAC ($r=0.568$, $p=0.027$), indicating a link between reducing power and phenolic content. TAC was significantly correlated with CUPRAC ($r=0.694$, $p=0.004$) showing that anthocyanins play a key role in the antioxidant capacity of passion fruit peel extracts.

3.2. Characterization of optimal extract: anthocyanin composition and erythrocyte cellular antioxidant capacity

3.2.1. Anthocyanin composition of PFP by HPLC

Fig. 2 displays the HPLC analysis of anthocyanins extracted from the optimal PFP extract. C3G was identified as the only anthocyanin in the extract, consistent with previous findings that confirm C3G as the dominant anthocyanin in PFP (Fonseca et al., 2022). The C3G content in the PFP extract was 0.52 ± 0.05 mg/g, aligning with reported values ranging from 0.029 to 5.78 mg/g (Herrera-Ramirez et al., 2020),

Table 1

Effects of ultrasound power and ethanol concentration on the chemical composition, and antioxidant capacity of purple passion fruit peel extracts.

Power (W)	Ethanol concentration (%)	TPC (mg GAE/g)	TAC (mg CYE/g)	CUPRAC (mg AAE/g)	Color intensity (a. u.)	Yellow pigments (%)	Red pigments (%)	Blue pigments (%)
300	60	7.04 ± 0.49^a	0.23 ± 0.02^b	24.41 ± 2.22^{bc}	1.70 ± 0.10^b	59.45 ± 0.16^b	29.68 ± 0.03^a	10.87 ± 0.14^c
300	80	5.26 ± 0.23^b	0.20 ± 0.01^b	12.68 ± 2.03^d	1.39 ± 0.04^c	69.12 ± 0.06^a	20.81 ± 0.02^e	10.07 ± 0.05^d
500	60	6.65 ± 0.12^a	0.20 ± 0.01^b	23.27 ± 0.98^c	2.01 ± 0.16^a	59.27 ± 0.10^b	29.43 ± 0.01^b	11.30 ± 0.11^b
500	80	6.47 ± 0.23^a	0.32 ± 0.07^a	27.45 ± 0.48^b	1.49 ± 0.02^c	68.43 ± 0.08^a	21.66 ± 0.03^d	9.91 ± 0.06^d
400	70	6.68 ± 0.07^a	0.20 ± 0.01^b	39.50 ± 1.58^a	1.68 ± 0.05^b	61.22 ± 0.37^c	27.11 ± 0.12^c	11.67 ± 0.26^a

Note: TPC=Total phenolic content, TAC=Total monomeric anthocyanin content, GAE= gallic acid equivalent, CYE=cyanidin-3-glucoside equivalent, AAE=ascorbic acid equivalent. Different superscript letters in the same column represent statistically different ($p < 0.05$) mean values. Quantitative data are the mean $\pm$ standard deviation ($n=4-6$).

Table 2

Regression coefficients derived from response surface modelling of experimental data illustrating the effects of ultrasound power and ethanol concentration on the chemical composition, instrumental color, and antioxidant capacity of purple passion fruit peel extracts.

