

Insights into the flavonoid composition and antioxidant activity of Jeramon, a novel Korean lemon cultivar

Chang-Dae Lee^a, Taesoo Kim^b, Sungwook Chae^{b,c,**}, Sanghyun Lee^{a,d,*}

^a Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

^b KM Convergence Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea

^c KM Korean Convergence Medicine, University of Science and Technology, Daejeon 34113, Republic of Korea

^d Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

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ABSTRACT

'Jeramon' is a newly developed Korean lemon cultivar bred from *Citrus limon* 'Frost Lisbon' × 'Meyer'. This study provides the first comprehensive phytochemical and functional characterization of 'Jeramon' across ripening stages. DPPH and ABTS assays consistently showed stronger antioxidant activity in peel than in flesh, corresponding to higher phytochemical contents. LC-MS/MS and HPLC/PDA analyses identified a distinctive flavonoid profile enriched in eriocitrin, rutin, and hesperidin, with hesperidin remaining stable during maturation. Unlike many citrus varieties, total phenolic and flavonoid levels remained relatively constant throughout ripening. The yellow peel accumulated the highest total concentration of the three key flavonoids. Correlation analysis indicated eriocitrin as a major contributor to antioxidant capacity. A dual antioxidant mechanism is proposed, involving direct radical scavenging and indirect modulation of carbonic anhydrase 4, supported by strong *in silico* binding of hesperidin. These findings highlight 'Jeramon' as a metabolically stable, high-value cultivar and a promising natural antioxidant source.

1. Introduction

Lemon (*Citrus limon* L.), a member of the genus *Citrus* in the family Rutaceae, is one of the most widely cultivated citrus fruits, ranking third in global citrus production (Choi, Kim, et al., 2023; Miran et al., 2016). It is valued for its sensory qualities and bioactive compounds, including vitamins, essential oils, and phenolics, which are widely used in industrial and functional food applications (Di Vaio et al., 2023; García-Salas et al., 2013; González-Molina et al., 2010). This combination of flavor and health-promoting properties has contributed to its widespread popularity (Kim et al., 2021). In South Korea, domestic production remains limited, with an estimated cultivation area of 49 ha yielding 636 tons, while the majority of lemons consumed in the country are imported. Import volumes have steadily increased from 3800 tons in 2003 to 22,914 tons in 2021 (Choi, Kim, et al., 2023).

The 'Jeramon' lemon cultivar was developed in South Korea through controlled pollination between *C. limon* 'Frost Lisbon' (seed parent) and *C. limon* 'Meyer' (pollen parent). Distinguished by its high acidity, pleasant fragrance, and extended flowering period, 'Jeramon' is a

promising candidate for domestic lemon production and potential functional applications (Choi, Kim, et al., 2023). Domestic lemons are prized for their freshness and reliability. However, nearly 90% of lemons consumed in Korea are imported, mainly from the United States and Chile. To endure lengthy transport and storage, imported fruits are commonly treated with wax or preservatives (Kang et al., 2023).

Previous investigations into global lemon cultivars have established that the distribution of key bioactive compounds, particularly flavanones such as eriocitrin and hesperidin, exhibits profound spatial compartmentalization between peel and flesh tissues (Xi et al., 2017), and is strongly influenced by the progressive stages of fruit maturation (Mcharek & Hanchi, 2017). Furthermore, comparative metabolomic studies have demonstrated distinct phytochemical profiles and antioxidant baseline capacities among established commercial cultivars like 'Eureka', 'Fino', and 'Lisbon' (Melgarejo et al., 2023). While preliminary studies have highlighted the antioxidant and phytochemical potential of 'Jeramon,' reporting that its extracts show strong radical-scavenging activity compared with other citrus varieties (Kang et al., 2023), Previous studies analyzed whole-fruit extracts and did not separately

* Corresponding author at: Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea.

** Corresponding author at: KM Convergence Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea.

E-mail addresses: kendall@kiom.re.kr (S. Chae), slee@cau.ac.kr (S. Lee).

examine peel and flesh tissues, evaluate changes during fruit maturation, or compare 'Jeramon' with other lemon cultivars (Choi, Kim, et al., 2023; Kang et al., 2023). Thus, comprehensive phytochemical profiling and functional evaluation remain limited.

To investigate its antioxidant potential, peel and flesh extracts were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. To support these findings, total phenolic content (TPC) and total flavonoid content (TFC) were determined. For further characterization, liquid chromatography-mass spectrometry (LC-MS/MS) and high-performance liquid chromatography (HPLC) were used to identify and quantify major flavonoids, including eriocitrin, rutin, and hesperidin. In addition, HPLC-based comparisons with lemon derived from the 'Eureka' cultivar were conducted to evaluate potential differences in flavonoid diversity and content. Correlation coefficient analysis was also performed to examine the relationship between individual compound concentrations and antioxidant capacity. As a complementary approach, network pharmacology and molecular docking were conducted to explore the functional relevance of key compounds, particularly those interacting with oxidative-related target proteins, given the traditional use of citrus peels for relieving oxidative stress in herbal medicine (Nishad et al., 2018; Onuma et al., 2017).

Therefore, this study sought to provide the first comprehensive phytochemical and functional characterization of 'Jeramon.' The DPPH and ABTS assays, along with quantification of TPC and TFC, were applied to its peel and flesh extracts. Through this stepwise approach, the present work not only establishes a detailed phytochemical and functional profile of 'Jeramon' but also highlights its compositional advantages over conventional lemons, supporting its potential for value-added applications in functional foods and nutraceutical development.

2. Materials and methods

2.1. Plant materials

'Jeramon' was developed by the Citrus Research Center, National Institute of Horticultural & Herbal Science, Rural Development Administration, Jeju, Republic of Korea. This cultivar was submitted for plant variety protection to the Korea Seed & Variety Service in 2016 and was officially registered on June 9, 2022 (registration No. 9060). Green 'Jeramon' fruits collected in November 2024 and yellow 'Jeramon' fruits collected in January 2025 were cultivated at Botari Farm, Bongseong-ri, Aewol-eup, Jeju, Republic of Korea (Fig. 1). The experiments were conducted using green 'Jeramon' peel (GJP), green 'Jeramon' flesh (GJF), yellow 'Jeramon' peel (YJP), yellow 'Jeramon' flesh (YJF), lemon peel (LP), and lemon flesh (LF). Lemon was derived from the 'Eureka' cultivar.

