

FUNCTIONAL GROUP IDENTIFICATION USING FTIR AND ANTHOCYANIN PROFILING BY HPLC IN PURPLE SWEET POTATO (*IPOMOEA BATATAS* L.)

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ABSTRACT

Background. Purple sweet potato (PSP) has attracted increasing scientific and industrial interest due to its distinctive purple flesh, which is primarily attributed to anthocyanins. Although PSP is produced in large quantities in Vietnam, comprehensive information on its functional groups and anthocyanin pigment composition remains limited. Therefore, characterization of functional groups and identification of anthocyanin pigments are essential to support both scientific research and industrial applications of this raw material.

Materials and methods. PSP tubers were collected from farms in Vinh Long Province, Vietnam. The tubers were washed, peeled, sliced, and steam-blanching at 100°C for 3 min, dried at 50°C for 8 h, and milled into powder. Functional groups were characterized using Fourier transform infrared (FTIR) spectroscopy. Anthocyanins were extracted with acidified methanol and analyzed using high-performance liquid chromatography (HPLC) with external standards for pigment identification and quantification.

Results. FTIR analysis revealed 14 characteristic absorption bands in PSP grown in Vietnam within the wavenumber range of 3288.63–418.55 cm⁻¹. These bands corresponded to hydroxyl, C–H/O–H/N–H, aromatic (C=O and C=C), ester C–O–C, glycosidic, and pyran ring functional groups, consistent with structural features typical of anthocyanin compounds. HPLC analysis detected three main anthocyanin pigments with retention times of 7.85, 8.77, and 9.93 min, identified as delphinidin, cyanidin, and peonidin, respectively. Their concentrations were 4.034 µg/g (2.2%), 28.490 µg/g (15.5%), and 151.206 µg/g (82.3%).

Conclusion. These results indicate that PSP is a promising source of anthocyanins and has strong potential for use as a functional food with health-promoting properties.

Keywords: anthocyanin pigments; Fourier transform infrared; functional groups; high-performance liquid chromatography (HPLC); purple sweet potato

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INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) has emerged as an important agricultural crop and is widely recognized as a nutritious food due to its high content of bioactive compounds, including β -carotene, anthocyanins, vitamins B1, B2, C, and E, as well as essential minerals such as Ca, Mg, K, and Zn (Liu et al., 2017). In recent years, sweet potato varieties with thick skins and purple flesh have garnered increasing interest from both industry and the scientific community due to their distinctive chemical composition. The purple coloration of sweet potato flesh has been conclusively attributed to the presence of anthocyanins, which are secondary metabolites responsible for color variations ranging from red to bluish-purple. Purple sweet potatoes (PSP) are particularly rich in anthocyanins, mainly composed of cyanidin- and peonidin-based compounds in monoacylated and diacetylated forms. Cyanidin, a major anthocyanin in PSP, has been reported to exhibit antimutagenic and anti-cancer activities, as well as antioxidant and anti-inflammatory properties. It has been shown to help prevent colon cancer (Lim et al., 2013) and alleviate conditions such as obesity, hyperglycemia, and oxidative stress (Zhang et al., 2009). Consequently, PSP has been recommended as a functional food and a potential pharmaceutical ingredient for improving health and maintaining normal physiological processes (Chandrasekara and Josheph Kumar, 2016).

Fourier transform infrared (FTIR) spectroscopy is one of the most widely used analytical techniques for molecular characterization due to its relatively low cost, ease of operation, and high reliability (Ricci et al., 2015). FTIR analysis is based on the absorption of infrared radiation by molecular bonds at characteristic wavenumbers corresponding to specific functional groups present in plant compounds (Lei and Sun, 2019). In addition, FTIR spectroscopy provides rapid, non-destructive analysis with minimal sample preparation, making it highly suitable for routine screening and quality control in the food industry. When combined with chemometric tools, FTIR has been extensively applied for authentication, adulteration detection, and compositional analysis of bioactive compounds (Rodríguez-Saona and Allendorf, 2011). The anthocyanin pigment profiles of PSP cultivated in China, Peru, and Korea have been reported by several researchers (Lim

et al., 2013; Li et al., 2019; Luo et al., 2018). Xu et al. (2024) identified at least 13 anthocyanin pigments in Chinese PSP using HPLC analysis, while Lee et al. (2013) reported 26 anthocyanins in Korean PSP. Similarly, Jesus et al. (2020) isolated a total of 16 anthocyanins from Brazilian PSP. However, despite the large-scale cultivation of PSP in Vietnam, comprehensive studies on its functional groups and anthocyanin pigment composition remain limited. Therefore, the application of FTIR spectroscopy combined with anthocyanin pigment identification is essential to enhance scientific understanding and support the industrial utilization of PSP-based materials and products. The findings of this study are expected to further demonstrate the potential of PSP as a functional food ingredient.

