



Comparative anthocyanin profiles and anti-inflammatory activities in strawberry cultivars (*Fragaria x ananassa* Duch)

Hong Suk Lee¹ · Joong-Hyuck Auh¹ 

Received: 22 October 2025 / Revised: 15 January 2026 / Accepted: 19 January 2026
© The Author(s) 2026

Abstract

This study compared and evaluated the metabolic profiles and anti-inflammatory activities of strawberry cultivars (*Fragaria x ananassa* Duch.) Janghee, Jukhyang, Seolhyang, Maehyang, and Merryqueen. Among the five cultivars, the anthocyanin-rich fractions of Jukhyang and Merryqueen contained higher total anthocyanin content than the other cultivars. Multivariate statistical analysis was performed on liquid chromatography-tandem mass spectrometry (LC–MS/MS) data from the metabolite profiling of the five varieties, identifying a total of 1075 metabolites. The hierarchical cluster analysis grouped them into CON (Janghee), Group 1 (Jukhyang and Seolhyang), and Group 2 (Maehyang and Merryqueen). Pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, which differed significantly among the groups, were quantified as signature metabolites. Anti-inflammatory activity using RAW 264.7 cells showed that Jukhyang exhibited the highest inhibitory effect on nitric oxide and inducible nitric oxide synthase expression. This study highlights differences in major metabolites among purified strawberry varieties and comparatively evaluated their distinct anti-inflammatory activities.

Keywords Strawberry · Anthocyanin-rich fraction · Metabolomics · Anti-inflammatory · RAW 264.7 cell

Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a globally popular crop valued for its sensory qualities (color, taste, and size) and nutritional content, particularly its high vitamin C and polyphenol levels (Urün et al., 2021). Recently, consumer interest in the health benefits of bioactive compounds in food products has increased. Among these, polyphenols in strawberries are particularly notable, with their bioactivities varying as secondary metabolites that influence both the sensory and nutritional qualities of the fruit. Key secondary metabolites in strawberries include ascorbic acid, anthocyanins, ellagic acid, and catechins, which are considered the primary bioactive compounds contributing to their health benefits (Reber et al., 2011).

Anthocyanins belong to the flavonoid group and are the bioactive compounds responsible for the color and health

benefits of strawberries. The health benefits of anthocyanins are attributed to their diverse bioactive properties, such as antioxidants (Elisia et al., 2007) and anti-inflammatory activities (Li et al., 2014). Anthocyanins in strawberries comprise approximately 90% pelargonidin-3-*O*-glucoside and 10% cyanidin-3-*O*-glucoside (Goiffon et al., 1999; Siri-jan et al., 2020). However, previous studies have shown variability in the contents of these major anthocyanin metabolites among different strawberry cultivars (Aaby et al., 2012; Min et al., 2020; Ristivojević et al., 2022). While breeding programs for strawberries have traditionally focused on agricultural value and sensory traits, consumer interest in cultivars rich in bioactive compounds, including anthocyanins, is increasing (Kim et al., 2015).

Macrophages play a protective role by recognizing and regulating inflammatory responses (Korns et al., 2011). Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, induces inflammation by increasing the production of cytokines (e.g., tumor necrosis factor- α (TNF- α), interleukin-1 α (IL-1 α)) and pro-inflammatory mediators (e.g., nitric oxide (NO), prostaglandin E₂ (PGE₂), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2)) within the immune system (Ren et al., 2020). Therefore, the inhibition of the

✉ Joong-Hyuck Auh
jhauh@cau.ac.kr

Hong Suk Lee
hongsuk2145@naver.com

¹ Department of Food Science and Biotechnology, Chung-Ang University, Anseong 17546, South Korea

LPS-activated inflammatory response is crucial for preventing inflammatory diseases (Zhou et al., 2017). Anthocyanins in strawberries demonstrated anti-inflammatory activity in LPS-induced RAW 264.7 macrophage cells (Duarte et al., 2018). However, most studies have focused on anthocyanins derived from a single cultivar, and differences in anthocyanin content among strawberry cultivars may result in varying levels of anti-inflammatory activity.

Metabolomic studies of various bioactive metabolites in strawberries have been conducted to evaluate their physiological activities. Liquid chromatography-mass spectrometry (LC-MS)-based metabolomics has successfully identified metabolite differences among various berry cultivars, including non-targeted metabolomic studies on variation of phenolics in cranberry *bokbunja* (Lee et al., 2013), (Wang et al., 2018), and strawberry (Olennikov et al., 2022), and has proven useful for analyzing strawberry metabolites. However, bioactivity evaluations may involve multiple secondary metabolites, not just anthocyanins. Additionally, comparative studies specifically focusing on anthocyanins, which are the primary metabolites in strawberries, across cultivars and studies comparing the anti-inflammatory activities of different cultivars are scarce.

In this study, anthocyanin-rich fractions were prepared by efficiently isolating and purifying the anthocyanin components from five strawberry cultivars commonly consumed in South Korea. These fractions were analyzed using LC-MS to identify the differences in anthocyanin metabolites among the cultivars, and metabolite profiling was performed using multivariate statistical analysis. Finally, the anti-inflammatory activity of the anthocyanin-rich fractions was evaluated in LPS-induced RAW 264.7 cells. This comparative analysis of the anthocyanin content in various strawberry cultivars provides valuable information for consumers, processors, and breeders.