2.2. Chemicals and apparatus

Ethanol (EtOH) ($\geq 95.0\%$ purity) used for extraction and HPLC-grade dimethyl sulfoxide (DMSO) were purchased from Samchun Chemicals (Pyeongtaek, Republic of Korea), and HPLC-grade methanol, The reagents for the colorimetric method, 2 N Folin-Ciocalteu phenol reagent, aluminum chloride hexahydrate (ReagentPlus®, $\geq 99\%$ purity), and sodium carbonate anhydrous ($\geq 99\%$ purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, (HPLC-grade), DPPH, and ABTS were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Absorbance was measured using an Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA). HPLC-grade water and acetonitrile (ACN) were obtained from Honeywell (Burdick and Jackson, Muskegon, MI, USA). The standard compounds, quercetin ($\geq 99\%$ purity), tannic acid ($\geq 99\%$ purity), eriocitrin (1) ($\geq 93\%$ purity), rutin (2) ($\geq 98\%$ purity), hesperidin (3) ($\geq 92\%$ purity), neohesperidin (4) ($\geq 97\%$ purity), scutellarein tetramethyl ether (5) ($\geq 98\%$ purity), 3,5,6,7,8,3',4'-heptamethoxyflavone (6) ($\geq 99\%$ purity), and 4'-methoxyflavone (7) ($\geq 95\%$ purity), were provided by the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Republic of Korea (Fig. 2). Freeze-drying was conducted using the FDU-1200 (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). HPLC analysis was performed using a Waters Alliance e2695 separation module (Milford, MA, USA) equipped with a Waters 2998 photodiode array detector (PDA) and an INNO C18 column (4.6 $\times$ 250 mm, 5 μ m). LC-MS/MS analysis was carried out using an ultra-high-performance liquid chromatography system (Ultimate 3000, Thermo Scientific, San Jose, CA, USA) coupled with a high-resolution mass spectrometer (Thermo Q-Exactive Orbitrap mass spectrometer, Thermo Fisher Scientific, San Jose, CA, USA) and a Waters Cortecs T3 column (150 mm $\times$ 2.1 mm, 1.6 μ m).

2.3. Preparation of samples and standard solutions for HPLC/PDA

The GJP, GJF, YJP, YJF, LP, and LF samples were freeze-dried to remove moisture and extracted using a reflux extractor with EtOH for 3 h in triplicate. The extracts were then filtered and concentrated under vacuum to obtain the solid residues for assay (Supplementary Table S1). EtOH extracts (GJP, GJF, YJP, YJF, LP, and LF) and compounds 1–7 were dissolved at concentrations of 30 mg/mL and 1 mg/mL, respectively, in MeOH. When insoluble in MeOH, DMSO was partially used as a co-solvent. All solutions were filtered through polyvinylidene difluoride membrane filters (0.2 μ m) from Hyundai Micro (Seoul, Republic of Korea), prior to HPLC/PDA analysis.

2.4. DPPH assay

The DPPH radical scavenging activity was assayed according to the method previously described method (Lee, Lee, et al., 2025). A stock solution of DPPH (2 mM) was prepared in EtOH and subsequently



Fig. 1. Samples of green 'Jeramon' (A), yellow 'Jeramon' (B), and lemon (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

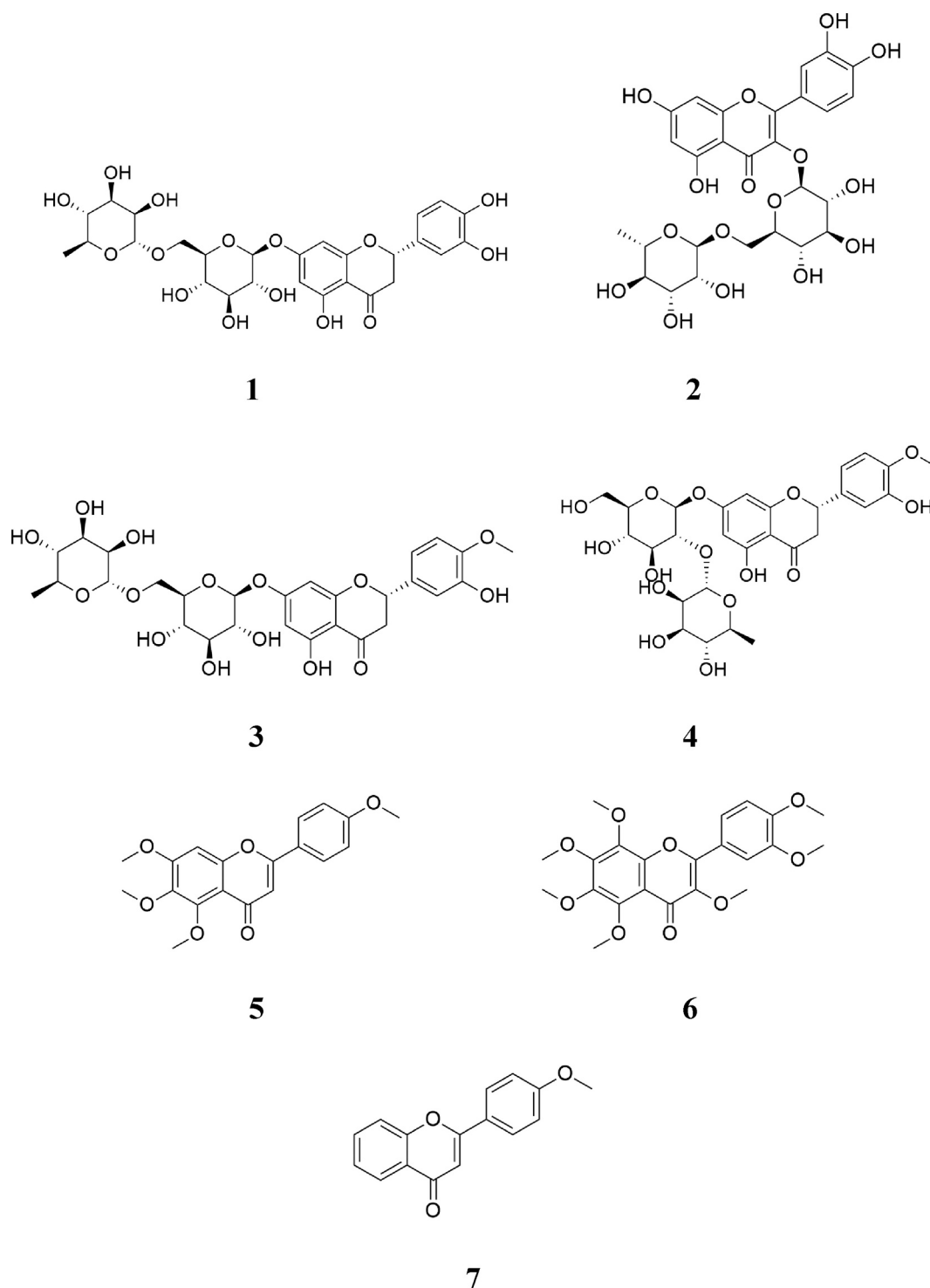


Fig. 2. Chemical structures of compounds 1–7. Compounds: eriocitrin (1), rutin (2), hesperidin (3), neohesperidin (4), scutellarein tetramethyl ether (5), 3,5,6,7,8,3',4'-heptamethoxyflavone (6), and 4'-methoxyflavone (7).

diluted to obtain a working solution with an absorbance of 0.8 at 514 nm. Stock solutions of the test samples were serially diluted to prepare a range of concentrations. A 10 μ L aliquot of each diluted sample was added to a 96-well plate, followed by 200 μ L of the DPPH working solution. Each measurement was performed in triplicate to ensure accuracy and reproducibility. The mixtures were thoroughly mixed using a microplate shaker and incubated at room temperature in the dark for 30 min. After the reaction, the absorbance of each sample was measured at 514 nm using a microplate reader. Ascorbic acid was used as the

standard at concentrations of 40, 80, 120, 160, and 200 μ g/mL.

