CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2025.008

A feasible process for recycling anthocyanins and pectin from the waste peels of purple passion fruit (*Passiflora edulis* Sims) as food additives

Tran Thanh Tuan[1], Nguyen Anh Tu[2], Le Thi Ngoc Huong[2], Dinh Thi Tu Ngan[2], Ngo Truong Ngoc Mai[3], Nguyen Thi Bich Thuyen[3], Ho Quoc Phong[3], Doan Van Hong Thien[3], and Nguyen Viet Nhan Hoa[3*]

[1]*Department of Advanced Materials Science & Engineering, Institute of Rare Metal, Mokpo National University, Chonnam 534-729, Korea*

[2]*Student at Faculty of Biological, Chemical and Food Technology, Can Tho University of Technology, Viet Nam*

[3]*Faculty of Chemical Engineering, College of Engineering, Can Tho University, Viet Nam*

**Corresponding author (nvnhoa.ctu.edu.vn)*

Article info.

Received 9 Aug 2024
Revised 5 Sep 2024
Accepted 15 Feb 2025

Keywords

Anthocyanins, anti-oxidants, food additives, natural food colorants, pectin, purple passion fruit peels

ABSTRACT

Purple passion fruit is widely cultivated in Vietnam and its peels are considered by-products or agricultural waste after processing, which could cause environmental issues. Notably, these peels contain some valuable components with high bioactivities and applicability, such as anthocyanins (natural colorants) and pectin. Therefore, this work proposes a process for the sequential recycling of anthocyanins and pectin from purple passion fruit waste, in which anthocyanin extraction conditions and their bioactivity by ultrasound-assisted solvent extraction were studied and the characteristics of the obtained pectin were analyzed. The results showed that approximately 95% anthocyanins (125.4 mg/100 g dried peels) were extracted under the best conditions such as 80% ethanol (v/v), 1:25 (g/mL) solid/liquid ratio, 40°C incubation temperature, and 10 minutes sonication time. The findings demonstrated the antioxidant and cytotoxic activity of KB epithelial cells of the anthocyanin extract. Additionally, 7.47% of pectin from the residues after extraction anthocyanins were extracted by citric acid with pH 2 at 87°C for 90 minutes. The pectin has 68.34% purity, and 57.14% of DE, and its structure was confirmed by FT-IR spectra. This study can be utilized to recover useful components from purple passion fruit peel waste, improving the fruit's value and reducing the environmental impacts of its peels.

1. INTRODUCTION

Purple passion fruit (*Passiflora edulis* Sims*)* is a climbing vine belonging to the *Passifloraceae* family and native to South American countries (Castillo et al., 2020). The fruit has been popularly cultivated in many other countries worldwide (e.g., Ecuador, New Zealand, Australia, India, and Vietnam) (Da Silva Nóbrega et al., 2017; Castillo et al., 2020; Viera et al., 2020). Passion fruit is known as a delicious, aromatic, and nutrient-rich one with high vitamins A and C and minerals (potassium, phosphorus, and iron) (Phamiwon & John, 2017; Thokchom & Mandal, 2017; Thomas et al., 2019). The fruit is used in fresh or processed products such as juices, jellies, and ice cream (Matheri et al., 2016;

Thokchom & Mandal, 2017; Castillo et al., 2020). According to the reported literature, the global demand for passion fruit production was estimated to reach about 1.5 million tons in 2017, and this tendency has increased in the next years (Castillo et al., 2020; Viera et al., 2020). Along these lines, a large amount of their seed and external peels (approximately half of the total mass of the fruit), like by-products in production processes are also generated as solid waste and if improperly treated, might become environmental contaminants (De Souza et al., 2018). It was noticeable that these peels contain many bioactive and valuable constituents such as flavonoids (anthocyanins and flavonols), phenolic acids, pectin, etc., which can be recovered, reused, and make many high-value products (Wen et al., 2008). Therefore, the peel waste of passion fruits can be utilized as supply resources for generating valuable products and can bring economic benefits as well as reduce the pressure on the environment.

Among the chemical constituents of the purple passion fruit peels, anthocyanins have attracted much interest due to their high amount, and potential applications in food industries like natural water-soluble colorants replacing synthetic dyes or in cosmetics, pharmaceuticals, and others. Anthocyanins have strong antioxidant activity, reducing inflammation, boosting the immune system, and anti-obesity properties with low toxicity, and high safety (Dhawan et al., 2004; De Queiroz et al., 2012; de Souza et al., 2018). However, the low stability of anthocyanins under environmental conditions such as light, pH, oxygen, high temperature, and the presence of metal ions was reported, which caused a decrease in their original colors and biological activity ions in the extraction processes or preservation (Castillo et al., 2020; Enaru et al., 2021; Saini et al., 2024). These unstable characteristics have hindered their extending applications on an industrial scale Liu et al., 2018; Castillo et al., 2020). In general, many methods have been employed to extract anthocyanins such as traditional solvent extraction, supercritical carbon dioxide extraction, deep eutectic solvent extraction, and some advanced ones such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), high-pressure liquid extraction (HPLE), pulsed electric fields (PEFE), high voltage electrical discharge (HVED). ultrasound-assisted enzymatic extraction, and ultrasonic-microwave-assisted extraction (Campalani et al., 2020; Farooq et al., 2020; Hawthorne et al., 2000; Oliveira Chaves et al., 2020). Among these methods, solvent extraction-assisted ultrasound seems to be a suitable solution due to its advantages, such as low cost, good repeatability, simple operation, effective extraction, and friendly environment (Anaya-Esparza et al., 2023). Apart from anthocyanins, these fruit peels also contain pectin, an important component with high value widely used in various fields such as a gelling agent and stabilizer in the food industry or biodegradable films, foams, and plasticizers, etc. (Mada et al., 2022). Some studies pointed out that the recovery of pectin from these peels could obtained by using inorganic and organic acid solutions (e.g., hydrochloric acid, sulfuric acid, citric acid, and acetic acid) and the pectin characteristics have potential applications in various industries (Dam & Nguyen, 2013; Freitas et al., 2020). Therefore, seeking effective and green processes for recovering valuable compounds like anthocyanins and pectin from agricultural waste products like purple passion fruit peels is necessary to ensure sustainable and safe supplies for industries, especially food industries.