MATERIAL AND METHODS

Material

Purple sweet potato (PSP) tubers were harvested from farms in Vinh Long Province, Vietnam. The tubers exhibited an average length of 12.95 ± 3.02 cm, a width of 7.54 ± 1.05 cm, and a weight of 287.8 ± 39.06 g. In addition, the hardness of the PSP tubers was measured as 97.00 ± 3.16 N.

Methods

Anthocyanin identification

Preparation of purple sweet potato powder

PSP tubers were sorted, washed with tap water, and drained. The tubers were peeled and sliced using a mandoline slicer (Zhejiang, China) to a thickness of approximately 2 mm. The slices were steam-blanching at 100°C for 3 min to prevent enzymatic browning. The blanched samples were dried in a drying oven (MEMMERT, UN260, Germany) at 50°C for 8 h to a moisture content of 6–7%. The dried slices were ground, sieved, and collected to obtain a particle size of 100 μ m. The resulting powder was packed in light-proof bags and stored at room temperature ($25 \pm 2^\circ\text{C}$) for FTIR and HPLC analysis.

Anthocyanin extraction

A 0.1 g sample of PSP powder was placed in a tube containing 4 mL of 50% aqueous methanol acidified with 2 M HCl. The mixture was sonicated for 20 min, followed by extraction in a water bath at 100°C and

120 rpm for 1 h. The extract was cooled in an ice bath for 15 min, vortex-mixed, and a 2 mL aliquot was transferred to a microcentrifuge tube. The sample was centrifuged at $10,000 \times g$ and 4°C for 8 min, and the resulting supernatant was filtered through a PTFE membrane filter (13 mm, $0.2 \mu\text{m}$; Advantec, USA) to obtain the final sample for analysis.

HPLC analysis

Anthocyanin content was quantified using a liquid chromatography system (UltiMate 3000 RS; Ulti-Mate, USA) equipped with a Waters XTerra C18 column ($5 \mu\text{m}$, $3.9 \times 150 \text{ mm}$, USA). The mobile phase consisted of 0.5% trifluoroacetic acid in water (A) and 100% acetonitrile (B). The gradient elution program was as follows: 0–2 minutes, 14% B; 2–4 minutes, 17% B; 4–7 minutes, 28% B; 7–10 minutes, 36% B; 10–12 minutes, 60% B; 12–15 minutes, 0% B. The flow rate was maintained at 0.6 mL/min , and detection was performed at 520 nm . Cyanidin, peonidin, pelargonidin chloride, malvidin chloride, and delphinidin

chloride (Extrasynthese Co., USA) were used as external standards. Anthocyanidin concentrations were calculated based on peak areas relative to calibration curves (0–60 ppm), with correlation coefficients (r^2) exceeding 0.99.

Fourier transform infrared spectroscopy technology

FTIR spectroscopy was used to analyze the functional groups present in PSP using an IR spectrometer (Shimadzu IR Affinity-1S, Japan).

RESULTS AND DISCUSSION

Determination of functional groups found in PSP powder

FTIR analysis revealed 14 absorption peaks for PSP powder within the wavenumber range of $3288.63\text{--}418.55 \text{ cm}^{-1}$ (Fig. 1). The broad band centered at 3288.63 cm^{-1} was mainly attributed to hydrogen-bonded O–H stretching vibrations. The band at