2.5. ABTS assay

The ABTS radical-scavenging activity was determined according to a previously described method (Lee, Yu, et al., 2025). The ABTS stock solution was prepared by dissolving ABTS (7.4 mM) and potassium persulfate ($K_2S_2O_8$, 2.6 mM) in deionized water. The stock solution was then diluted to achieve an absorbance of 1.0 at 734 nm. Stock solutions

of the samples were serially diluted with deionized water to prepare various concentrations. A 10 μL aliquot of each diluted sample was placed in a 96-well plate, and 200 μL of the ABTS working solution was added to each well. Each test was performed in triplicate to ensure accuracy and consistency. The mixtures were thoroughly mixed using a microplate shaker and incubated at room temperature in the dark for 30 min. After the reaction, the absorbance of each sample was measured at 734 nm using a microplate reader. Ascorbic acid was used as the standard at concentrations of 40, 80, 120, 160, and 200 $\mu\text{g/mL}$. For the blank, deionized water was used in place of the ABTS working solution.

2.6. Determination of TPC

The TPC of GJP, GJF, YJP, and YJF was assessed using the Folin–Ciocalteu colorimetric method with slight modifications (Athanasiadis et al., 2024). Sample stock solutions were serially diluted to obtain final concentrations ranging from 0.5 to 2.0 mg/mL. A volume of 60 μL from each dilution was transferred into individual wells of a 96-well microplate, followed by the addition of 40 μL of Folin–Ciocalteu reagent (2 N). Subsequently, 100 μL of 7.5% (w/v) sodium carbonate solution was added to each well. The plate was gently agitated using a microplate shaker to ensure complete mixing, and the mixtures were incubated in the dark at room temperature for 30 min. Absorbance was then measured at 760 nm using a microplate spectrophotometer. To generate the standard calibration curve, tannic acid solutions were prepared at concentrations ranging from 7.813 to 125 ppm.

2.7. Determination of TFC

The TFC of GJP, GJF, YJP, and YJF was determined using an aluminum chloride colorimetric assay with slight modifications from a previously reported method (Athanasiadis et al., 2024). Sample stock solutions were serially diluted to achieve final concentrations ranging from 1.0 to 4.0 mg/mL. For the assay, 100 μL of each diluted sample was dispensed into the wells of a 96-well microplate, followed by the addition of 100 μL of 2% (w/v) aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) solution. The mixtures were gently agitated using a microplate shaker and incubated in the dark at room temperature for 10 min. After incubation, absorbance was measured at 430 nm using a microplate reader. Quercetin was used as the standard, and calibration solutions were prepared at concentrations ranging from 3.906 to 62.5 ppm.

2.8. LC-MS/MS conditions

As previously described (Lee, Lee, et al., 2025), the separation process was carried out at a column temperature of 45 $^{\circ}\text{C}$. The mobile phase consisted of water (eluent A: 0.1% formic acid) and ACN (eluent B: 0.1% formic acid). The gradient started at 3% B, increased to 15% B over 15 min, then rose to 100% B over the next 35 min, and was held at 100% for an additional 5 min, resulting in a total run time of 55 min. The column was then re-equilibrated to 3% B for 5 min. The flow rate was maintained at 0.25 mL/min. For mass spectrometric analysis, a Thermo Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was used to perform both MS and MS/MS detection under electrospray ionization conditions in positive and negative modes. The mass range was set from m/z 100 to 1500, with a resolution of 70,000 (MS1). Spray voltage was set at 3.5 kV (positive mode) and 2.5 kV (negative mode), with a capillary temperature of 320 $^{\circ}\text{C}$. Sheath gas, auxiliary gas, and sweep gas were set to 50, 10, and 1 arb units, respectively. MS/MS fragmentation was performed using stepped normalized collision energies of 10, 30, and 50.

2.9. HPLC/PDA conditions

The HPLC/PDA conditions were established based on a previously

reported method (Lee, Yu, et al., 2025). A reverse-phase HPLC/PDA system was used for quantitative analysis. Gradient elution was performed using 0.5% acetic acid (solvent A) and ACN (solvent B) as the mobile phase. The gradient conditions were as follows: 0–8 min (13% B), 12 min (20% B), 20 min (23% B), 30 min (50% B), 35–40 min (100% B), and 42–57 min (13% B). The flow rate was maintained at 1 mL/min, and the column temperature was set at 30 $^{\circ}\text{C}$. The detection wavelength was 254 nm.

2.10. Calibration curve

HPLC standards were analyzed at five or six different concentrations to construct the calibration curve (Supplementary Table S2). Linearity was determined based on the correlation coefficient (r^2). The compound contents in the samples were quantified using this curve. Calibration was established from the relationship between peak area (Y) and concentration (X, $\mu\text{g/mL}$). Results were expressed as mean $\pm$ standard deviation (SD, $n = 3$). For the determination of TPC and TFC, calibration curves were constructed using tannic acid and quercetin as standards, respectively (Supplementary Table S3). The results were expressed as tannic acid equivalents (TAE) and quercetin equivalents (QE).

2.11. Identification of target genes and overlap with disease targets

Potential protein targets for molecular docking were predicted using the SwissTargetPrediction tool (<http://www.swisstargetprediction.ch>) (Daina et al., 2017) and the SuperPred web server (<https://prediction.ch.arite.de>) (Nickel et al., 2014), in combination with the GeneCards database (<https://www.genecards.org>) (Stelzer et al., 2016). SwissTargetPrediction utilized structural similarity based on SMILES input, while SuperPred applied machine learning to predict protein targets and disease associations. The GeneCards database was queried using the keyword “oxidative” to retrieve disease-associated genes. Predicted protein targets were compared with oxidative-related genes using Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/>) to identify overlapping, disease-relevant targets.

2.12. Protein-protein interaction (PPI) network analysis

Interactions among the identified targets were examined using the STRING database (<https://string-db.org/>) (Szklarczyk et al., 2023), with *Homo sapiens* selected as the species. PPI data were filtered using a high-confidence threshold (≥ 0.4). Both experimental and predicted interactions, including co-expression and curated sources, were considered. Network analysis focused on identifying hub proteins by evaluating network centrality metrics such as degree, betweenness, and clustering coefficients.

2.13. Key target screening

For visualization and further analysis, the interaction networks were imported into Cytoscape (Shannon et al., 2003) using the STRING plugin. A confidence threshold of 0.4 was applied. The NetworkAnalyzer tool in Cytoscape was used to compute centrality metrics, including degree, betweenness, and closeness. Proteins with the highest scores were selected as key regulatory nodes within the network.

2.14. Gene ontology (GO) and pathway enrichment analysis

Gene function and pathway enrichment analyses were performed using the Metascape web platform (<https://metascape.org/>) (Zhou et al., 2019). Statistically significant terms were identified using the hypergeometric test with Benjamini-Hochberg p -value correction across multiple databases, including GO, Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and Molecular Signatures Database (MSigDB). Enriched terms were automatically grouped into non-

redundant clusters based on a Kappa similarity score (threshold 0.3), with each cluster represented by the most significant term (lowest *p*-value).