With the abovementioned, a process for recovering anthocyanins and pectin as a food additive from purple passion fruit peel waste was developed in this work. First, the extraction of anthocyanins was carried out using ethanol solution and assisted ultrasound. The effect of extraction parameters including ethanol concentration, incubation temperature, ultrasonic time, and solid/liquid ratio on anthocyanin extraction yield was investigated. Furthermore, the antioxidant activity and cytotoxicity test for the KB cell line of the anthocyanin extract were assayed. Pectin from residues after separating anthocyanins was extracted using a citric acid solution and the chemical structure and properties of the pectin was confirmed by FT-IR analysis and identified. These research findings will provide crucial information for considering the recovery of valuable products from agricultural byproducts.

2. MATERIALS AND METHOD

2.1. Sample preparation

Peel waste of fresh purple passion fruit was randomly collected from fruit stores located in Can Tho City (Vietnam). These selected peels were cleansed with tap water and then cut into small pieces (1.0 cm x1.0 cm). To prevent fungi and mold attacks, these peel pieces were soaked in 10 wt.%

NaCl solution and dried at 50°C in the oven for 48 hours to reach a moisture content of around 10 wt.%. The dried peels were milled to powder with a smaller particle than 2.0 mm particle size (Fig. 1) and stored in the dark at 4°C as materials for all extraction experiments.

Fig. 1. Fresh (a) and dried (b), and finely ground peel waste (c) of purple passion fruit.

2.2. Chemicals

All chemicals in this work were purchased from Xilong Scientific Co., Ltd., China with analytical grade. Aqueous solutions such as ethanol (C_2H_6O, ≥ 99.7%), sodium acetate (CH_3COONa, ≥ 99.0%), acetic acid (CH_3COOH, ≥ 99.5%), potassium chloride (KCl, ≥ 99.5%), and hydrochloric acid (HCl, ~36%) were diluted or dissolved in a distilled water to desired concentrations for extracting and quantifying anthocyanins after the extraction process. Other chemicals like citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$, ≥ 99.5%), calcium chloride anhydrous ($CaCl_2$, ≥ 96.0%), sodium hydroxide (NaOH, ≥ 96.0%), and silver nitrate ($AgNO_3$, ≥ 99.98%) were employed for recovering pectin from the residues after extracting anthocyanins.

2.3. Extraction experiments

2.3.1. Ultrasound-assisted extraction of anthocyanins

A specific amount of the dried peels (1.0 g) was added into an Erlenmeyer flask (50 mL) containing an ethanol solution with a certain ethanol concentration. The mixture reaction was sonicated using an ultrasonic bath with 180W, 40 kHz (GT Sonic Ultrasonic Cleaner, Guangdong GT Ultrasonic Co., Ltd., China) at a specific temperature and time. After the required time, the mixtures were centrifuged (Hettich EBA 280, Germany). The solution was filtered out of the solids (residues) using filter paper to determine the extraction yield of anthocyanins. To affect extraction, parameters such as ethanol concentration (60 ÷ 99.7%(v/v), incubation temperature (30÷60°C), ultrasonic time (5 ÷ 20 minutes), and solid-liquid ratio (1/10 ÷ 1/25 g/mL) on the extraction yield of anthocyanins was surveyed. Each experiment in this study was replicated three times to calculate errors of around 5%.

2.3.2. Pectin extraction from residues

Experiments for extracting pectin from residues after separating anthocyanins were performed according to the report described by Dam and Nguyen with some minor revision (Dam and Nguyen, 2013). Namely, the residues and citric acid solution mixture at pH 2.0 with 1:30 (g/mL) of the solid-to-liquid ratio was stirred at 87°C for 90 minutes using a heating magnetic stirrer (ARE, Velp, Italia). After the reaction, the solution was separated from the solid by hot filtering using filter paper. Then, the pectin from the resulting filtrate was precipitated by adding ethanol with a 1: 2 (v/v) volume ratio, gently stirring, and leaving for 3.0 hours at 4°C. The raw pectin was collected and dried at 60°C in an oven to a constant weight (24 hours).

2.4. Analytical methods

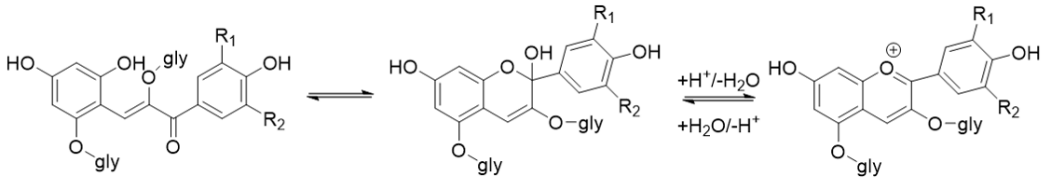
2.4.1. Quantitative for anthocyanins

Total monomeric anthocyanins from the extracts were measured by UV-Vis spectrophotometer (Biochrom Ltd., UK) at 520 nm and 700 nm using the pH differential method (Lee et al., 2005; Teng et al., 2020). This method is based on the color change of anthocyanins under different pH due to their structural change, in which a colored form at pH 1.0 and a colorless one at pH 4.5 (see **Scheme 1)**. The concentration of anthocyanins was calculated as milligrams (mg) of cyanidin-3-*O*-glucoside equivalent (C3gE) per 100 g of dried materials.