Factors	Regression Coefficients	Standard Error	t-Value	p-Value	−95 % Confidence Limit	+95 % Confidence Limit
Total Phenolic Content (TPC)						
Mean/Interc.	20.884	2.811	7.430	<0.001	13.659	28.109
(1) Ultrasound Power (W)	−0.027	0.007	−3.997	0.010	−0.045	−0.010
(2) Ethanol (%)	−0.220	0.040	−5.525	0.003	−0.322	−0.117
1 by 2	<0.001	<0.001	4.369	0.007	<0.001	0.001
R ²	0.875					
R ² adj	0.812					
Total Anthocyanin Content (TAC)						
Mean/Interc.	0.7	0.118	5.5	0.003	0.3	1.0
(1) Ultrasound Power (W)	−0.001	0.000	−4.912	0.004	−0.002	−0.001
(2) Ethanol (%)	−0.007	0.002	−4.362	0.007	−0.012	−0.003
1 by 2	<0.001	<0.001	5.582	0.003	<0.001	0.000
R ²	0.860					
R ² adj	0.790					
Cupric Ion Reducing Antioxidant Capacity (CUPRAC)						
Mean/Interc.	115.2	13.326	8.6	<0.001	81.0	149.5
(1) Ultrasound Power (W)	−0.198	0.032	−6.124	0.002	−0.281	−0.115
(2) Ethanol (%)	−1.513	0.188	−8.025	<0.001	−1.997	−1.028
1 by 2	0.003	<0.001	7.421	0.001	0.002	0.005
R ²	0.312					
R ² adj	<0.001					
Color Intensity (CI)						
Mean/Interc.	2.936	0.324	9.1	<0.001	2.104	3.768
(1) Ultrasound Power (W)	0.001	<0.001	2.807	0.038	<0.001	0.002
(2) Ethanol (%)	−0.025	0.004	−6.203	0.002	−0.035	−0.014
R ²	0.872					
R ² adj	0.835					
Yellow Pigments (YP)						
Mean/Interc.	28.3	0.738	38	<0.001	26.4	30.2
(1) Ultrasound Power (W)	0.005	0.002	2.977	0.031	0.001	0.010
(2) Ethanol (%)	0.516	0.010	49	<0.001	0.490	0.543
1 by 2	<0.001	<0.001	−4.380	0.007	<0.001	<0.001
R ²	0.942					
R ² adj	0.913					
Red Pigments (RP)						
Mean/Interc.	62.4	0.223	280	<0.001	61.8	63.0
(1) Ultrasound Power (W)	−0.019	0.001	−34.6	<0.001	−0.020	−0.017
(2) Ethanol (%)	−0.533	0.003	−169	<0.001	−0.541	−0.524
1 by 2	<0.001	<0.001	37.668	<0.001	<0.001	<0.001
R ²	0.970					
R ² adj	0.956					
Blue Pigments (BP)						
Mean/Interc.	10.4	0.118	88	<0.001	10.1	10.7
(1) Ultrasound Power (W)	0.011	0.001	18.88	<0.001	0.009	0.012
1 by 2	<0.001	<0.001	−19.905	<0.001	<0.001	<0.001
R ²	0.626					
R ² adj	0.519					

depending on factors such as extraction methods and fruit origin (Medina et al., 2017). Variations in C3G levels can be attributed to differences in extraction techniques (Młynarczyk et al., 2018), climatic conditions (Przybylska-Balcerek et al., 2021), and the ripening stage of the fruit during harvest (dos Reis et al., 2018). Interestingly, C3G accounts for 97 % of the TAC in PFP, emphasizing its role as a key bioactive compound in this by-product.

3.2.2. Erythrocyte cellular antioxidant capacity of PFP

The optimal PFP extract's antioxidant capacity in protecting erythrocytes from AAPH-induced oxidative stress was evaluated by measuring intracellular reactive oxygen species (ROS) levels (Fig. 3). Erythrocytes are particularly vulnerable to oxidative damage due to their high polyunsaturated fatty acid (PUFA) content and involvement in hemoglobin redox reactions (Cruz et al., 2021). As shown in Fig. 3, PFP extract dilutions (20- and 40-fold) significantly reduced intracellular ROS levels in erythrocytes compared to positive and negative controls and quercetin standards (25, 50, and 100 mg/L). Specifically, ROS inhibition by the PFP extract at 20-fold (56.3 ± 1.2 %) and 40-fold (62.3 ± 6.8 %) dilutions was lower than that observed for quercetin standards at 25 mg/L (95.1 ± 3.7 %), 50 mg/L (77.4 ± 11.8 %), and 100

mg/L (67.0 ± 8.6 %), as well as the negative control (68.9 ± 2.3 %) and the positive control (100 ± 3 %).

The enhanced antioxidant capacity of the PFP extract may be attributed to the efficient extraction of lipophilic antioxidants, such as flavonoids, which protect the red blood cell membrane from oxidative stress (de Moura et al., 2022; Heijnen et al., 2002). Additionally, anthocyanins, particularly in their natural form, protect erythrocytes from oxidative stress more effectively than synthetic anthocyanins (Charif et al., 2021; Garcia and Blesso, 2021). Furthermore, the ability of the extract to permeate the erythrocyte membrane likely contributed to its suppression of AAPH-induced ROS generation (Roche et al., 2009).