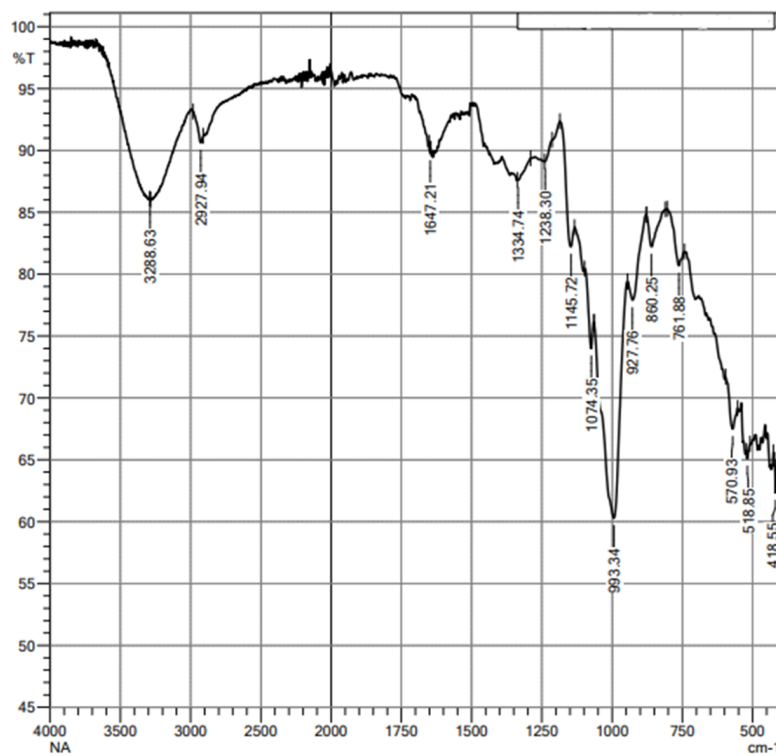


Fig. 1. FTIR spectrum of PSP powder

Table 1. Functional group absorption peaks identified in PSP powder

N _o .	Peak, cm ⁻¹	Intensity	Functional groups	N _o .	Peak, cm ⁻¹	Intensity	Functional groups
1	418.55	62.00	Core-benzene	8	1074.35	74.00	C-O-C stretching (ether or glycosidic bonds)
2	518.85	65.00	Core-benzene	9	1145.72	82.10	C-O-C stretching (ether or glycosidic bonds)
3	570.93	67.40	Core-benzene	10	1238.30	89.00	C-O-C stretching (ether or glycosidic bonds)
4	761.88	81.10	C-H	11	1334.74	87.40	Aromatic
5	860.25	82.00	C-H	12	1647.21	89.50	Aromatic
6	927.76	77.50	C-H	13	2927.94	91.00	Aldehyde (C=O-H)
7	993.34	60.40	C-H	14	3288.63	86.20	Carbon hydroxyl (C-OH)

2927.94 cm⁻¹ corresponded primarily to C–H stretching vibrations, with possible contributions from O–H and N–H groups, which are commonly present in plant matrices containing polysaccharides and proteins. In the fingerprint region, absorption bands associated with carbonyl (C=O), aromatic C=C, and ether or glycosidic (C–O–C) linkages are characteristic of the flavonoid backbone and glycosylated structures of anthocyanins, indicating the presence of anthocyanin-related functional groups within the PSP matrix (Bhushan et al., 2023).

The most diagnostic absorption bands distinguishing different ionic forms of anthocyanins were observed between 1700 cm⁻¹ and 1000 cm⁻¹. Infrared spectra arise from stretching and bending vibrations of molecular bonds, where stronger bonds and lighter atoms vibrate at higher frequencies (Bhushan et al., 2023). The absorption bands at 1145.72 cm⁻¹ and 1074.35 cm⁻¹ were attributed to C–O–C stretching vibrations of esters or glycosidic bonds, while the band at 1238.30 cm⁻¹ corresponded to pyran ring stretching, a feature characteristic of flavonoid compounds. The absorption bands at 1334.74 cm⁻¹ and 1647.21 cm⁻¹ were associated with cyanidin-related ring stretching and vibrational dispersion.

The presence of hydrocarbon (C–H) bonds and benzene ring vibrations at absorption wavelengths of 418.55, 518.85, 570.93, 761.88, 860.25, 927.76, 993.34 cm⁻¹, supported by aromatic groups absorptions at 1074.35 and 1145.72 cm⁻¹, indicated the presence of malvidin glucoside and cyanidin glycoside groups (Saati et al., 2021). As summarized in Table 1, the functional groups identified included core-benzene,

C–H (occurring in greater number than other functional groups), C–O–C stretching (ether or glycosidic bonds), aromatic structures, aldehyde (C=O–H), and carbon hydroxyl (C–OH) groups.