2.15. Molecular docking simulation

Blind docking was conducted using the 3D structures of ligands and proteins. The three most abundant compounds **1–3** were selected as ligands, and their SMILES codes were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). These codes were used as input for the SwissTargetPrediction database (<http://www.swisstargetprediction.ch/>) to identify potential protein targets. Proteins predicted to bind the ligands were compared with stomach-related proteins retrieved from the UniProt database (<https://www.uniprot.org/>). A total of 22 proteins overlapped between the datasets, indicating that they are both functionally relevant to the stomach and potential binding partners for the ligands. The 3D structures of these proteins were downloaded from the RCSB Protein Data Bank (<https://www.rcsb.org/>) and prepared using the Dock Prep tool in UCSF Chimera. Docking was then performed using AutoDock Vina (version 1.2.7) via UCSF Chimera (version 1.19) (Eberhardt et al., 2021). Docking poses with the lowest binding energy and an RMSD value of 0 were considered reliable.

2.16. Statistical analysis

All experiments were conducted in triplicate to enhance reproducibility and minimize statistical variability. Results are presented as mean $\pm$ SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) to evaluate the effects of independent variables and their interactions. When significant differences were observed, Tukey's multiple comparison test was applied as a *post hoc* analysis. Prior to statistical testing, data were assessed for normality to ensure appropriate test selection. All analyses were conducted using GraphPad Prism version 8.0.2 (GraphPad Software, Boston, MA, USA), and a *p*-value of less than 0.05 was considered statistically significant. Correlation analysis between constituent contents and antioxidant capacity was performed using MetaboAnalyst (version 6.0) (Pang et al., 2024).

3. Results

3.1. TPC and TFC analysis

GJP (190.62 mg TAE/g), YJP (159.90 mg TAE/g), and LP (174.91 mg TAE/g) exhibited relatively high TPC levels, with no statistically significant difference among them ($p > 0.05$). Similarly, no significant difference was observed among GJF (81.06 mg TAE/g), YJF (89.58 mg TAE/g), and LF (93.53 mg TAE/g). These results indicate that the peel (GJP, YJP, and LP) contains higher phenolic levels than the flesh (GJF, YJF, and LF), regardless of ripening stage. As for TFC, YJP (9.44 mg QE/g) had the highest mean value, followed by GJP (7.68 mg QE/g), GJF

(6.50 mg QE/g), LP (5.12 mg QE/g), YJF (3.23 mg QE/g), and LF (1.17 mg QE/g). However, while no statistically significant differences were found between GJP and GJF, or between GJP and YJP ($p > 0.05$), significant variations were observed in other stages (YJP > YJF and LP > LF). This indicates that unlike TPC, flavonoid content is more dynamically influenced by the interaction of tissue type and fruit maturity (Fig. 3).

3.2. LC-MS/MS analysis

Using tandem MS and mass-to-charge ratios, the primary peaks of YJP and GJP were tentatively characterized based on web-based databases and multiple spectral libraries (Supplementary Fig. S1). This analysis led to the tentative identification of the following compounds: eriocitrin (**1**), rutin (**2**), hesperidin (**3**), neohesperidin (**4**), scutellarein tetramethyl ether (**5**), 3,5,6,7,8,3',4'-heptamethoxyflavone (**6**), and 4'-methoxyflavone (**7**) (Supplementary Table S4 and S5). Total ion chromatograms are presented in Supplementary Fig. S1. LC-MS/MS analysis of samples revealed a distinct flavonoid profile, with eriocitrin (**1**) and hesperidin (**3**) tentatively suggested as major compounds. Notably, a comparison between YJP and GJP revealed that the detected compounds were nearly identical across both samples. However, it should be noted that this analysis was limited to YJP and GJP. Given that phytochemical composition can vary depending on tissue type or maturity (Mansour, 2019), further studies are needed to determine whether similar compound profiles exist in other parts of the flesh. Although this limitation restricts a more comprehensive understanding, particularly since lemons are known to contain other classes of compounds such as amino acids, the current findings still offer valuable insights into the phytochemical characteristics of 'Jeramon' (Ledesma-Escobar et al., 2015). Notably, the distinctive profile observed suggests that 'Jeramon' may differ from other citrus cultivars in its metabolite composition, offering a useful reference for future comparative studies among lemon varieties.

3.3. HPLC/PDA analysis

The phytochemical composition of the peel and flesh of yellow 'Jeramon', green 'Jeramon', and lemon was analyzed and compared (Table 1). Eriocitrin (**1**) was more abundant in YJP (49.06 mg/g DW) than in LP (10.83 mg/g DW). However, in the flesh, no statistically significant difference was observed between YJF and LF, indicating comparable levels in the edible portion. Rutin (**2**) levels were slightly higher in yellow lemon, but this difference was minor and did not alter the overall trend favoring 'Jeramon' in terms of compound richness. Hesperidin (**3**) was consistently higher in 'Jeramon' than in lemon, both in peel and flesh. In YJP, hesperidin (**3**) content reached 20.47 mg/g DW, substantially exceeding that of LP (5.26 mg/g DW). Similarly, YJF contained 4.97 mg/g DW, compared to 3.22 mg/g DW in LF. Furthermore, a direct comparison between the green and yellow maturation stages of 'Jeramon' revealed unique developmental dynamics. Unlike

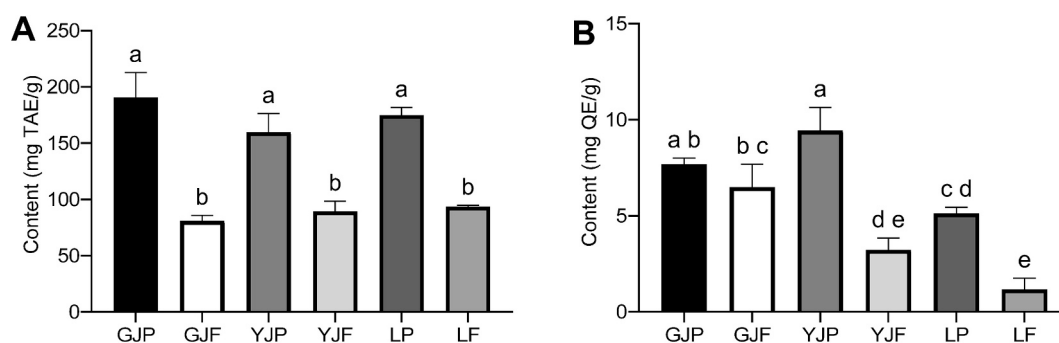


Fig. 3. TPC (A) and TFC (B) of GJP, GJF, YJP, YJF, LP, and LF.

^{a-e} Statistical differences within the same graph were determined by Tukey's test. Values not sharing a common letter are significantly different ($p < 0.05$).

Table 1
Content of compounds 1–7.

Sample	Standard compound (mg/g DW)							Total
	1	2	3	4	5	6	7	
GJP	11.05 ± 0.347 ^b	0.61 ± 0.019 ^d	20.32 ± 0.641 ^a	tr	tr	tr	tr	31.98
GJF	7.13 ± 0.042 ^c	0.37 ± 0.014 ^e	8.63 ± 0.051 ^b	ND	ND	ND	ND	16.13
YJP	49.06 ± 1.855 ^a	0.67 ± 0.026 ^c	20.47 ± 0.023 ^a	tr	tr	tr	ND	70.20
YJF	4.79 ± 0.109 ^c	0.22 ± 0.005 ^f	4.97 ± 0.132 ^c	ND	ND	ND	ND	9.98
LP	10.83 ± 0.167 ^b	1.97 ± 0.006 ^a	5.26 ± 0.023 ^c	ND	ND	ND	ND	18.06
LF	5.60 ± 0.323 ^c	1.08 ± 0.001 ^b	3.22 ± 0.019 ^d	ND	ND	ND	ND	9.9

tr = trace, ND = not detected. ^{a-f} Statistical differences within the same column were determined by Tukey's test. Values not sharing a common letter are significantly different ($p < 0.05$).