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad (1)$$

$$a = \frac{A \times M \times DF \times 100000 \times V_s}{\varepsilon \times L \times m} \quad (2)$$

where: A, A_{520}, and A_{700} are absorbance, absorbance at 520 nm, and absorbance at 700 nm, respectively; M is anthocyanin molecular weight of cyanindin-3-O-glucoside (449.2 g/mol); ε is coefficienS of molar extinction of cyaniding-3-glucoside (26900 $L \cdot mol^{-1} \cdot cm^{-1}$); L = cell path length (1 cm), DF = dilution factor; m = weight of sample (g); V_s = solvent volume (mL); a is mass of anthocyanins per 100 g the dried material powders.



Scheme 1. Change in the structural forms of anthocyanins at different pH values

To estimate the content of total anthocyanins in the purple passion fruit peel waste (a_T), 1 g of the materials was sonicated several times with 80% (v/v) ethanol for 10 minutes at 30°C until the content of anthocyanins in the final extract was below the limit of detection (LOD). Our research indicated that the content of total anthocyanins in the material powder was determined to be 132.03 mg cyanidin-3-O-glucoside per 100 g of the dried material through four continuous extraction stages of ethanol solutions.

The extraction percentage of the anthocyanins (%*E*) was calculated as follows: Eq.3, in which a_E is the mass of anthocyanins in the extract.

$$\%E = \frac{a_E}{a_T} \times 100\% \quad (3)$$

2.4.2. Evaluating bioactivity of the crude extract containing anthocyanins

DPPH method: The antioxidant activity of the crude extract containing anthocyanins from the passion fruit peels under optimal extraction conditions was ascertained by its capacity to inhibit 1,1-diphenyl-2-picrylhydrazyl (DPPH, $C_{18}H_{12}N_5O_6$, 97.0%, Sigma-Aldrich, USA) using UV-VIS measurements at 517 nm and Curcumin was used as a controlled substance to calculate EC_{50} (Marxen et al., 2007).

Cytotoxic activity: The KB human cancer cell line provided by ATCC (the American Type Culture Collection) was applied to evaluate the cytotoxicity of the crude extract. These cells were cultured in a medium, including 10% fetal bovine serum (FBS) and DMEM (Dulbecco's Modified Eagle Medium) and incubated under standard conditions such as 5% CO_2; 37°C; 98% humidity; absolute sterility (Scudiero et al., 1988; Freshney, 2015). The cytotoxicity of the crude extract was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, $C_{18}H_{16}BrN_5S$, ≥ 98.0%, Sigma-Aldrich, USA) assay by optical absorption at 540 nm and Ellipticine ($C_{17}H_{14}N_2$, ≥99.0%, Sigma-Aldrich, USA) was used as a reference.

All assays were measured at the Vietnam Academy of Science and Technology.

2.4.3. Pectin quantification ,and characterization

Pectin extraction yield: The extraction percentage of pectin (%E_{pectin}) from the residues was calculated as Eq. 3, in which m_{pectin} and $m_{residues}$ are the mass of dried pectin and residues, respectively.

$$\%E_{pectin} = \frac{m_{pectin}}{m_{residues}} \times 100\% \quad (4)$$

FT-IR spectra: The structure of the pectin was analyzed by Fourier transform infrared spectroscopy (FTIR, Shimadzu, IRAffinity-1s WL) in the 500 to 4000 nm range, where its characteristic functional groups were shown.

The pure level of pectin: The purity of pectin was estimated as calcium pectate according to the reported literature (Mui, 2001). Namely, the mixture of 0.15 g of the crude pectin and 100 mL of 1.0 N NaOH solution was stirred for 7.0 hours at room temperature to achieve complete saponification. Then, 50 mL of 0.1 N acetic acid was added to the saponified solution, following 50 mL of 2.0 N $CaCl_2$ solution. The mixture was slowly stirred and left for 1.0 hr. After that, the mixture was gently boiled for 5 minutes. The calcium pectate precipitate was collected by filtration and was washed with hot distilled water until no Cl^- ions remained in the filtrate using 1% (wt/wt) $AgNO_3$ solution as the reagent test. The collected calcium pectate was dried at 105°C to constant weight. The purity of pectin was calculated as follows:

$$P(\%) = \frac{m_{calcium\ pectate} \times 0.92}{m_{crude\ pectin}} \times 100 \quad (5)$$

where P(%) is the pure level of pectin; $m_{calcium\ pectate}$ and $m_{crude\ pectin}$ are weights of calcium pectate and raw pectin, respectively.