3.3. Application of optimal extract in a functional food model

3.3.1. Bioactivity and in vitro static digestion simulation

Table 3 presents the TPC, TAC, FRAP, and DPPH results for undigested and digested PFP-enriched gummies and the control. The undigested control gummies exhibited no detectable TAC, while the undigested PFP-enriched gummies had a TAC of 0.18 ± 0.07 mg CYE/30 g gummy. After the *in vitro* digestion, the TAC of the control gummies remained undetectable. In contrast, the TAC of the PFP-enriched

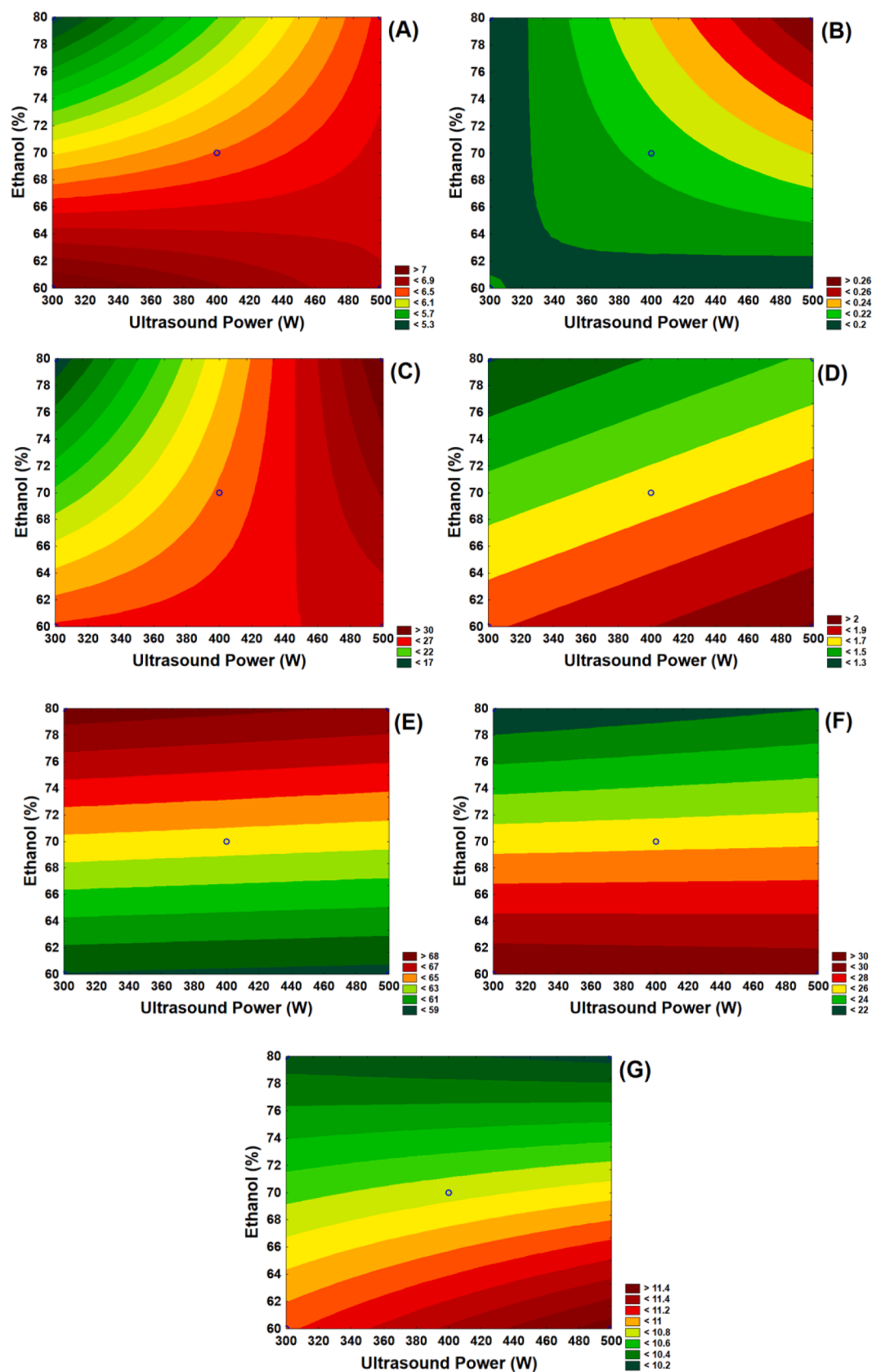


Fig. 1. Effect of ethanol concentration (%) and ultrasonic power (W) on total phenolic content (A), total anthocyanin content (B), cupric-ion reducing antioxidant capacity (C), color intensity (D), yellow pigments (E), red pigments (F), and blue pigments (G) of purple passion fruit peel.