Compounds: eriocitrin (1), rutin (2), hesperidin (3), neohesperidin (4), scutellarein tetramethyl ether (5), 3,5,6,7,8,3',4'-heptamethoxyflavone (6), and 4'-methoxyflavone (7).

typical citrus cultivars where phenolic concentrations frequently suffer rapid degradation during the transition from the immature green to the fully mature yellow stage, 'Jeramon' showcased remarkable metabolic stability. Eriocitrin (1) levels surged dramatically from the green stage (GJP: 11.05 mg/g DW) to the mature yellow stage (YJP: 49.06 mg/g DW). Concurrently, hesperidin (3) remained impeccably stable across ripening (GJP: 20.32 mg/g DW vs. YJP: 20.47 mg/g DW). This indicates

that 'Jeramon' possesses a highly stabilized secondary metabolite biosynthetic pathway, allowing it to preserve or even enhance its high-value bioactive constituents as the fruit matures.

3.4. Identification of associated targets

Potential targets were identified by querying the keyword

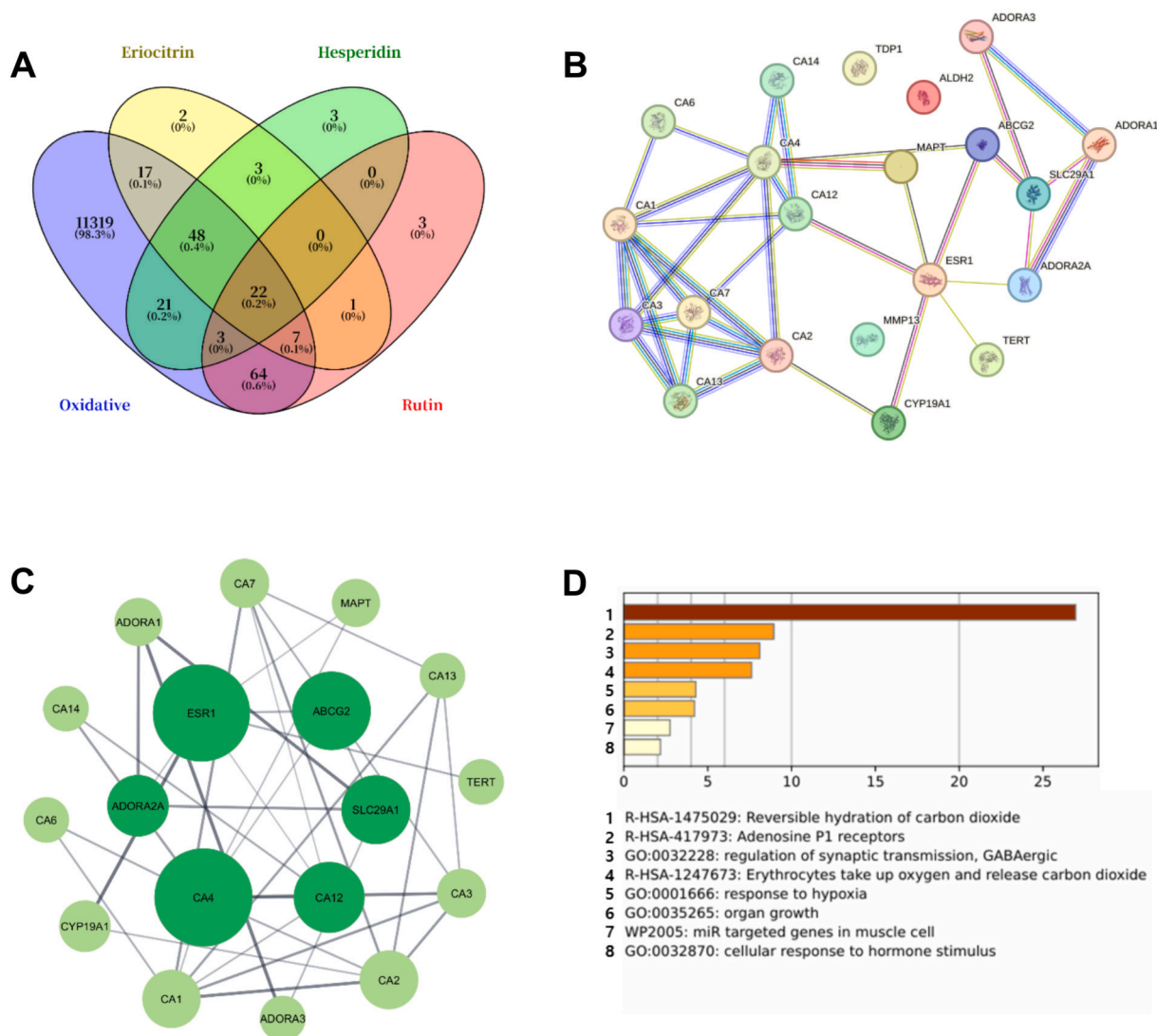


Fig. 4. Venn diagram showing the overlap between guanosine and IBD-related targets (A); STRING PPI network of the disease targets (B); circular layout of the same PPI network for improved visualization (C); and GO and pathway enrichment analysis (D).

“oxidative” against the GeneCards database, resulting in 11,501 oxidative-related targets. Additionally, 100 targets for each of compounds 1–3 were predicted using the SwissTargetPrediction tool and the SuperPred web server. After standardization, 22 overlapping targets were identified (Fig. 4A). The discovery of these 22 common protein targets suggests that compounds 1–3 may influence multiple pathways relevant to oxidative processes.

3.5. PPI network visualization and enrichment clustering

To explore the biological interactions among the 22 overlapping proteins, a PPI network was constructed using the STRING database (Fig. 4B). The resulting network was imported into Cytoscape for topological analysis. Centrality parameters, particularly betweenness centrality, were calculated to identify key regulatory proteins. This analysis highlighted carbonic anhydrase 4 (CA4) as a core target with a high centrality value (Fig. 4C). Mapping the targets of compounds 1–3 within the PPI network provided insights into the biological significance of these proteins. These core targets were subsequently prioritized for molecular docking studies based on their central roles in the network and their relevance to oxidative pathophysiology. Fig. 4D shows the results of pathway enrichment analysis for these core targets. The bars are colored in a gradient to represent *p*-values of increasing statistical significance. As illustrated in the bar graph, the most significantly enriched biological pathway was “reversible hydration of carbon dioxide,” providing a strong rationale for investigating the carbonic anhydrase enzyme family as a key molecular target in subsequent analyses.

3.6. Molecular docking simulation

To validate the interaction between the flavonoids and the key target protein identified from network analysis, molecular docking simulations were conducted on CA4. The visualized docking poses revealed that eriocitrin (1), rutin (2), and hesperidin (3) exhibited strong potential binding affinities within the catalytic active site of CA4 (Fig. 5). The

Table 2

Molecular docking results of compounds 1–3 with the target protein.