Esterification index (DE): The DE of pectin was determined by the titrimetric method (Hosseini et al., 2016). 0.1 g of the pectin was dissolved in the

solution of 2 mL of ethanol 96% and 20 mL of distilled water. The mixture was titrated with 0.1N NaOH using phenolphthalein (Xilong Scientific Co., Ltd., China) as an indicator to obtain volume V_1 (mL). Then, 10 mL of NaOH 0.5 N was added to the mixture after titration, which was shaken well, and left at room temperature for 90 minutes. After the required period, 12 mL of HCl 0.5 N was added to the mixture and shaken well until the pink color disappeared. Finally, the mixture was titrated with 0.1N NaOH to obtain the volume V_2 (mL).

$$DE = \frac{V_2}{V_1+V_2} \times 100\% \quad (6)$$

3. RESULTS AND DISCUSSION

3.1. Anthocyanin extraction from purple passion fruit peel waste

3.1.1. Effect of ethanol concentration

Anthocyanins are glucosides of the anthocyanidins and have strongly polar properties. Hence, polar solvents like methanol, ethanol, acetone, water, or their mixtures are commonly used to extract them from materials (Tena & Asuero, 2022). Considering in terms of extraction effectiveness and toxicity of these solvents, mixtures of ethanol and water were chosen to extract anthocyanins from purple passion fruit peel wastes in this work.

To investigate the effect of the dosage of ethanol on the extraction rate of anthocyanins, the ethanol concentration in the mixture was varied from 60 to 99.7% (v/v), and other extraction parameters including solid/liquid ratio, incubation temperature, and sonication time were fixed at 1:20 (g/mL), 40°C, and 15 minutes, respectively. In Fig. 2, the extraction percentage of anthocyanins significantly increased from 35.4 to 70.82% as rising ethanol concentration from 60 to 80% (v/v) and then gradually decreased to 35.41% at 99.7% ethanol (v/v). Increasing the viscosity of the extraction solvent owing to the dissolution of soluble carbohydrates like pectin from the materials at low ethanol concentrations could hinder the liberation of anthocyanins due to reducing the propagation of particles in the ultrasonic field and the level of cavitation (Arruda et al., 2017). Meanwhile, the decrease in the polarity of the extraction solvent at high ethanol concentrations reduced the dissolution of anthocyanins. Therefore, an ethanol concentration of 80% (v/v) was chosen for the following investigations.

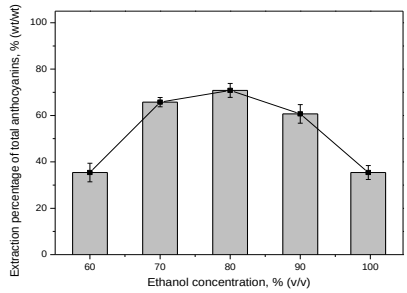


Fig. 2. Effect of ethanol concentration on anthocyanins from passion fruit peels ([C_2H_5OH] = 60-100% (v/v); incubation temperature = 40°C; solid/liquid ratio = 1:20 (g/mL), sonication time = 15 minutes)

3.1.2. Effect of incubation temperature

Raising the temperature during the ultrasonic process could lower the viscosity of the extraction solvent, leading to rose plant cell disruption and compounds from the cells dissolving into the solvent more easily (Yusaf, 2015). In addition, the mass transfer of the compounds from the materials to the extraction solvent was lifted. However, some compounds with bad thermal stability, like anthocyanins could be degraded at high temperatures (Oancea, 2021). Hence, the effect of incubation temperature on the extraction of anthocyanins was considered in the range of 30 to 60°C with fixing 80% (v/v) ethanol concentration, 1:20 (g/mL) solid/liquid ratio, and 15 minutes sonication time. As presented in Fig. 3, the extraction percentage of anthocyanins increased significantly from 55.5% to 70.8% when the temperature changed from 30 to 40°C, following sharply dropping to 20.2% at 60°C. Thus, 40°C temperature is the best condition for the extraction of anthocyanins.

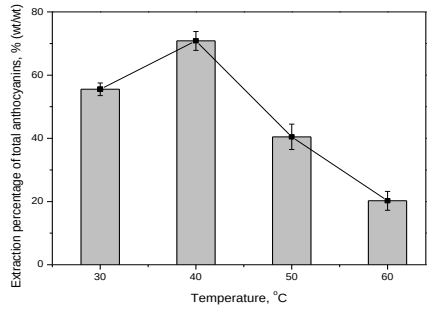


Fig. 3. Effect of temperature on anthocyanins from the passion fruit peels ([C_2H_5OH] = 80 % (v/v); incubation temperature = 30÷60°C; solid/liquid ratio = 1:20 (g/mL); sonication time = 15 minutes)

3.1.3. Effect of sonication time

The extraction time plays a vital role in the recovery process because of its extraction performance and cost production. Although prolonging the sonication time can cause the extraction efficiency of anthocyanins, these compounds can be degraded by temperature, oxygen, or light (Yan et al., 2023). So, the sonication time for the extraction of anthocyanins from the materials was done for 5 to 20 minutes, and the extraction parameters were maintained at the ethanol concentration of 80% (v/v), solid/liquid ratio of 1:20 (g/mL), and incubation temperature of 40°C. The results reveal that the highest anthocyanin extraction efficiency was 80.94% after 10 minutes of ultrasonic treatment (Fig. 4). This demonstrates that 10 minutes of sonication time was enough for the anthocyanin extraction.