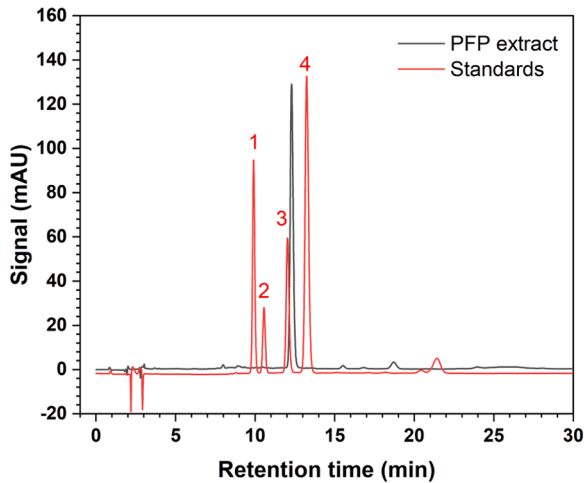


Fig. 2. HPLC chromatogram identification at 520 nm of anthocyanins in purple passion fruit peel extract (PFP), along with four anthocyanin standards: (1) delphinidin-3-glucoside, (2) delphinidin-3-rutinoside, (3) cyanidin-3-glucoside, and (4) cyanidin-3-rutinoside.

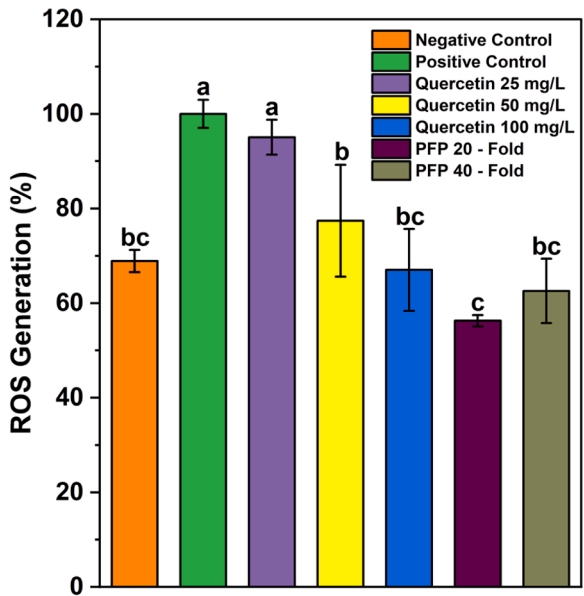


Fig. 3. Effect of the optimal purple passion fruit peel (PFP) extract on intracellular reactive oxygen species (ROS) generation induced by 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) in human erythrocytes.

gummies showed a slight increase to 0.19 ± 0.02 mg CYE/30 g gummy, corresponding to a bioaccessibility of 112 %, indicating that gastrointestinal conditions may enhance the release of anthocyanins. The reason why anthocyanins exhibited high bioaccessibility can be partially attributed to the protective effect of specific compounds in the food matrix, notably soluble fibers and gelatin proteins/peptides, which interacted with them to enhance their stability during digestion (Dantas et al., 2019). For TPC, the undigested control gummies (16.06 ± 0.43 mg GAE/30 g gummy) and PFP-enriched gummies (16.61 ± 0.41 mg GAE/30 g gummy) were comparable. However, after digestion, the TPC of the PFP-enriched gummies dramatically increased to 27.10 ± 0.40 mg GAE/30 g gummy (bioaccessibility 163 %), significantly ($p < 0.05$) outperforming the digested control gummies (15.07 ± 1.89 mg GAE/30 g gummy; bioaccessibility 94 %). This increase indicates that gastrointestinal digestion facilitates the release or stabilization of bound phenolic compounds, as observed in similar studies with passion fruit

Table 3
Impact of *in vitro* digestion on the chemical composition and antioxidant activity of PFP-enriched gummies.