Materials and methods

Chemicals and reagents

Janghee (JA), Jukhyang (JH), Seolhyang (SH), Maehyang (MH), and Merryqueen (MQ) strawberries were purchased from a local market in South Korea. The fresh fruits of these five strawberry cultivars were freeze-dried and stored in a deep freezer at -80°C until further analysis. Detailed information on the strawberry cultivars is provided in Table 1. Amberlite XAD-7HP, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), and LPSs from *Escherichia coli* were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-glucose Dulbecco's modified Eagle's medium (DMEM) and Dulbecco's phosphate-buffered saline (DPBS) were obtained from Biowest (Nuaille, France). Penicillin-streptomycin (P/S), fetal bovine serum (FBS), and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) were purchased from Gibco (Grand Island, NY, USA). Ethyl acetate was purchased from Samchun Chemical Co. (Pyeongtaek, Korea). Methanol was purchased from Daejung Chemicals (Seoul, South Korea). Formic acid, MS-grade water, and acetonitrile were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside were purchased from ChemFaces (Wuhan, China).

Isolation and purification of the anthocyanin-rich fraction

Strawberries were freeze-dried and milled before use, and each strawberry powder sample (5 g) was extracted with 100 mL of 80% acidic methanol (0.1% HCl) by bath sonication (Branson Ultrasonics, 3150 R-DTH sonicator, Branson, MO, USA) for 30 min. The extract was centrifuged at $5000\times g$ for 10 min, and the supernatant was filtered using Whatman No. 1 qualitative filter paper (GE Healthcare Life Sciences, Little Chalfont, UK). The filtrate was concentrated to 36°C

Table 1 Information and total anthocyanin content of strawberry cultivars

Commercial name	Parentage	Origin	Abbr	Total anthocyanin content (mg Cyanidin-3- <i>O</i> -glucoside/g)
Janghee (Akihime)	Kunowase x Nyoho	Japan (1992)	JA	55.77 ± 0.17^d
Jukhyang	Red Pearl x Maehyang	Korea (2012)	JH	73.37 ± 0.10^a
Seolhyang	Akihime x Red Pearl	Korea (2005)	SH	49.09 ± 0.00^e
Maehyang	Tochinomine x Akihime	Korea (2001)	MH	64.68 ± 0.19^c
Merryqueen	Seolhyang x Maehyang	Korea (2017)	MQ	72.42 ± 0.25^b

Results were obtained in triplicate ($n=3$) and expressed as mean $\pm$ standard deviation *SD*. Different letters indicate significant differences ($p < 0.05$)

using a rotary vacuum evaporator (Eyela, Tokyo, Japan). The concentrated sample was reconstituted with water, and the reconstituted strawberry concentrate was subjected to three sequential partitioning steps using ethyl acetate to prepare the aqueous phase. The aqueous phase was loaded onto a glass column tube (2.2 cm × 30 cm) packed with an ion-exchange resin (Amberlite XAD-7HP; particle size, 20–60 mesh). The column was washed with 1 L of distilled water to remove sugars and organic acids. The anthocyanins were eluted using 300 mL of methanol (0.1% HCl). The eluted anthocyanins were concentrated at 36 °C using a rotary vacuum evaporator (Eyela, Tokyo, Japan) and freeze-dried to obtain anthocyanin-rich fractions from the five strawberry varieties. The extracts were stored in a freezer at −50 °C until use.

Total anthocyanin content (TAC)

TAC was determined using the pH differential method. The anthocyanin-rich fraction (1 mg/mL) dissolved in DMSO was further diluted in 25 mM potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). The samples were then incubated at 25 °C for 30 min. After the reaction, the absorbance of each sample was measured at 520 nm and 700 nm, and the content was determined as follows:

$$\text{Total anthocyanin content} \left(\text{mg} \frac{\text{C3OG}}{\text{g}}, \text{cyanidin-3-O-glucoside equivalent} \right) = \frac{A \times MW \times DF \times V \times 1000}{\epsilon} \times L$$

where $A = [(A_{520\text{nm}} - A_{700\text{nm}})]_{(\text{pH } 1.0)} - [(A_{520\text{nm}} - A_{700\text{nm}})]_{(\text{pH } 4.5)}$, MW is the 449.2 g/mol molecular weight of cyanidin-3-O-glucoside, DF is the dilution factor of solution, V is the volume of extracting solution, 1000 is factor for conversion from g to mg, ϵ is the 26,900 L/mol/cm (extinction coefficient for cyaniding-3-O-glucoside) and L is path length.

LC-MS/MS analysis

The anthocyanin-rich fractions from the five strawberry cultivars were analyzed using a Vanquish UHPLC system coupled with a Q Exactive Orbitrap mass spectrometer equipped with a DAD/multiwavelength detector (VF-D11-A, Thermo Fisher Scientific) and a heated electrospray ionization (HESI) interface. Chromatographic separation was performed using a Thermo Hypersil Gold AQ C18 column (100 × 2.1 mm, 1.9 μm). The mobile phase comprised 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). A gradient program was applied as follows: 5% B for 0–4 min, 5–40% B for 4–30 min, 40–100% B for 30–31 min, 100% B for 31–31.5 min, 100–5% B for 31.5–32 min, and 5% B for 32–35 min

to re-equilibrate the system. The flow rate was set at 0.3 mL/min, the injection volume was 5 μL , and the column oven temperature was maintained at 36 °C. A full MS scan was performed in positive ion mode with a scan range of 100–1500 m/z and spray voltage of 3.80 kV.

LC-MS/MS data processing

The LC-MS/MS data were processed using Compound Discoverer 3.3 software (Thermo Fisher Scientific). The analysis parameters were set as follows: precursor mass range, 100–1500 Da; retention time range, 0–31 min; minimum peak intensity, 1×10^7 ; and alignment mass tolerance, 5 ppm. All raw data were filtered based on spectral properties using a signal-to-noise ratio threshold of > 3, followed by feature grouping parameters set to a retention time tolerance of ≤ 0.2 min. Metabolite annotation was performed against public spectral databases including MoNA (<https://mona.fiehnlab.ucdavis.edu/>), MassBank (www.massbank.jp), and PubChem (<https://pubchem.ncbi.nlm.nih.gov>). Identification confidence followed Metabolomics Standards Initiative (MSI) standards (Level 1: pelargonidin-3-O-glucoside and cyanidin-3-O-glucoside confirmed with authentic standards from ChemFaces; Level

2: all other Table 2 metabolites via spectral matching). Heatmaps for visual comparison of sample differences were generated using Compound Discoverer 3.3.