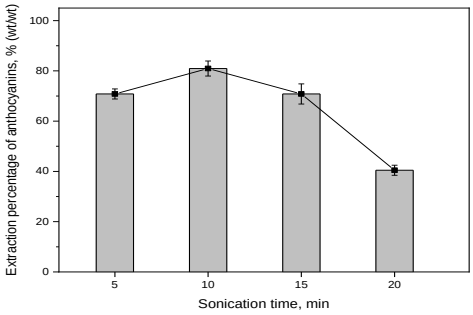


Fig. 4. Effect of sonication time on anthocyanins from the passion fruit peels ([C_2H_5OH] = 80 % (v/v); incubation temperature = 40°C, solid/liquid ratio = 1:20 (g/mL), sonication time = 5÷20 minutes)

3.1.4. Effect of solid/liquid ratio

In general, the use of more extraction solvents could boost the extraction efficiency of compounds from the materials due to enhanced mass transfer and chemical solubility. However, utilizing a lot of the solvents could limit the evaporation of the liquid, resulting in a reduction in the formation of air bubbles, leading to a decrease in the extraction efficiency (Liu et al., 2018). Therefore, solid/liquid ratios in this study were varied from 1:10 to 1:25 (g/mL). All experiments were performed at 80% (v/v) ethanol concentration, 40°C incubation temperature, and 10 minutes sonication time. The results in Fig. 5 indicated that the extraction percentage of anthocyanins gradually increased as the dosage of the extraction solvent rose. Namely, the extraction yield of anthocyanins increased from 68.0 to 94.9% with varying solid/liquid ratios from 1:10 to 1:25 g/mL. The results confirmed that the complete extraction of anthocyanins from the materials could be achieved by increasing the dosage of solvent extraction. Yet, the cost of the process should be considered.

From the obtained results, the best conditions for extracting anthocyanins from the materials with assisted ultrasonic wave were 80% (v/v) ethanol concentration, 40°C incubation temperature, 1:25 g/mL solid/liquid ratio, and 10 minutes of sonication time. The complete extraction of anthocyanins could be attained by adjusting the solid/liquid ratio or applying counter-current extraction with multiple stages. The purification of anthocyanins from the crude extract could succeed using chromatography methods or separation membranes (Tan et al., 2022).

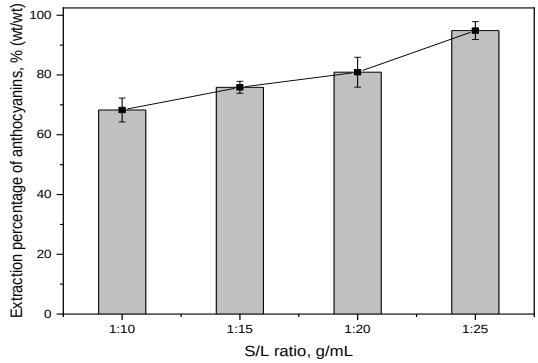


Fig. 5. Effect of solid/liquid ratio on the extraction of anthocyanins from passion fruit peels ([C_2H_5OH] = 80 % (v/v); incubation temperature = 40°C; solid/liquid ratio = 1:10 ÷1:25 (g/mL); sonication time =10 minutes)

3.2. Bioactive properties of the raw extract containing anthocyanins

The bioactivities of the plant extracts like the antioxidant activity significantly depend on various factors like the raw materials, storage conditions, extraction methods, etc. Hence, in this work, the antioxidant activity of the anthocyanin extract from the purple passion fruit peel waste was tested. In addition, KB epidermoid cancer cell toxicity of this extract was also tested, which has rarely been published before. Tables 1 and 2 show the results of bioactive assays of the extract at different concentrations. The EC_{50} value of the extract was higher than that of curcumin and did not exhibit activity at concentrations below 256 µg/mL. Meanwhile, the higher concentrations of the extract displayed a better percentage of inhibition of the KB

cell line. However, the extract's IC_{50} value was far higher than the IC_{50} value of Ellipticine and did not exhibit activity at doses below 256 µg/mL. Based on these assay results, the antioxidant activity and KB cell toxicity of the anthocyanins extract from the purple passion fruit peel wastes were insignificant compared with references to the positive control.

Table 1. Results of the antioxidant activity of the extract containing anthocyanins from purple passion fruit peel

Sample	Tested concentration (µg/mL)	% free radical scavenging	EC_{50} (µg/mL)
Anthocyanin extract	256	11	>256
	64	0	
	16	0	
	4	0	
Curcumin (positive control)	32	95	7.64±0.5
	8	52	
	2	19	
	0.5	7	

Table 2. Results of the cytotoxic activity of anthocyanin extract from purple passion fruit peels

Sample	Concentration (µg/ml)	Percentage inhibition of KB cell line (%)
Anthocyanin extract	256	19
	64	15
	16	9
	4	2
	IC_{50}	>256
Certified reference material (Ellipticine)	IC_{50}	0.43±0.02

3.3. Pectin extraction from the residues and its characteristics

To consider the pectin recovery ability from the passion fruit peels after the anthocyanin extraction process, the pectin from the residues was extracted by using citric acid solution under conditions at pH 2 with a 1:30 (g/mL) of the solid/liquid ratio at 87°C for 90 minutes. Our studies showed that the extraction percentage of pectin was 7.47% with 68.34% purity and 57.14% of the degree of esterification (DE) rate higher than 50% and is grouped as high methyl ester pectin (Liew et al., 2018).

To confirm the structure of the pectin, FT-IR spectra were recorded in the range of 500 to 4000 nm, where its characteristic functional groups were shown. As presented in **Fig. 6**, the broad peak at 3439 cm^{-1} and the weak peak at 2951 cm^{-1} are characterized by the stretching vibration of O-H and C-H of pectin (Chen et al., 2014). The peaks at 1723 cm^{-1} and 1621 cm^{-1} were generated by the groups of esterified carboxyl (–COOR) and ionized carboxyl (–COO), respectively. The "fingerprint region" of polysaccharides was 800-1200 cm^{-1}, in which bands between 1103 cm^{-1} and 1016 cm^{-1} were related to furanose and α–and β–pyranose rings in pectin samples (Xu et al., 2022). These results agreed well with the previous literature on the structure of the pectin extracted from passion fruit peels (Liang et at., 2022). Thus, high-methoxyl pectin was obtained from the residues after extracting anthocyanins from the passion fruit peels and fulfills a requirement to be considered as a commercially available food-grade pectin (Canteri et al., 2005).