SAMPLE	TAC (mg C3G/30 g gummy)	TPC (mg GAE/30 g gummy)	FRAP (mg AAE/30 g gummy)	DPPH (mg AAE/30 g gummy)
Undigested Negative- control gummy	ND*	16.06 ± 0.43 ^b	1.05 ± 0.86 ^c	0.99 ± 0.12 ^c
<i>In vitro</i> digested negative- control gummy	ND*	15.07 ± 1.89 ^b	1.83 ± 0.29 ^c	0.20 ± 0.06 ^d
Undigested PFP- enriched gummy	0.18 ± 0.07 ^a	16.61 ± 0.41 ^b	5.74 ± 0.26 ^b	5.58 ± 0.06 ^a
<i>In vitro</i> digested PFP-enriched gummy	0.19 ± 0.02 ^a	27.10 ± 0.40 ^a	7.23 ± 0.15 ^a	4.68 ± 0.72 ^b
*Bioaccessibility (%)				
PFP-enriched gummy	112	163	128	84

Note: Quantitative data are expressed in mean $\pm$ standard deviation ($n = 4$), in which different superscript letters in the same column represent statistically different ($p < 0.05$) mean values. C3G: cyanidin-3-glucoside equivalents, GAE: Gallic acid equivalents, FRAP: Ferric Reducing Antioxidant Power, AAE: Ascorbic Acid Equivalents. *Bioaccessibility (%) = (*In vitro* digested gummy/Undigested gummy)*100 %. ND*: Not detectable.

juices, which reported improved bioaccessibility of anthocyanins and phenolics post-digestion (Xie et al., 2021).

A notable difference was observed in the FRAP assay. The PFP-enriched gummies had significantly higher undigested FRAP values (5.74 ± 0.26 mg AAE/30 g gummy) than the negative control (1.05 ± 0.86 mg AAE/30 g gummy). Following digestion, FRAP values increased in both samples. The PFP-enriched gummies reached 7.23 ± 0.15 mg AAE/30 g gummy (bioaccessibility 128 %), surpassing their pre-digestion value, while the control gummies increased to 1.83 ± 0.29 mg AAE/30 g gummy (bioaccessibility 174 %). Although the control showed higher bioaccessibility, the PFP-enriched gummies maintained significantly higher FRAP values before and after digestion. Similar trends were observed in studies using passion fruit leaf and juice extracts, where the solubility of phenolic compounds improved during digestion, leading to higher antioxidant capacity (Aguillon-Osma et al., 2019). Lima et al. (2024) also demonstrated increased bioaccessibility of TAC, TPC, and FRAP of blackcurrant press cake post-digestion, attributing it to the enzymatic breakdown of complex compounds.

Interestingly, the DPPH radical scavenging activity displayed a different trend. Undigested PFP-enriched gummies exhibited a significantly higher DPPH value (5.58 ± 0.06 mg AAE/30 g gummy) than the control (0.99 ± 0.12 mg AAE/30 g gummy). However, digestion decreased DPPH activity for both samples, with the digested PFP-enriched gummies reducing to 4.68 ± 0.72 mg AAE/30 g gummy (bioaccessibility 84 %). This reduction may be due to structural modifications of anthocyanins during digestion, such as deglycosylation, which might render them less effective in scavenging free radicals (Gui et al., 2023). In contrast, the digested control gummies dramatically decreased to 0.20 ± 0.06 mg AAE/30 g gummy (bioaccessibility 20 %). This decrease in antioxidant capacity may be attributed to the hypothesis that refined sweeteners have lower antioxidant levels compared to unrefined products by the end of simulated digestion (Scrob et al., 2022).