Multivariate statistical analyses were conducted using the SIMCA software (version 18.0; Umetrics, Umeå, Sweden). Principal component analysis (PCA) was conducted to evaluate the differences between groups, and hierarchical cluster analysis (HCA) was performed to assess sample similarity. Differences between groups were evaluated by constructing an orthogonal partial least squares discriminant analysis (OPLS-DA) model. Metabolites contributing to group discrimination were identified based on variable importance in projection (VIP) values ≥ 1.5 and $p < 0.01$.

Quantification

A parallel reaction monitoring scan was performed under the same conditions as for the LC-MS/MS analysis to quantify the primary metabolites. The anthocyanin-rich fractions of the five strawberry cultivars were scanned at m/z 433.1132 and 449.1078, followed by MS² to quantify pelargonidin-3-O-glucoside and cyanidin-3-O-glucoside, respectively.

Table 2 Tentative identification of metabolites in each group

Groups	<i>m/z</i>	RT (min)	Tentative identification	MS/MS fragments	Subclass	VIP values	Error (ppm)	References
1	433.1132	8.93	Pelargonidin-3- <i>O</i> -glucoside	271.0598, 272.0632, 121.0283	Anthocyanin	15.92	0.64	MoNA*
	475.1236	10.72	Pelargonidin 3-(6''-acetylglucoside)	271.0598, 272.0632, 121.0284	Anthocyanin	3.45	0.23	(Aaby et al., 2012; Talpur et al., 2017)
2	449.1078	8.66	Cyanidin-3- <i>O</i> -glucoside	287.0548, 288.0582, 137.0235	Anthocyanin	7.45	-0.75	MoNA*
	535.1082	9.74	Cyanidin-3- <i>O</i> -malonylglucoside	287.0547, 288.0581, 137.0232	Anthocyanin	5.39	-0.04	(Aaby et al., 2012; Razgonova et al., 2025)

Results were obtained in six replicates and expressed as mean $\pm$ standard deviation *SD*; Different letters denote significant differences ($p < 0.01$). *RT* retention time

*MoNA (Massbank of North America): <https://mona.fiehnlab.ucdavis.edu/>

Cell culture

RAW 264.7, a macrophage cell line, was obtained from the Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin–streptomycin, and 1% HEPES in an incubator maintained at 37 °C with 5% CO₂. Subcultures were performed by washing the cells with DPBS, detaching them using a scraper, and seeding 2.5×10^6 cells in 10 cm cell culture dishes. Cells with fewer than 30 passages were used in all the experiments.

Evaluation of cytotoxicity

The cytotoxicity of the anthocyanin-rich fraction against RAW 264.7 cells was determined using the MTT assay. First, 96-well cell culture plates were seeded at a density of 4×10^4 cells per well and incubated for 24 h. The cells were then treated with the anthocyanin-rich fraction diluted to 50 and 100 $\mu\text{g/mL}$ in DMEM and incubated for 20 h. MTT (1 mg/mL, 20 μL) dissolved in DPBS was added to the treated wells and incubated for 4 h. Subsequently, the DMEM in all wells was removed, and 200 μL of DMSO was added to dissolve the formazan crystals. The absorbance of the dissolved formazan crystals was measured at 570 nm using a microplate reader (SpectraMax M2, Molecular Devices, San Jose, CA, USA). The results were compared with those of the control to determine cell viability.

Evaluation of anti-inflammatory activity

Measurement of nitric oxide (NO) production

The NO production level of the anthocyanin-rich fraction for RAW 264.7 cells was determined as previously described (Kim et al., 2019). RAW 264.7 cells were seeded into 96-well cell culture plates and incubated for 24 h. The medium was then removed, and the samples were treated with LPS (100 ng/mL) and incubated for 24 h. The cell culture medium was harvested from each well. Subsequently, 50 μL of harvested medium and 50 μL of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid) were added to a 96-well plate and incubated in the dark for 10 min. Then, 50 μL of NED solution (0.1% *N*-1-naphthyl ethylenediamine dihydrochloride in water, Kanto Chemical, Japan) was added and incubated in the dark for 10 min. After the reaction, the absorbance was measured at 540 nm, and the NO levels of all samples were determined by comparison with that of LPS-induced cells.

Expression level of iNOS

Western blotting was performed using a previously established method (Kim et al., 2019) to determine the expression level of the inflammatory biomarker iNOS. RAW 264.7 cells at a density of 1×10^6 were seeded in 6-cm culture dishes and incubated for 18 h. The medium was then

removed, and all plates except the control were treated with LPS (100 ng/mL). Meanwhile, all plates except the control and the LPS-treated plates were treated with the anthocyanin-rich fraction (100 µg/mL) and incubated for 24 h. At the end of the incubation period, the cells were washed with PBS and harvested using PBS containing 0.1% protease inhibitor. The harvested cells were centrifuged at $13,000 \times g$ for 5 min. After centrifugation, the resulting cell pellet was resuspended in RIPA buffer containing 1% protease inhibitor and incubated at 4 °C for 10 min. After centrifugation at $13,000 \times g$ for 15 min, 40 µg of protein was separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% bovine serum albumin (BSA) solution in Tris-buffered saline with 0.1% Tween 20 (TBST) for 1 h at 25 °C. The primary antibodies (β -actin (Santa Cruz Biotechnology, Dallas, TX, USA) and iNOS (Cell Signaling Technology, Beverly, MA, USA)) were incubated overnight at 4 °C. After incubation, the membranes were incubated with secondary antibodies (anti-mouse IgG, horseradish peroxidase-linked (Cell Signaling Technology) and anti-rabbit IgG, horseradish peroxidase-linked (Cell Signaling Technology)) for 1 h at 25 °C. Protein expression levels were visualized using Amersham ImageQuant 800 (Cytiva, Marlborough, MA, USA) and quantified using ImageQuant TL (ver. 10.2; Cytiva).