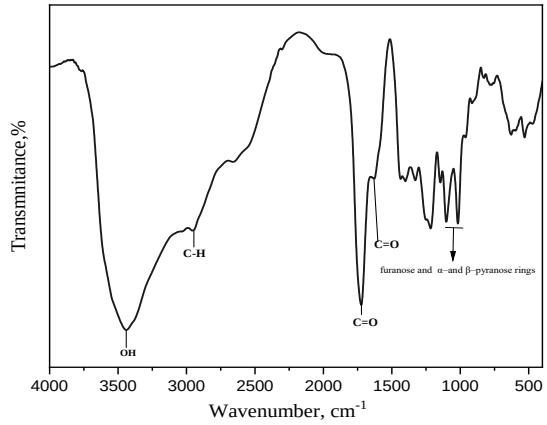


Fig. 6. FTIR spectrum of pectin extracted from the residues of purple passion fruit peel waste after extracting anthocyanins

3.4. Proposed process

From the obtained results, a feasible flowsheet for recovering anthocyanins and pectin from the purple passion fruit peel waste was proposed (Fig. 7). First, the collected passion fruit peel waste underwent a pretreatment process, including cleaning, cutting into species, soaking into NaCl solution, drying at 50°C, and grinding to material powders. Second, anthocyanins from the material powders were extracted by ethanol solution with assisted ultrasound under conditions such as 80% ethanol (v/v), 25 g/mL solid/liquid ratio, 40°C incubation temperature, and 10 minutes of sonication time.

Then, the anthocyanin extract is obtained from the extraction solution by evaporating the extraction solvent and following the purification process for pure anthocyanins. Finally, Pectin from residues after the extraction process of anthocyanins was recovered by using the citric acid solution at pH 2 at 87°C and precipitated with 96% ethanol in 1:2 (v/v) ratio. The flow sheet illustrates that anthocyanins can be fully extracted from the materials using ultrasound assistance in a short time and at low temperatures, minimizing the degradation of these natural colorants. Additionally, ethanol is utilized as an environmentally friendly and efficient solvent that can be reused after each extraction process. Moreover, obtained pectin can be applicable in the food industry. Thus, with its advantages, this processed process could be considered in recovering valuable constituents like anthocyanins and pectin from agricultural byproducts or wastes like the purple passion fruit peels, which could enhance economic values and decrease the burden on the environment.

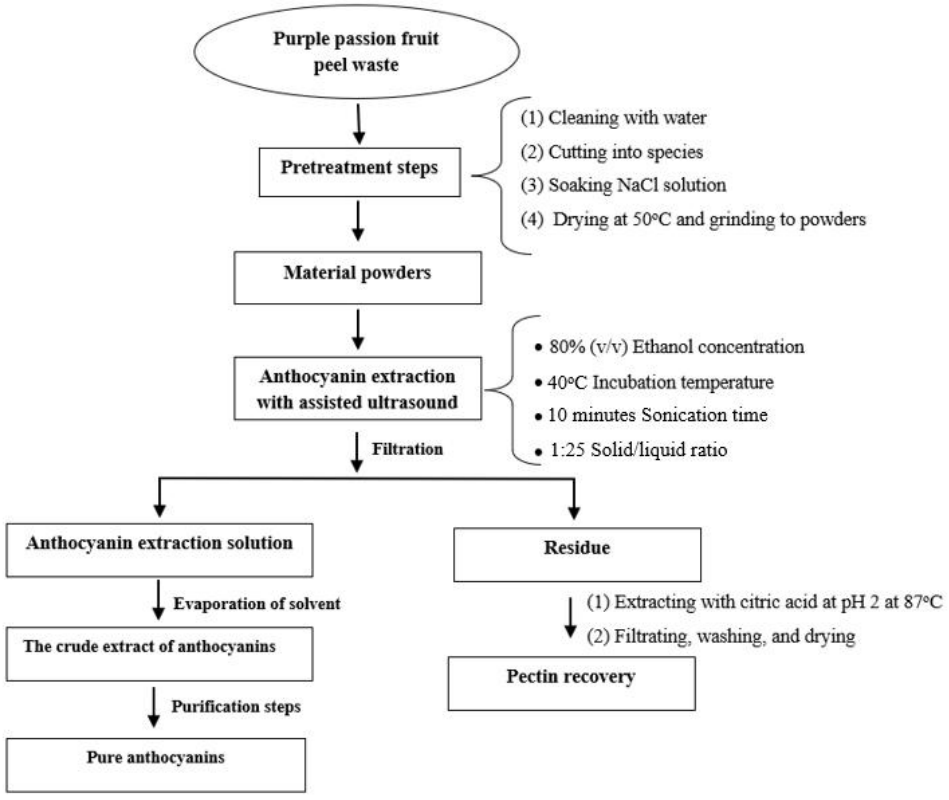


Fig. 7. Flow sheet of anthocyanin and pectin recovery from purple passion fruit peel waste