The bioaccessibility of phenolic compounds in PFP-enriched gummies can also be influenced by the interactions between the antioxidant compounds and other ingredients in the gummy formulation, such as xylitol, inulin, honey, and gelatin, as well as the effects of heat during preparation. For example, gelatin can enhance the stability of polyphenols through covalent interactions, contributing to better gelation and antioxidant retention during digestion (Giménez et al., 2013; Karim and Bhat, 2009). Similarly, inulin, has been reported to alter the

solubility and release of phenolic compounds, potentially stabilizing and enhancing their bioaccessibility during gastrointestinal digestion (Fonteles et al., 2021). However, thermal processing, which is necessary for gummy preparation, can promote phenolics' solubility and include structural modifications, leading to either enhanced or diminished antioxidant capacity, depending on the specific formulation (Toydemir et al., 2022). Further research is needed to elucidate the specific mechanism by which these interactions influence the bioaccessibility and stability of anthocyanins and other phenolic compounds in gummy formulations.

3.3.2. Sensory analysis

Out of 51 participants (45 % male, 55 % female) aged between 18 and 60 years old, all residing in Ireland, the sensory evaluation was conducted for control and PFP-enriched gummies using a 9-point hedonic scale to assess color, taste, texture, and overall impression (Table 4). The control gummies received significantly higher scores for color (7.61 ± 1.47) and taste (6.80 ± 1.43) compared to the PFP-enriched gummies, which scored 4.78 ± 1.75 and 5.02 ± 1.10 , respectively ($p < 0.05$). There was no significant difference for texture, with control gummies scoring 6.43 ± 1.86 and PFP-enriched gummies scoring 5.94 ± 2.07 . Similarly, the overall impression score of the control gummies (6.86 ± 1.48) was significantly higher than that of the PFP-enriched gummies (5.25 ± 1.73 , $p < 0.05$).

The acceptability index (AI) was calculated based on overall impression scores, yielding 76 % for control gummies and 66 % for PFP-enriched gummies. The control gummies exceeded the commonly accepted threshold of 70 % for satisfactory consumer acceptance (Arruda et al., 2016). The PFP-enriched gummies fell below this benchmark, indicating the need for further improvement, particularly in color and taste (Costell et al., 2010). These differences in sensory perception align with the lower consumer purchase intention for PFP-enriched gummies. Although no significant off-flavours were reported by the panel, optimizing the formulation, especially for taste and color, could enhance consumer appeal and acceptance.

4. Conclusions

This study demonstrated the potential of PFP extracts for functional food applications. Response Surface Methodology revealed that ultrasound power and ethanol concentration significantly influenced the extraction of bioactive compounds, with optimal conditions yielding extracts rich in phenolics and anthocyanins. C3G was identified as the dominant anthocyanin, and the extracts exhibited robust antioxidant capacity, protecting erythrocytes from oxidative stress. PFP-enriched gummies showed enhanced phenolic and anthocyanin bioaccessibility and antioxidant capacity post-digestion, though sensory analysis indicated a need for improvements in taste and color. These findings highlight the potential of PFP extracts in functional food development and emphasize the importance of further research to enhance sensory attributes, optimize formulations, and study their integration into various food applications.

Ethical statement

The biological protocol using human blood was approved by the UL Science and Engineering Research Ethics Committee (approval #2023_02_01_S&E). A written consent was obtained from the blood donor.

The sensory analysis was approved by the UL Science and Engineering Research Ethics Committee (approval #2024_07_03_S&E). A written consent was obtained from the consumers

CRedit authorship contribution statement

Sopida Sakulrang: Writing – original draft, Investigation, Formal

Table 4

Sensory evaluation of negative-control and passion fruit peel-enriched gummy, expressed in color, taste, texture, and overall impression.

Sample	Color	Taste	Texture	Overall impression	Acceptability index (%)
Negative-control gummy	7.61 ± 1.47 ^a	6.80 $\pm$ 1.43 ^a	6.43 $\pm$ 1.86 ^a	6.86 $\pm$ 1.48 ^a	76 ^a
Passion fruit peel-enriched gummy	4.78 ± 1.75 ^b	5.02 $\pm$ 21.10 ^b	5.94 $\pm$ 2.07 ^b	5.25 $\pm$ 1.73 ^b	66 ^b

Note: Data are expressed in mean $\pm$ standard deviation, in which different letters in the same column represent a significant difference at a 95 % confidence interval ($p < 0.05$).

*Acceptability index = (Overall impression score/ the highest score of overall impression)*100.

analysis. **Mutasem Razem:** Writing – original draft, Investigation, Formal analysis. **Nima Mohammadi:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Daniel Granato:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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