Statistical analysis

All experiments were conducted in triplicate ($n=3$ biological replicates), with each replicate derived from independent strawberry plant samples or distinct RAW 264.7 cell culture wells to capture physiological variability; results are presented as mean $\pm$ standard deviation. Statistical analyses were performed using SPSS 12.0 software (Statistical Package for the Social Sciences; IBM SPSS Statistics, Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, USA). One-way analysis of variance was used with Tukey's HSD post hoc test and Kruskal–Wallis test to determine significant differences at $p < 0.05$; spearman correlation analysis was performed between cyanidin-3-*O*-glucoside quantification and iNOS expression.

Results and discussion

Extraction yields and TAC

The extraction yields of the five strawberry cultivars were calculated as mg per g of dry weight. Extraction yields were as follows: JA (5.71 ± 0.61 mg/g), JH (6.3 ± 0.30 mg/g), SH

(5.5 ± 1.02 mg/g), MH (6.8 ± 0.43 mg/g), and MQ (8.0 ± 1.20 mg/g). The TAC of the anthocyanin-rich fractions of the five strawberry cultivars, determined according to the pH differential method, is shown in Table 1. The TAC of the anthocyanin-rich fraction was highest for JH (73.37 mg C3OG/g), followed by MQ (72.42 mg C3OG/g). SH (49.09 mg C3OG/g) had the lowest value among the five varieties.

Anthocyanin pigments in strawberries are primarily responsible for the flesh color and are located on the surface. As a source of anti-inflammatory agents, it is important to identify the different activities of anthocyanins in strawberries according to cultivar. All five strawberry cultivars used in this study are among the most consumed and produced cultivars in Korea, and are suitable for metabolite analysis and comparative evaluation of bioactivity (Lim et al., 2016). In addition to anthocyanins, strawberries contain metabolites that affect their bioactivity, such as ellagic acid, ellagitannins, and quercetin glycosides. These components, in addition to anthocyanins, were detected in crude extracts, making it challenging to compare the bioactivity of anthocyanins in strawberries. Amberlite XAD-7HP showed the highest adsorption capacity during the extraction and purification of anthocyanins from red cabbage in a previous study (Chandrasekhar et al., 2012). It was also successfully used for the purification of anthocyanins from jamun (*Syzygium cumini* L.) in a previous study (Jampani et al., 2014). Isolating and purifying anthocyanins from strawberries using Amberlite XAD-7HP helped separate complex strawberry components, resulting in higher purity and increasing the accuracy of the study.

The TAC results indicated that JA had a higher TAC content than SH, which is consistent with the results of previous studies (Kim and Shin 2015). Differences in TAC content among cultivars have been attributed to variations in the content of major anthocyanin metabolites (Chaves et al., 2017). The significant differences in anthocyanin content among the five cultivars suggested that different metabolomic patterns may exist among the cultivars during the anthocyanin profiling process.

Metabolomic comparison of anthocyanin-rich fractions

The five strawberry cultivars were subjected to metabolomic profiling using ultra-high performance liquid chromatography (UHPLC)-HESI Orbitrap-MS/MS. Multivariate statistical analysis was performed to identify different metabolite patterns among the cultivars. Heatmap analysis of the 1075 metabolites in the total metabolome confirmed that there were differences in the relative metabolite contents between strawberry cultivars (Fig. 1A). PCA scores of the five strawberry cultivars were visualized using principal components 1 (PC1) and

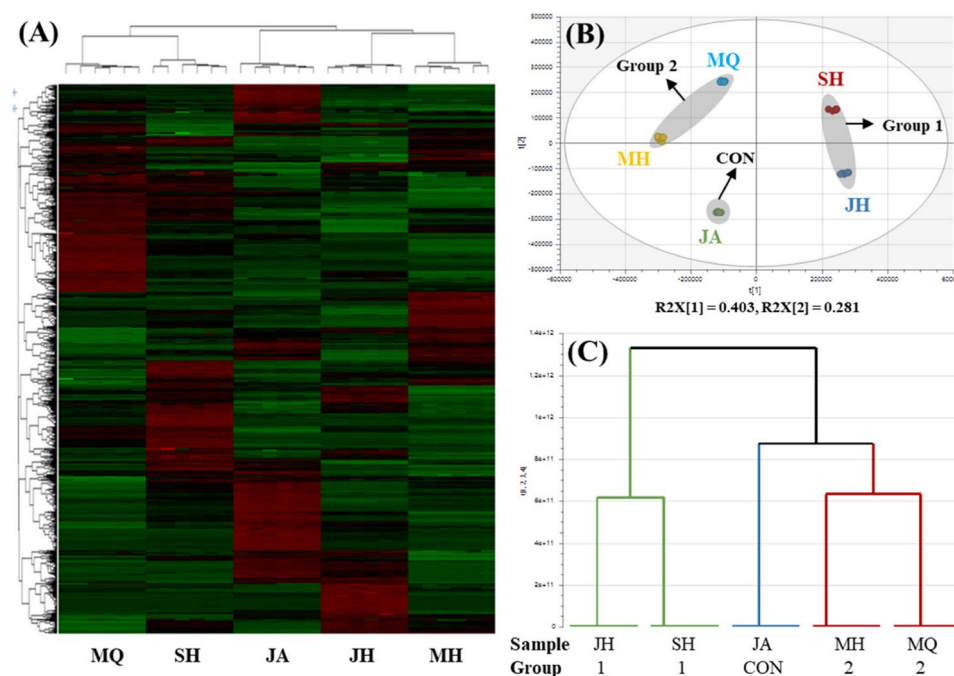


Fig. 1 Multivariate statistical analysis of anthocyanin-rich fractions from five strawberry cultivars. LC–MS profiling was conducted in six biological replicates ($n=6$) to enhance statistical power for PCA and other multivariate analyses, thereby capturing cultivar-specific metabolite variability. **(A)** Heatmap analysis of metabolites in anthocyanin-rich fractions from five cultivars. Each column represents one cultivar, and each row corresponds to one of the 1075 detected

metabolites. Light green indicates relatively low content, while red indicates relatively high content. **(B)** Principal component analysis (PCA) score plot showing separation of JA, MH, and MQ from JH and SH along PC1, which explains 40.3% of the dataset variance. **(C)** Hierarchical cluster analysis (HCA) grouping of cultivars based on similarities in metabolite profiles