4. CONCLUSION

The recovery of anthocyanins and pectin from the purple passion fruit peel waste was studied. The effect of factors on the extraction yield of anthocyanins by ethanol solution with assisted ultrasound was done. The studied results indicated that 94.9% of anthocyanins were extracted under the best conditions of 80% (v/v) ethanol, 1:25 solid/liquid ratio, 40°C incubation temperature, and 10 minutes of sonication time. The virtue of assisted ultrasound is to bring a short extraction procedure and less energy consumption. The antioxidant activity and epithelial cell cytotoxicity of the extract containing anthocyanins were assayed. The recovery of pectin from the residues after extracting anthocyanins attained 7.47% with 68.34% purity and 57.14% of DE, which was classified as high methyl ester pectin. From the obtained results, a process for recovering anthocyanin and pectin from purple passion fruit peel waste was proposed. This study gives a lot of dense perspectives on enhancing passion fruit peel value in terms of economy and environment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Anaya-Esparza, L. M., Aurora-Vigo, E. F., Villagrán, Z., Rodríguez-Lafitte, E., Ruvalcaba-Gómez, J. M., Solano-Cornejo, M. Á., Zamora-Gasga, V. M., Montalvo-González, E., Gómez-Rodríguez, H., & Aceves-Aldrete, C. E. (2023). Design of Experiments for optimizing ultrasound-assisted extraction of bioactive compounds from plant-based sources. *Molecules*, *28*(23), 7752.

Arruda, H. S., Pereira, G. A., & Pastore, G. M. (2017). Optimization of extraction parameters of total phenolics from Annona crassiflora Mart.(Araticum) fruits using response surface methodology. *Food Analytical Methods*, *10*, 100-110.

Campalani, C., Amadio, E., Zanini, S., Dall'Acqua, S., Panozzo, M., Ferrari, S., De Nadai, G., Francescato, S., Selva, M., & Perosa, A. (2020). Supercritical CO_2 as a green solvent for the circular economy: Extraction of fatty acids from fruit pomace. *Journal of CO_2 Utilization*, *41*, 101259.

Castillo, N. R., Ambachew, D., Melgarejo, L. M., & Blair, M. W. (2020). Morphological and agronomic variability among cultivars, landraces, and genebank accessions of purple passion fruit, Passiflora edulis f. edulis. *HortScience*, *55*(6), 768-777.

Canteri, M., Fertonani, H., Waszczynskyj, N., & Wosiacki, G. (2005). Extraction of pectin from Apple-pomace. *Brazilian Archives of Biology and Technology* 48(1), 259–266.

Chen, J., Wang, Z., Li, G., & Guo, R. (2014). The swimming speed alteration of two freshwater rotifers Brachionus calyciflorus and Asplanchna brightwelli under dimethoate stress. *Chemosphere*, *95*, 256-260.

Dam, S. M., & Nguyen, D. V. (2013). Optimization of pectin extraction from fruit peel of purple passion fruit (Passiflora Edulis Sims) in Vietnam. *Acta Horticulturae*, *989*, 279-284.

Da Silva Nóbrega, D., Peixoto, J. R., Vilela, M. S., Faleiro, F. G., Gomes, K. d. P. S., de Sousa, R. M. d. D., & Nogueira, I. (2017). Agronomic descriptors and ornamental potential of passion fruit species. *Ornamental Horticulture*, *23*(3), 357-362.

De Queiroz, M. d. S. R., Janebro, D. I., da Cunha, M. A. L., Medeiros, J. d. S., Sabaa-Srur, A. U., Diniz, M. d. F. F., & Dos Santos, S. C. (2012). Effect of the yellow passion fruit peel flour (Passiflora edulis f. flavicarpa deg.) in insulin sensitivity in type 2 diabetes mellitus patients. *Nutrition Journal*, *11*, 1-7.

De Souza, C. G., Rodrigues, T. H., e Silva, L. M., Ribeiro, P. R., & de Brito, E. S. (2018). Sequential extraction of flavonoids and pectin from yellow passion fruit rind using pressurized solvent or ultrasound. *Journal of the Science of Food and Agriculture*, *98*(4), 1362-1368.

Dhawan, K., Dhawan, S., & Sharma, A. (2004). Passiflora: A review update. *Journal of Ethnopharmacology*, *94*(1), 1-23.

Enaru, B., Drețcanu, G., Pop, T. D., Stănilă, A., & Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, *10*(12), 1967.

Farooq, S., Shah, M. A., Siddiqui, M. W., Dar, B., Mir, S. A., & Ali, A. (2020). Recent trends in extraction techniques of anthocyanins from plant materials. *Journal of Food Measurement and Characterization*, *14*, 3508-3519.

Freitas, C. M. P., Sousa, R. C. S., Dias, M. M. S., & Coimbra, J. S. R. (2020). Extraction of Pectin from Passion Fruit Peel. *Food Engineering Reviews*, *12*, 460–472.

Freshney, R. I. (2015). *Culture of animal cells: a manual of basic technique and specialized applications*. John Wiley & Sons.

Herrera-Ramirez, J., Meneses-Marentes, N., & Tarazona Díaz, M. P. (2020). Optimizing the extraction of anthocyanins from purple passion fruit peel using response surface methodology. *Food Measure*, *14*, 185–193.

Hawthorne, S. B., Grabanski, C. B., Martin, E., & Miller, D. J. (2000). Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix. *Journal of Chromatography A*, *892*(1-2), 421-433.

Hosseini, S. S., Khodaeian, F., & Yarmand, M. S. (2016). Effect of acid extraction conditions on yield and quality characteristics of pectin from sour orange peel. *Iranian Journal of Biosystems Engineering*, *47*(2), 231-242.

Lee, J., Durst, R., & Wrolstad, R. (2005). AOAC official method 2005.02: total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method. *Official methods of analysis of AOAC International*, *2*.