Protein	Ligand	Affinity (kcal/mol)	Number of H bonds
CA4	1	−7.23	10
	2	−7.86	2
	3	−8.58	5

Compounds: eriocitrin (1), rutin (2), and hesperidin (3).

calculated binding affinities were −8.58 kcal/mol for hesperidin (3), −7.86 kcal/mol for rutin (2), and −7.23 kcal/mol for eriocitrin (1) (Table 2). These negative binding energies, particularly the strong affinity of hesperidin (3), suggest that the major flavonoids in ‘Jeramon’ may effectively interact with and modulate CA4 at the molecular level.

3.7. DPPH and ABTS assays with ‘Jeramon’ and compounds 1–3

The antioxidant capacities of GJP, GJF, YJP, and YJF were assessed using DPPH and ABTS radical-scavenging assays, expressed as IC₅₀ values (mg/mL) (Fig. 6; Supplementary Table S6). In both assays, peel-derived extracts (GJP and YJP) exhibited stronger radical-scavenging activity than flesh-derived extracts (GJF and YJF), as indicated by consistently lower IC₅₀ values. Specifically, DPPH IC₅₀ values for GJP and YJP were 17.46 mg/mL and 20.30 mg/mL, whereas GJF and YJF showed markedly higher values of 36.50 mg/mL and 43.36 mg/mL, respectively. A similar trend was observed in the ABTS assay, with GJP and YJP showing IC₅₀ values of 5.10 mg/mL and 3.64 mg/mL, significantly lower than those of GJF (30.37 mg/mL) and YJF (29.92 mg/mL).

The antioxidant activities of compounds 1–3 were also evaluated using DPPH and ABTS assays (Table 3). Rutin (2) exhibited the strongest activity with IC₅₀ values of 0.46 mg/mL (DPPH) and 0.21 mg/mL (ABTS), followed by eriocitrin (1) with 0.61 mg/mL and 0.34 mg/mL, respectively. Hesperidin (3) showed the weakest effect, with IC₅₀ values of 2.34 mg/mL (DPPH) and 0.53 mg/mL (ABTS).

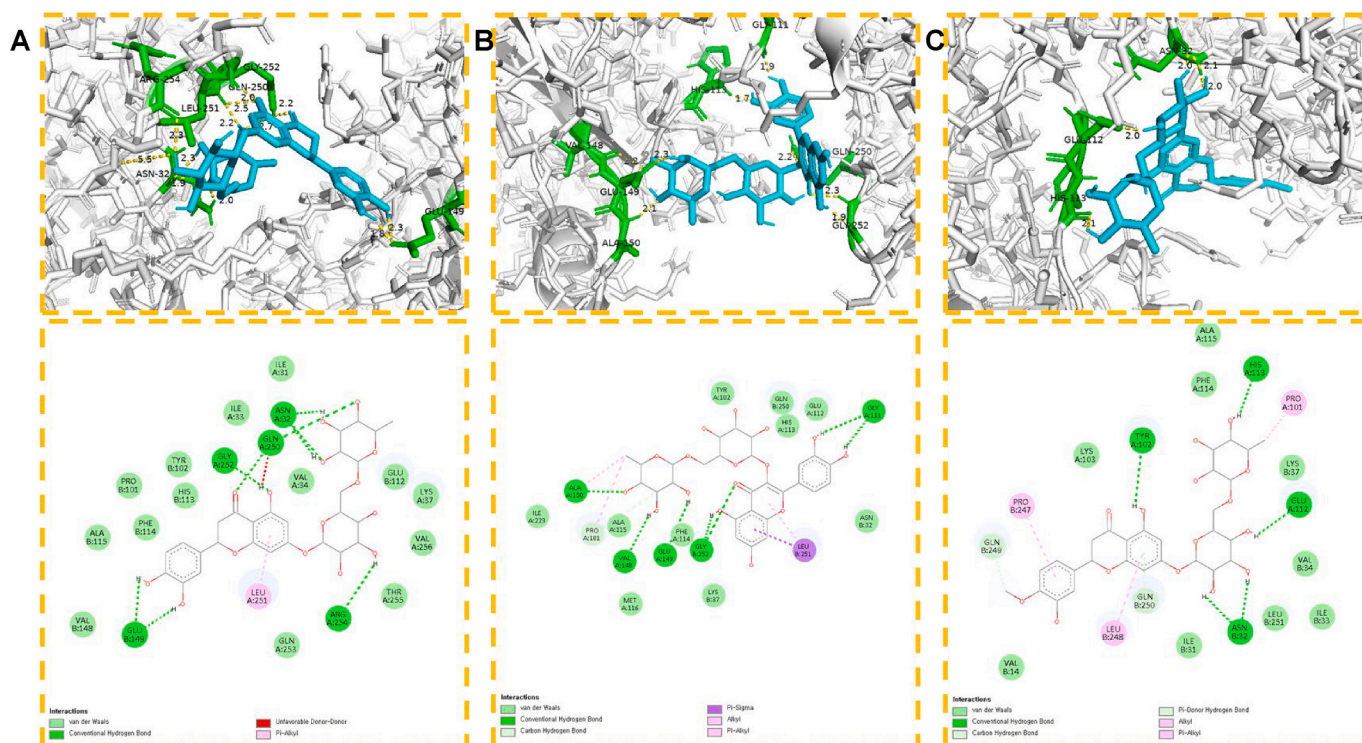


Fig. 5. Molecular docking simulation of interactions between CA4 and the three compounds: eriocitrin (A), rutin (B), and hesperidin (C). The compounds are shown primarily as cyan stick structures located within the catalytic core domain of CA4. Dashed yellow lines indicate hydrogen bonds.

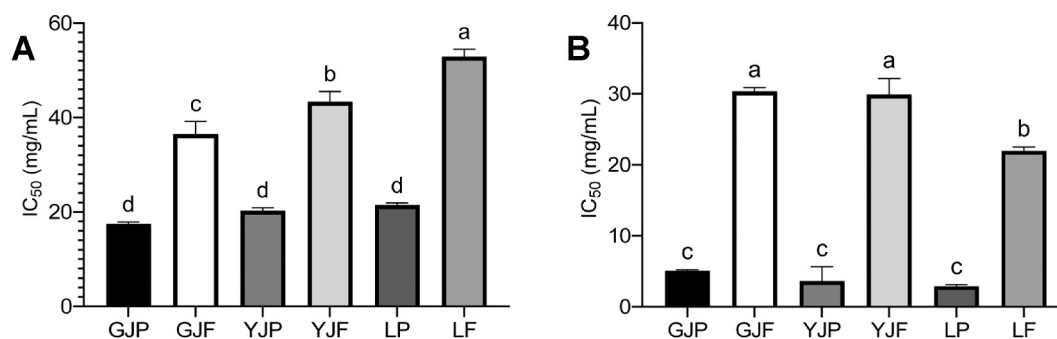


Fig. 6. IC₅₀ values for DPPH (A) and ABTS (B) radical-scavenging activities of GJP, GJF, YJP, YJF, LP, and LF.