2 (PC2), which accounted for 40.3% and 28.1% of the variance, respectively. The five cultivars were divided into three clusters (JA, MQ, and MH; SH and JH) based on PC1 (Fig. 1B). HCA was used to group cultivars with similar metabolite patterns (Fig. 1C). The cultivars were grouped as Control (CON) containing JA, Group 1 containing JH and SH, and Group 2 containing MH and MQ. All other strawberry cultivars were derived from the JA cultivar line, and JA was selected as the control for the subsequent classification of metabolites to identify differences between groups.

We identified the major bioactive compounds in the anthocyanin family, including pelargonidin-3-*O*-glucoside, pelargonidin-3-(6''-acetylglucoside), cyanidin-3-*O*-glucoside and cyanidin-3-*O*-malonylglucoside. However, pelargonidin-3-rutinoside, a major anthocyanin in strawberries identified in previous studies, was not a signature metabolite for any group in this study (da Silva et al., 2007; Xu et al., 2019). The primary reason is that, in this study, pelargonidin-3-rutinoside was not classified as one of the key metabolites contributing to the differences among groups during the anthocyanin profiling process.

Metabolomic profiles in the anthocyanin-rich fraction

The differentially expressed metabolites in each group were tentatively identified (Table 2). The compound differentially present in Group 1 (JH and SH) confirmed the differential presence of pelargonidin-3-*O*-glucoside (m/z 433.1132), and pelargonidin 3-(6''-acetylglucoside) (m/z 475.1236) when compared to the CON group. Group 2 (MH and MQ) showed the differential presence of cyanidin-3-*O*-glucoside (m/z 449.1078), and cyanidin-3-*O*-malonylglucoside (m/z 535.1082) when compared to the CON group.

Quantification of signature metabolites

Pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside, the major anthocyanin metabolites in each group, were quantified using LC–MS/MS. Pelargonidin-3-*O*-glucoside, which was differentially present in Group 1, was quantified at 170.22 mg/g in JH and 170.45 mg/g in SH.

Table 3 Contents of signature metabolites in the anthocyanin-rich fraction

Sample	Pelargonidin-3- <i>O</i> -glucoside (mg/g)	Cyanidin-3- <i>O</i> -glucoside (mg/g)
JA	103.10 ± 0.29 ^c	2.81 ± 0.08 ^e
JH	170.22 ± 1.35 ^a	8.04 ± 0.07 ^c
SH	170.45 ± 1.74 ^a	3.30 ± 0.03 ^d
MH	84.73 ± 1.21 ^d	8.26 ± 0.02 ^b
MQ	124.82 ± 0.79 ^b	9.98 ± 0.08 ^a

Results were obtained in triplicate and expressed as mean ± standard deviation (SD); Different letters denote significant differences ($p < 0.05$)

Cyanidin-3-*O*-glucoside, differentially present in Group 2, was identified as the most abundant compound in MQ (9.98 mg/g), followed by that in MH (8.26 mg/g) (Table 3).

These metabolites have demonstrated anti-inflammatory properties in previous studies and play key roles in inflammatory signaling pathways, particularly in the inhibition of NO production (Bowen-Forbes et al., 2010; Pergola et al., 2006). Cyanidin-3-*O*-glucoside is a common anthocyanin found in fruits and vegetables with purple, red, and blue colorations (Miyazawa et al., 1999). Previous studies have shown that cyanidin-3-*O*-glucoside in fruits of the genus *Rubus* effectively modulates inflammatory responses by inhibiting NO production, iNOS expression, and NF- κ B expression, while increasing I- κ B expression, suggesting anti-inflammatory activity (Jung et al., 2014). Pelargonidin-3-*O*-glucoside, the primary anthocyanin responsible for the red color of strawberries, inhibits the production of NO, TNF- α , and IL-6 in a concentration-dependent manner, blocks inflammatory signaling by inhibiting JNK phosphorylation in the MAPK pathway, and prevents I κ B- α degradation in the NF- κ B pathway (Duarte et al., 2018; Xu et al., 2018). The quantitative values of cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside measured in the experiment showed a much higher pelargonidin-3-*O*-glucoside content than cyanidin-3-*O*-glucoside. These results are consistent with those of a previous study, which reported that the total anthocyanin content of pelargonidin-3-*O*-glucoside in strawberries was higher than that of cyanidin-3-*O*-glucoside (Goiffon et al., 1999).