HPLC analysis of anthocyanin pigments in PSP

Anthocyanin pigments in PSP were detected and quantified using HPLC (Fig. 2). The anthocyanins were eluted within a retention time (RT) range of 1–15 min (Fig. 2b). A total of 17 major peaks exhibiting characteristic anthocyanin absorption at 520 nm were monitored. The chromatographic properties and relative peak areas of the detected compounds are presented in Table 2. Anthocyanin identification was based on retention time relative to that of the external standard. Peak n_o. 6 exhibited a retention time of 7.85 min corresponding to delphinidin (7.853 min), peak n_o. 9 showed a retention time of 8.77 min, matching cyanidin (8.773), while peak n_o. 12 displayed a retention time of 9.93 min, corresponding to peonidin (9.933). These three compounds represented the major anthocyanins detected, with peak area percentages of 1.45%, 14.03%, and 63.72%, respectively. Although several anthocyanins were detected, delphinidin glucoside (peak 6), cyanidin glucoside (peak 9), and peonidin glucoside (peak 12) were predominant in PSP.

Purple sweet potatoes are commonly classified as cyanidin-rich or peonidin-rich based on the cyanidin-to-peonidin ratio, which influences flesh color and potentially functional properties. Sweet potatoes with higher peonidin content typically exhibit light red to red flesh, whereas increasing cyanidin content results in purple to deep-purple coloration. Structurally,

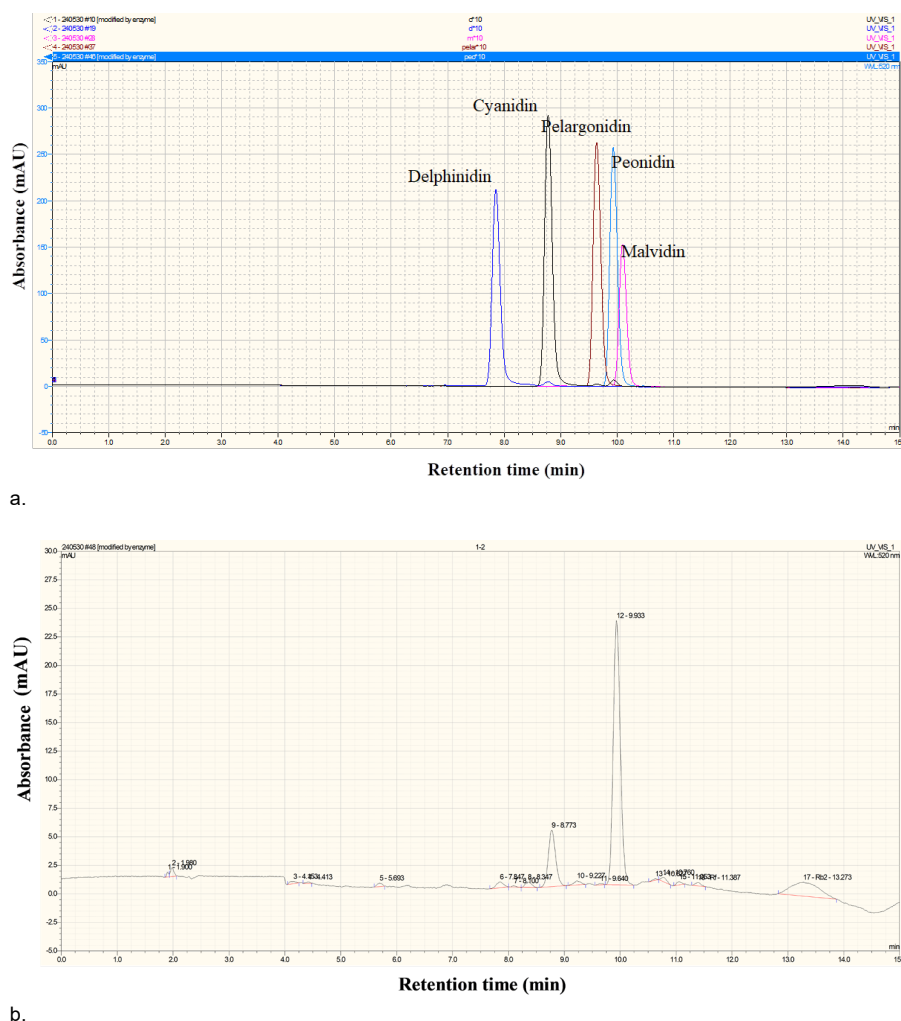


Fig. 2. Chromatograms of standard anthocyanin pigments (a) and HPLC profile of anthocyanins extracted from PSP (b) detected at 520 nm