^{a-d} Statistical differences within the same graph were determined by Tukey's test. Values not sharing a common letter are significantly different ($p < 0.05$).

Table 3

IC₅₀ values for DPPH and ABTS radical-scavenging activities of compounds 1–3.

Compound	DPPH (IC ₅₀ , mg/mL)	ABTS (IC ₅₀ , mg/mL)
1	0.61 ± 0.01 ^b	0.34 ± 0.01 ^b
2	0.46 ± 0.02 ^b	0.21 ± 0.01 ^c
3	2.34 ± 1.07 ^a	0.53 ± 0.03 ^a

^{a-c} Statistical differences within the same column were determined by Tukey's test. Values not sharing a common letter are significantly different ($p < 0.05$). Compounds: eriocitrin (1), rutin (2), and hesperidin (3).

3.8. Correlation coefficient analysis

To further elucidate the contributions of individual compounds to the observed antioxidant activities, correlation analyses were performed between IC₅₀ values and major flavonoid contents. Interestingly, although hesperidin (3) also exhibited negative correlations, only the association between ABTS IC₅₀ and eriocitrin (1) reached statistical significance ($r = -0.58$, $p < 0.05$) (Fig. 7).

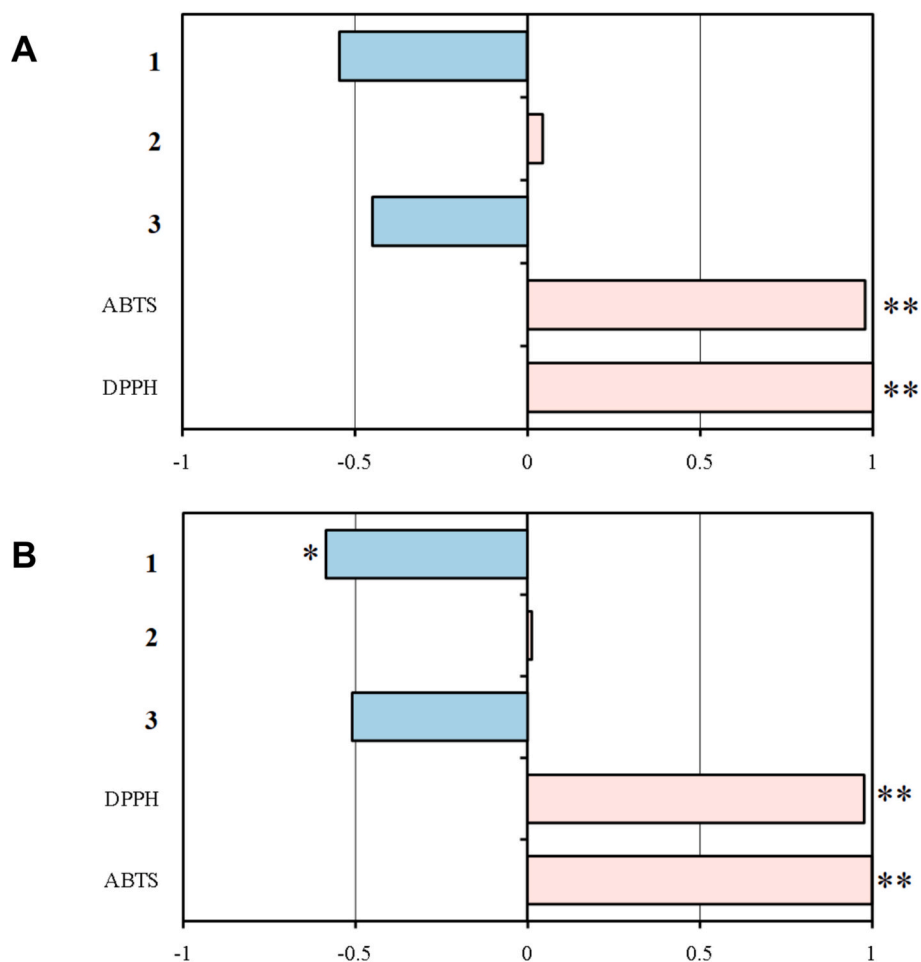


Fig. 7. Correlation of DPPH (A) and ABTS (B) activities with eriocitrin (1), rutin (2), and hesperidin (3). *, $p < 0.05$, **, $p < 0.001$.

4. Discussion

4.1. 'Jeramon' peel as a rich source of phenolic compounds

This study provides the first detailed analysis of TPC and TFC in the peel and flesh of the 'Jeramon' cultivar at different ripening stages. Variations in TPC and TFC have been reported across lemon cultivars. The TPC and TFC of the pulp or flesh of Lisbon cultivar, the variety from which 'Jeramon' was derived, were analyzed in comparison to other lemon cultivars in a previous study (Liu et al., 2024). Lisbon had higher values than other cultivars, except Fino. The results of the present study were significantly higher, more than twice the amount, except for GJF. On the other hand, the TFC values are consistent with those of Liu et al. (2024), wherein TFC are lower than TPC. Their values were also in similar range as that of the present study. These results could be attributed to the differences in the method employed and the standards used.

It was found that the peel of 'Jeramon' generally contains higher TPC than the flesh, while TFC differences are less consistent across ripening stages. These results align with previous reports on citrus fruits, wherein the TPC of various citrus fruits was compared (Costanzo et al., 2022; Czech et al., 2021). Their results were consistent across the different citrus fruits, wherein the peel had significantly higher TPC compared to the flesh and whole fruits. Although the absence of a significant peel–flesh difference in green 'Jeramon' suggests possible cultivar-specific or ripening stage-related variations.

Collectively, these insights highlight a complex interplay between fruit quality traits, metabolic regulation, and environmental adaptability. Importantly, our analysis shows considerable variation in TPC and TFC among samples. Previous studies on lemon have demonstrated that ethylene regulates carotenoid and anthocyanin biosynthesis in a harvest stage-dependent manner, and it is plausible that similar ethylene-mediated shifts in phytochemical accumulation also occurred in 'Jeramon,' contributing to the observed variation in metabolite profiles (Liu et al., 2021). In citrus, ethylene signaling is known to interact with phenylpropanoid metabolism, which governs phenolic and flavonoid biosynthesis, suggesting that hormonal regulation during ripening may partially explain the observed variability. These observations provide a foundation for future studies linking metabolite profiles, genetic regulation, and fruit quality traits in 'Jeramon,' ultimately informing strategies to optimize both nutritional value and marketability.

The analysis also revealed that maturation did not significantly alter TPC or TFC levels in 'Jeramon,' contrasting with the typical reduction of these compounds during fruit ripening (Mansour, 2019). In many citrus fruits, phenolic concentrations decrease with maturation. For example, in navel orange, TPC drops from 18.83 mg gallic acid equivalents (GAE)/g dry weight (DW) in young fruit to 5.69 mg GAE/g DW at commercial maturity ($p \leq 0.01$), which is attributed to the breakdown of soluble phenolic acids and fluctuations in bound fractions during growth (Hou et al., 2021). Recent quantitative assessments in the literature report that standard lemon fruit tissues typically reach a maximum tannin concentration of merely 7.4 mg TAE/g, with leaf extracts yielding 5.9 mg TAE/g (Ali et al., 2024). In contrast, 'Jeramon' exhibited relatively stable TPC and TFC levels throughout maturation, suggesting either sustained phenolic biosynthesis or reduced metabolic degradation during ripening. This stability may result from a slower degradation rate, potentially linked to the unique chemical composition of the phenolic and flavonoid fractions of 'Jeramon.' Furthermore, these stable levels may indicate more effective preservation of these compounds compared to other citrus cultivars. The consistent TPC and TFC in 'Jeramon' could offer benefits for its antioxidant properties, which are critical for its nutritional value and health-promoting effects.