Cytotoxicity of anthocyanin-rich fraction in RAW 264.7 cells

An MTT assay was performed to evaluate the cytotoxicity of the anthocyanin-rich fractions from different strawberry cultivars. All anthocyanin-rich fractions were tested at concentrations of 50 and 100 μ g/mL. The results indicated that cell viability remained above 80% across all

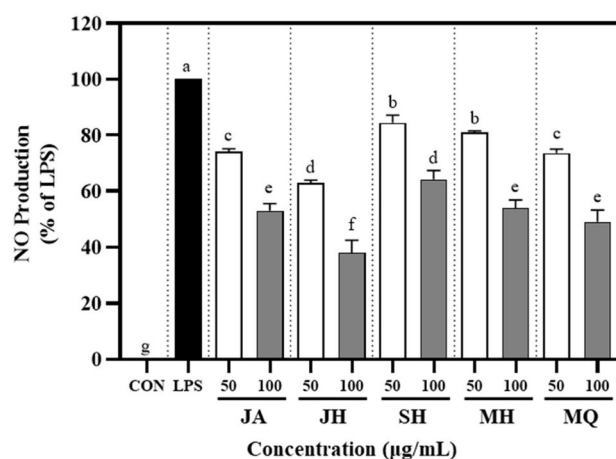


Fig. 2 Effect of anthocyanin-rich fractions on LPS-induced NO production in RAW 264.7 cells. Cells were treated with 50 or 100 μ g/mL of each sample and 100 ng/mL LPS for 24 h. Subsequently, 50 μ L of the harvested cell culture medium was mixed with 50 μ L of sulfanilamide solution (1% sulfanilamide in 5% phosphate buffer) and incubated for 10 min. Subsequently, 50 μ L of NED solution was added, followed by an additional 10 min incubation. Results are expressed as the percentage relative to LPS-stimulated cells. Different letters indicate significant differences ($p < 0.05$) JA Janghee, JH Jukhyang, SH Seolhyang, MH Maehyang, MQ Merryqueen

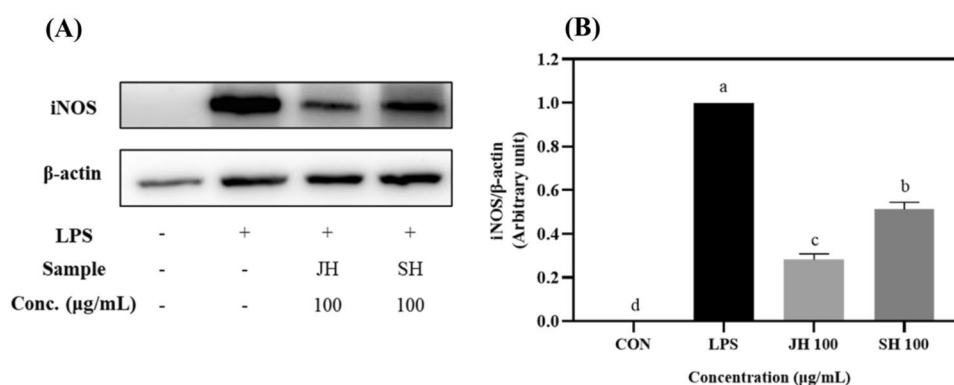
concentrations, although a slight decrease in viability was observed with increasing concentrations. Based on these findings, subsequent experiments were conducted using concentrations up to 100 μ g/mL.

Anti-inflammatory activity of anthocyanin-rich fraction

LPS-induced RAW 264.7 cells were treated with the anthocyanin-rich fractions to evaluate their inhibitory effects on NO production. The anthocyanin-rich fractions were tested at concentrations of 50 and 100 μ g/mL, as determined by the MTT assay. All samples significantly inhibited NO production in a concentration-dependent manner compared to that in LPS-treated cells (Fig. 2). The inhibition rates at the maximum concentration of 100 μ g/mL were as follows: JA (53%), JH (38%), SH (64%), MH (54%), and MQ (49%), with JH demonstrating a stronger inhibitory effect than the other varieties. Based on the NO production results, western blot analysis was performed to assess the ability of JH, which induced the lowest NO production, and SH, which induced the highest NO production, to inhibit iNOS expression. At 100 μ g/mL, both JH and SH anthocyanin-rich fractions inhibited iNOS expression in LPS-induced RAW 264.7 cells compared to LPS alone (Fig. 3B).

Regarding the anti-inflammatory effect, all cultivars inhibited NO production at a maximum concentration of 100 μ g/mL without inducing cytotoxicity. Additionally,

Fig. 3 Comparative inhibition of iNOS expression by JH and SH anthocyanin-rich fractions. RAW 264.7 cells were treated with 100 $\mu\text{g/mL}$ of JH and SH fractions for 24 h, and iNOS expression was analyzed using western blotting. Both cultivars significantly reduced iNOS expression compared to that in the LPS group. Different letters indicate significant differences ($p < 0.05$)



western blot analysis showed that JH exhibited greater iNOS inhibition than SH. JH demonstrated the strongest anti-inflammatory activity compared to the other cultivars, which may be attributed to the higher presence of pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside compared to the other cultivars. However, during LC–MS profiling, JH and SH formed the same group with similar metabolomic patterns, but showed the most significant differences in anti-inflammatory activity. The quantitative values of pelargonidin-3-*O*-glucoside in JH and SH were 170.22 mg/g and 170.45 mg/g, respectively, with no significant difference observed between the two cultivars. By contrast, cyanidin-3-*O*-glucoside levels were 8.04 mg/g in JH and 3.30 mg/g in SH, demonstrating an approximate 2.4-fold difference. Spearman correlation analysis revealed cyanidin-3-*O*-glucoside strongly correlated with iNOS suppression ($\rho = -0.829$), providing further evidence that higher cyanidin-3-*O*-glucoside content enhances anti-inflammatory efficacy, which may contribute to JH's superior anti-inflammatory activity. Previous studies have indicated that cyanidin-3-*O*-glucoside possesses potent anti-inflammatory effects (Jung et al., 2014; Ma et al., 2015). Although pelargonidin-3-*O*-rutinoside and pelargonidin-3-*O*-malonylglucoside, which are prominent strawberry anthocyanins, were not identified as signature metabolites distinguishing the groups, their anti-inflammatory potential has been reported in earlier studies (Bowen-Forbes et al., 2010; Gasparrini et al., 2017). Therefore, it is necessary to consider the impact of variation in these minor metabolites. Future investigations quantifying anti-inflammatory anthocyanin metabolites, including pelargonidin-3-*O*-rutinoside and pelargonidin-3-*O*-malonylglucoside, will help strengthen the reliability of experimental findings.