Liang, Y., Yang, Y., Zheng, L., Zheng, X., Xiao, D., Wang, S., Ai B., & Sheng, Z. (2022). Extraction of Pectin from Passion Fruit Peel: Composition, Structural Characterization and Emulsion Stability. *Foods*, *11*(24), 3995.

Liew, S., Chin, N., & Yusof, Y. (2018). Extraction and characterization of pectin from passion fruit peels. *Agriculture and Agricultural Science Procedia*, *2*, 231–236.

Liu, M., Su, Y. J., Lin, Y. L., Wang, Z. W., Gao, H. M., Li, F., Wei, X. Y., & Jiang, H. L. (2018). Optimization of green extraction of anthocyanins from purple passion fruit peels by response surface

methodology. *Journal of Food Processing and Preservation*, *42*(10), e13756.

Liu, Y., Liu, Y., Tao, C., Liu, M., Pan, Y., & Lv, Z. (2018). Effect of temperature and pH on stability of anthocyanin obtained from blueberry. *Journal of Food Measurement and Characterization*, *12*, 1744-1753.

Mada, T., Duraisamy, R., & Guesh, F. (2022). Optimization and characterization of pectin extracted from banana and papaya mixed peels using response surface methodology. *Food Science & Nutrition*, *10*(4),1222-1238.

Marxen, K., Vanselow, K. H., Lippemeier, S., Hintze, R., Ruser, A., & Hansen, U.-P. (2007). Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, *7*(10), 2080-2095.

Matheri, F., Mwangi, M., Runo, S., Ngugi, M., Kirubi, D., Teya, F., Mawia, A., Kioko, F., & Kamau, D. (2016). Phenotypic characterization of selected kenyan purple and yellow passion fruit genotypes based on morpho-agronomic descriptors. *Adv Crop Sci Tech*, *4*(226), 2.

Mui, N. V. (2001). *Biochemical Practice*. National University Hanoi Publisher.

Oancea, S. (2021). A review of the current knowledge of thermal stability of anthocyanins and approaches to their stabilization to heat. *Antioxidants*, *10*, 1337.

Oliveira Chaves, J., Corrêa de Souza, M., Capelasso da Silva, L., Lachos-Perez, D., Torres-Mayanga, P. C., da Fonseca Machado, A. P., Forster-Carneiro, T., Vázquez Espinosa, M., Velasco González de Peredo, A., & Fernández Barbero, G. (2020). Extraction of Flavonoids From Natural Sources Using Modern Techniques. *Frontiers in Chemistry*, *8*, 507887.

Phamiwon, Z., & John, S. (2017). Phytochemical investigation and antioxidant activities of Passiflora edulis (passion fruit) leaves from the Ukhrul district, Manipur, India. *World Journal of Pharmaceutical Research*, *6*(14), 793-801.

Saini, R. K., Khan, M. I., Shang, X., Kumar, V., Kumari, V., Kesarwani, A., & Ko, E.-Y. (2024). Dietary Sources, Stabilization, Health Benefits, and Industrial Application of Anthocyanins—A Review. *Foods*, *13*(8), 1227.

Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofziger, T. H., Currens, M. J., Seniff, D., & Boyd, M. R. (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer research*, *48*(17), 4827-4833.

Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S., Xue, H. (2022). Extraction and purification of anthocyanins: A review. *Journal of Agriculture and Food Research*, 8, 100306.

Teng, Z., Jiang, X, He, F., & Bai, W. (2020). Qualitative and quantitative methods to evaluate anthocyanins. *eFood*, *1*(5), 339 - 346.

Tena, N., & Asuero, A. G. (2022). Antioxidant capacity of anthocyanins and other vegetal pigments: modern assisted extraction methods and analysis. *Antioxidants*, *11*(7), 1256.

Thokchom, R., & Mandal, G. (2017). Production preference and importance of passion fruit (Passiflora edulis): A review. *Journal of Agricultural Engineering and Food Technology*, *4*(1), 27-30.

Thomas, B., Vithiya, B., Prasad, T., Mohamed, S., Magdalane, C. M., Kaviyarasu, K., & Maaza, M. (2019). Antioxidant and photocatalytic activity of aqueous leaf extract mediated green synthesis of silver nanoparticles using Passiflora edulis f. flavicarpa. *Journal of nanoscience and nanotechnology*, *19*(5), 2640-2648.

Viera, W., Brito, B., Zambrano, E., Ron, L., Merino, J., Campaña, D., & Álvarez, H. (2020). Genotype x environment interaction in the yield and fruit quality of passion fruit germplasm grown in the Ecuadorian Littoral. *International journal of fruit science*, *20*(S3) S1829-S1844.

Wen, L., Mao, H., Zhang, Y., & Li, Y. (2008). Compositions and antioxidant activity of Passiflora edulis rind. *Food Science*, *29*, 54-58.

Xu, F., Zhang, S., Waterhouse, G.I.N., Zhou, T., Du, Y., Sun-Waterhouse, D., & Wu, P. (2022). Yeast fermentation of apple and grape pomaces affects subsequent aqueous pectin extraction: Composition, structure, functional and antioxidant properties of pectins. *Food Hydrocoll*, *133*, 107945.

Yan, S., Li, Y., Liu, J., Si, D., & Zhang, X. (2023). Guideline for extraction, qualitative, quantitative, and stability analysis of anthocyanins. *eFood*, *4*(1), e59.

Yusaf, T. (2015). Evaluating the effect of heat transfer on cell disruption in ultrasound processes. *Annals of microbiology*, *65*, 1447-1456.