cyanidin exhibits stronger antioxidant activity than peonidin due to its higher number of hydroxyl groups (Xu et al., 2015). Previous studies have also reported peonidin as the dominant anthocyanin in PSP (Truong et al., 2010; Lim et al., 2013; Li et al., 2019; Xu et al., 2024)

Xu et al. (2024) reported that the major anthocyanins in PSP consisted primarily of cyanidin and peonidin glycosides. Li et al. (2019) similarly suggested that PSP pigments comprise multiple anthocyanin compounds mainly based on cyanidin and peonidin in monoacylated and diacetylated forms. In contrast, Luo et al. (2018) identified peonidin 3-caffeoyl-feruloyl

sophoroside-5-glucoside and peonidin 3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside as the major anthocyanins. Truong et al. (2010) identified 17 anthocyanins in the ‘Stokes Purple’ and ‘NC 415’ cultivars using HPLC-DAD/ESI-MS/MS, with five predominant compounds: cyanidin 3-caffeoylsophoroside-5-glucoside, peonidin 3-caffeoylsophoroside-5-glucoside, cyanidin 3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside, peonidin 3-caffeoyl-p-hydroxybenzoyl-sophoroside-5-glucoside, and peonidin-caffeoyl-feruloylsophoroside-5-glucoside.

The chemical structures and molar concentrations of the three major anthocyanins identified in this

Table 2. HPLC analysis of anthocyanins in PSP extract

Peak label	Retention time, min	Peak area, %	Peak label	Retention time, min	Peak area, %
1	1.90	0.34	10	9.23	0.95
2	1.98	1.00	11	9.64	0.23
3	4.15	0.50	12	9.93	63.72
4	4.41	0.20	13	10.63	0.23
5	5.69	0.62	14	10.76	0.82
6	7.85	1.45	15	11.05	0.53
7	8.10	0.16	16	11.39	0.66
8	8.35	1.16	17	13.27	13.38
9	8.77	14.03			

study are presented in Table 3. Cyanidin (magenta and crimson), peonidin (magenta), and delphinidin (purple, mauve, and blue) contribute significantly to the coloration of PSP cultivated in Vietnam. These anthocyanins differ in the position and degree of methylation of hydroxyl groups. Peonidin glucoside was the predominant anthocyanin, with a concentration of 151.206 µg/g, accounting for 82.3% of the total anthocyanin content. Cyanidin glucoside was the second most abundant (28.490 µg/g; 15.5%), followed by delphinidin glucoside (4.034 µg/g; 2.2%). As the number and length of conjugated double bonds in anthocyanin structures increase, color intensity also increases (Hellmann et al., 2021).

Table 3. Chemical structure and molar concentration of major anthocyanins identified in PSP

Anthocyanin	Cyanidin	Delphinidin	Peonidin	Total
R ₁	OH	OH	OCH ₃	
R ₂	OH	OH	OH	
R ₃	H	OH	H	
Molar concentration, µg/g	28.490	4.034	151.206	183.730
Ratio, %	15.5	2.2	82.3	100

CONCLUSIONS

This study comprehensively characterized anthocyanin pigments in purple sweet potato (PSP) grown in Vinh Long, Vietnam, highlighting their potential for rational utilization and processing. FTIR analysis revealed 14 broad spectral peaks, confirming the presence of characteristic functional groups associated with anthocyanin compounds. HPLC analysis identified cyanidin, peonidin, and delphinidin as the major anthocyanins present in PSP. Owing to its intense natural pigmentation and plant-based origin, PSP demonstrates strong potential as a natural food colorant for applications in bakery products, beverages, and confectionery. Beyond color enhancement, PSP may serve as a source of bioactive compounds with potential health benefits, supporting its development as a functional food ingredient. In the future, sweet potato cultivation in Vietnam is expected to expand to meet the growing demand for functional food production from this important agricultural crop.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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