Cultivar-specific mechanisms linking strawberry anthocyanin content to anti-inflammatory activity

Previous metabolomics studies on strawberries using LC–MS have revealed phenolic variations among cultivars, showing that ellagitannins and quercetin glycosides, in

addition to anthocyanins, contribute to overall bioactivity (Olenikov et al., 2022; Wang et al., 2018). In the present study, the distinct clustering patterns observed in PCA and HCA analyses among cultivars support previous research demonstrating substantial inter-cultivar variation in anthocyanin composition across 27 strawberry varieties (Aaby et al., 2012). Consistent with those results, pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside were identified as the predominant anthocyanins in strawberries (Goiffon et al., 1999).

The anti-inflammatory effects of strawberry anthocyanins have been primarily associated with suppression of NO production and iNOS expression. Pelargonidin-3-*O*-glucoside has been shown to inhibit I κ B- α degradation and JNK phosphorylation, thereby attenuating downstream activation of NF- κ B and AP-1 transcription factors triggered by TLR4 signaling (Duarte et al., 2018). Similarly, cyanidin-3-*O*-glucoside downregulates iNOS and pro-inflammatory cytokines through inhibition of the NF- κ B and MAPK pathways (Jung et al., 2014; Ma et al., 2015). The higher anti-inflammatory activity observed in the JH cultivar can thus be attributed to these molecular mechanisms. Furthermore, strawberry extracts have been reported to suppress iNOS and COX-2 expression by reducing LPS-induced NO production and activating AMPK signaling (Gasparrini et al., 2017). Therefore, the high anthocyanin content in the JH cultivar appears to reduce NO production by 38% through similar regulatory pathways.

In conclusion, the present study characterized the diversity of anthocyanin composition among strawberry cultivars and compared their anti-inflammatory activities. This study provides a comprehensive understanding of the anti-inflammatory activity of strawberry cultivars, particularly the anthocyanin-rich fraction. It offers an important foundation for selecting strawberry cultivars containing bioactive compounds with significant anti-inflammatory activity, or for future cultivar improvement. However, this study was limited to NO and iNOS as primary biomarkers. Future studies should evaluate additional inflammatory markers, such as prostaglandin E_2 (PGE_2),

cyclooxygenase-2 (COX-2), and matrix metalloproteinase-9 levels. Furthermore, incorporating in vivo models will be essential to fully elucidate the anthocyanin-mediated anti-inflammatory mechanisms in strawberries and validate the in vitro findings.

Acknowledgements This research was supported by the Chung-Ang University Graduate Research Scholarship (Academic Scholarship for College of Biotechnology and Natural Resources), and the authors thank the BT Research Facility Center of Chung-Ang University.

Funding Open Access funding enabled and organized by Chung-Ang University.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Aaby K, Mazur S, Nes A, Skrede G. Phenolic compounds in strawberry (*Fragaria x ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry* 132: 86–97 (2012)
- Bowen-Forbes CS, Zhang Y, Nair MG. Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of Food Composition and Analysis* 23: 554–560 (2010)
- Chandrasekhar J, Madhusudhan M, Raghavarao K. Extraction of anthocyanins from red cabbage and purification using adsorption. *Food and Bioproducts Processing* 90: 615–623 (2012)
- Chaves VC, Calvete E, Reginatto FH. Quality properties and antioxidant activity of seven strawberry (*Fragaria x ananassa* duch) cultivars. *Scientia Horticulturae* 225: 293–298 (2017)
- da Silva FL, Escribano-Bailón MT, Alonso JJP, Rivas-Gonzalo JC, Santos-Buelga C. Anthocyanin pigments in strawberry. *LWT-Food Science and Technology* 40: 374–382 (2007)
- Duarte LJ, Chaves VC, dos Santos Nascimento MVP, Calvete E, Li M, Ciraolo E, Ghigo A, Hirsch E, Simões CMO, Reginatto FH. Molecular mechanism of action of pelargonidin-3-*O*-glucoside, the main anthocyanin responsible for the anti-inflammatory effect of strawberry fruits. *Food Chemistry* 247: 56–65 (2018)
- Elisia I, Hu C, Popovich DG, Kitts DD. Antioxidant assessment of an anthocyanin-enriched blackberry extract. *Food Chemistry* 101: 1052–1058 (2007)
- Gasparrini M, Forbes-Hernandez TY, Giampieri F, Afrin S, Alvarez-Suarez JM, Mazzoni L, Mezzetti B, Quiles JL, Battino M. Anti-inflammatory effect of strawberry extract against LPS-induced stress in RAW 264.7 macrophages. *Food and Chemical Toxicology* 102: 1–10 (2017)
- Goiffon J-P, Mouly PP, Gaydou EM. Anthocyanic pigment determination in red fruit juices, concentrated juices and syrups using liquid chromatography. *Analytica Chimica Acta* 382: 39–50 (1999)
- Jampani C, Naik A, Raghavarao K. Purification of anthocyanins from jamun (*Syzygium cumini* L.) employing adsorption. *Separation and Purification Technology* 125: 170–178 (2014)
- Jung H, Kwak H-K, Hwang KT. Antioxidant and antiinflammatory activities of cyanidin-3-glucoside and cyanidin-3-rutinoside in hydrogen peroxide and lipopolysaccharide-treated RAW 264.7 cells. *Food Science and Biotechnology* 23: 2053–2062 (2014)
- Kim Y-J, Shin Y. Antioxidant profile, antioxidant activity, and physicochemical characteristics of strawberries from different cultivars and harvest locations. *Journal of the Korean Society for Applied Biological Chemistry* 58: 587–595 (2015)
- Kim SK, Kim DS, Kim DY, Chun C. Variation of bioactive compounds content of 14 oriental strawberry cultivars. *Food Chemistry* 184: 196–202 (2015)
- Kim G-D, Lee JY, Auh J-H. Metabolomic screening of anti-inflammatory compounds from the leaves of *Actinidia arguta* (Hardy Kiwi). *Foods* 8: 47 (2019)
- Korns D, Frasch SC, Fernandez-Boyanapalli R, Henson PM, Bratton DL. Modulation of macrophage efferocytosis in inflammation. *Frontiers in Immunology* 2: 57 (2011)
- Lee DY, Heo S, Kim SG, Choi H-K, Lee H-J, Cho S-M, Auh J-H. Metabolomic characterization of the region- and maturity-specificity of *Rubus coreanus* Miquel (Bokbunja). *Food Research International* 54: 508–515 (2013)
- Li L, Wang L, Wu Z, Yao L, Wu Y, Huang L, Liu K, Zhou X, Gou D. Anthocyanin-rich fractions from red raspberries attenuate inflammation in both RAW 264.7 macrophages and a mouse model of colitis. *Scientific Reports* 4: 6234 (2014)
- Lim S, Hwang H, Shin Y. Physicochemical properties and antioxidant activities of 'Seolhyang' and 'Janghee' strawberries from different ripening stages grown in Korea. *Journal of the East Asian Society of Dietary Life* 26: 80–87 (2016)
- Ma M-M, Li Y, Liu X-Y, Zhu W-W, Ren X, Kong G-Q, Huang X, Wang L-P, Luo L-Q, Wang X-Z. Cyanidin-3-*O*-glucoside ameliorates lipopolysaccharide-induced injury both *in vivo* and *in vitro* suppression of NF- κ B and MAPK pathways. *Inflammation* 38: 1669–1682 (2015)
- Min K, Yi G, Lee JG, Kim HS, Hong Y, Choi JH, Lim S, Lee EJ. Comparative transcriptome and metabolome analyses of two strawberry cultivars with different storability. *PLOS ONE* 15: e0242556 (2020)
- Miyazawa T, Nakagawa K, Kudo M, Muraishi K, Someya K. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *Journal of Agricultural and Food Chemistry* 47: 1083–1091 (1999)
- Olennikov DN, Khandy MT, Chirikova NK. Oriental strawberry metabolites: LC–MS profiling, antioxidant potential, and post-harvest changes of *Fragaria orientalis* fruits. *Horticulturae* 8: 975 (2022)
- Pergola C, Rossi A, Dugo P, Cuzzocrea S, Sautebin L. Inhibition of nitric oxide biosynthesis by anthocyanin fraction of blackberry extract. *Nitric Oxide* 15: 30–39 (2006)
- Razgonova MP, Okhlopko ZM, Nawaz MA, Egorova PS, Golokhvast KS. Supercritical extraction and identification of bioactive compounds in *Dryopteris fragrans* (L.) Schott. *Pharmaceuticals* 18: 299 (2025)

- Reber JD, Eggett DL, Parker TL. Antioxidant capacity interactions and a chemical/structural model of phenolic compounds found in strawberries. *International Journal of Food Sciences and Nutrition* 62: 445–452 (2011)
- Ren J, Su D, Li L, Cai H, Zhang M, Zhai J, Li M, Wu X, Hu K. (2020) Anti-inflammatory effects of Aureusidin in LPS-stimulated RAW 264.7 macrophages via suppressing NF- κ B and activating ROS-and MAPKs-dependent Nrf2/HO-1 signaling pathways. *Toxicology and Applied Pharmacology* 387: 114846
- Ristivojević P, Lekić N, Cvijetić I, Krstić Đ, Andrić F, Milojković-Opšenica D, Morlock GE. Effect-directed profiling of strawberry varieties and breeding materials via planar chromatography and chemometrics. *Molecules* 27: 6062 (2022)
- Sirijan M, Pipattanawong N, Saeng-On B, Chaiprasart P. Anthocyanin content, bioactive compounds and physico-chemical characteristics of potential new strawberry cultivars rich in anthocyanins. *Journal of Berry Research* 10: 397–410 (2020)
- Talpur M, Talpur F, Balouch A, Nizamani S, Surhio M, Shah M. Analysis and characterization of anthocyanin from phalsa (*Grewia asiatica*). *MOJ Food Process Technol* 5: 00127 (2017)
- Urün I, Attar SH, Sönmez DA, Gündeşli MA, Ercişli S, Kafkas NE, Bandić LM, Duralija B. Comparison of polyphenol, sugar, organic acid, volatile compounds, and antioxidant capacity of commercially grown strawberry cultivars in Turkey. *Plants* 10: 1654 (2021)
- Wang Y, Vorsa N, Harrington PdB, Chen P. Nontargeted metabolomic study on variation of phenolics in different cranberry cultivars using uplc-im-hrms. *Journal of Agricultural and Food Chemistry* 66: 12206–12216 (2018)
- Xu Y, Hu D, Li Y, Sun C, Chen W. An effective method for preparation of high-purity pelargonidin-3-*O*-glucoside from strawberry and its protective effect on cellular oxidative stress. *Journal of Chromatography B* 1072: 211–220 (2018)
- Xu Y, Xie L, Xie J, Liu Y, Chen W. Pelargonidin-3-*O*-rutoside as a novel α -glucosidase inhibitor for improving postprandial hyperglycemia. *Chemical Communications* 55: 39–42 (2019)
- Zhou Y, Wang J, Yang W, Qi X, Lan L, Luo L, Yin Z. Bergapten prevents lipopolysaccharide-induced inflammation in RAW 264.7 cells through suppressing JAK/STAT activation and ROS production and increases the survival rate of mice after LPS challenge. *International Immunopharmacology* 48: 159–168 (2017)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.