4.2. Comparative flavonoid profiling among green and yellow 'Jeramon' in major flavonoids

The LC-MS/MS results for 'Jeramon' are consistent with those reported in other lemon cultivars (Mateus et al., 2024), reinforcing the potential of lemon byproducts as valuable sources of bioactive compounds with health-promoting properties. The composition of the 'Jeramon' peel and flesh at two distinct ripening stages is summarized in Table 1. Eriocitrin (1) has been reported to improve gut microbiota by reducing harmful bacteria associated with dysbiosis and increasing beneficial strains (Ramos et al., 2023).

Recent studies indicate that rutin (2) may help prevent muscle loss by inhibiting pathways involved in muscle breakdown (Hah et al., 2023). Moreover, rutin (2) has been identified as a major constituent in various medicinal plants, such as *Cyperus exaltatus* (Choi, Lee, et al., 2023) and *Lonicera japonica* (Ma, 2024), highlighting its widespread occurrence in phytomedicine. Beyond its antioxidant and anti-inflammatory properties, novel formulations such as rutin (2) nanocrystals have demonstrated enhanced antibacterial, anticancer, and enzyme inhibitory activities, overcoming its limited solubility and bioavailability (Choi, Lee, et al., 2023).

Hesperidin (3) is well known for mitigating cardiovascular risk factors and has been identified as a citrus-derived flavonoid with potential neuroprotective activity against tau hyperphosphorylation in Alzheimer's disease models (Khorasanian et al., 2023; Kim et al., 2025). Collectively, our findings indicate that compounds 1–3 contribute significantly to the functional potential of byproducts such as the peel, which is often discarded after consumption.

Considering the total content of all three compounds, YJP exhibited the highest overall concentration, followed by GJP, GJF, and YJF, indicating that both tissue type and ripening stage influence cumulative phytochemical content. Representative chromatograms of standards and samples are shown in Supplementary Fig. S2 and Fig. S3. Quantitative HPLC/PDA analysis further confirmed the prominence of eriocitrin (1) and hesperidin (3) in 'Jeramon,' supporting the LC-MS/MS results. These findings are consistent with prior studies on lemons, reinforcing the notion that these two flavonoids are key bioactive markers in lemon-based products (Athanasiadis et al., 2024).

4.3. Network pharmacology

The network pharmacology analysis revealed a considerable overlap between oxidative stress-related targets and the predicted targets of the major flavonoids in the samples. This overlap suggests that these compounds may exert biological activity through multi-target mechanisms rather than a single molecular pathway, which is consistent with pleiotropic effects commonly reported for dietary polyphenols (Chen et al., 2025). CA4, a Zn^{2+} metalloenzyme, was identified as a key potential antioxidant target. Its primary role is maintaining acid-base equilibrium, and disruption of this function can lead to local acidosis, which enhances reactive oxygen species (ROS) generation and oxidative stress (Alfarouk et al., 2020; Sulaiman et al., 2022). Flavonoids are known to inhibit CA isoforms by chelating the Zn^{2+} active site and forming hydrogen bonds (Karioti et al., 2016). Gene ontology and pathway enrichment analysis supported this interpretation as the enriched pathways were primarily associated with carbon dioxide hydration, hormone signaling, and cellular responses to oxidative stress, all of which are processes that can be influenced by polyphenolic antioxidants (Basharat et al., 2025).

4.4. CA4 modulation as an antioxidant mechanism

Molecular docking analysis was subsequently conducted to investigate the binding interaction between CA4 and compounds 1–3 (Sangkaew et al., 2020). All three were accommodated within the catalytic pocket of CA4 and formed multiple stabilizing interactions with

amino acid residues in the active site region. In the case of eriocitrin (1), hydrogen bonding interactions were observed with residues such as ASN32, GLN250, and GLU149, while additional van der Waals bonds were observed with hydrophobic residues, including LEU251 and VAL24, which contributed to stabilization of the ligand-protein complex. Rutin (2) demonstrated an extensive interaction network involving hydrogen bonds with residues such as FLN111 and GLU149, as well as π - π interactions between the aromatic rings of the flavonoid and hydrophobic residues, including LEU251, which suggests a strong binding affinity within the catalytic domain. Similarly, hesperidin (3) exhibited hydrogen bonding interactions with residues including GLU112 and TYR102, together with hydrophobic contacts involving LEU251 and VAL14, which further stabilized its orientation within the binding pocket. These interactions are characteristic of protein-polyphenol complexes, where the multiple hydroxyl groups present in flavonoid structures act as hydrogen bond donors and acceptors while the aromatic rings contribute to hydrophobic π - π stacking interactions (Mutlu et al., 2025; Yilmaz et al., 2022). Similar mechanisms have been widely reported for phenolic compounds interacting with proteins in food systems and biological targets (Fredsgaard et al., 2026).

Among the three compounds, rutin (2) appeared to form the most extensive interaction network with CA4 probably due to its larger glycosylated structure and higher number of hydroxyl groups, which increases its capacity to establish multiple hydrogen bonds within the active site (Lin et al., 2025; Yan et al., 2024). The strong binding interactions observed for these flavonoids have physiological relevance as CA4 plays a key role in carbon dioxide hydration and acid-base homeostasis, processes that are closely linked to metabolic regulation and oxidative stress responses (Hopkins et al., 2022; Occhipinti & Boron, 2019).

Although rutin (2) exhibited the most extensive interaction network within the CA4 binding pocket, including multiple hydrogen bonds and π - π interactions, its concentration in the analyzed samples was relatively low compared with eriocitrin (1) and hesperidin (3). In contrast, eriocitrin (1) and hesperidin (3) were the predominant flavonoids detected, particularly in 'Jeramon' peel, where their concentrations were substantially higher than those of rutin (2). The ability of these flavonoids to form multiple hydrogen bonds and hydrophobic interactions with biologically relevant proteins such as CA4 suggests potential roles in oxidative stress modulation and metabolic regulation. Grounded in this biochemical and *in silico* rationale, the antioxidant capacity of 'Jeramon' extracts was experimentally validated using DPPH and ABTS assays. By stabilizing the extracellular microenvironment pH, these flavonoids suppress acidic stress that promotes ROS-generating enzymatic reactions (Alfarouk et al., 2020). Therefore, the antioxidant potential of 'Jeramon' likely arises from a synergy between chemical radical neutralization and enzymatic microenvironment stabilization.

4.5. Potent antioxidant activity of 'Jeramon' peel extracts

Among all samples, GJP showed the lowest IC₅₀ value in the DPPH assay, while YJP exhibited the strongest activity in the ABTS assay, reflecting some assay-dependent variation in antioxidant potency. These results indicate that antioxidant activity is concentrated in the peel, likely due to higher levels of polyphenolic and other redox-active compounds in the outer tissue layers (Fig. 6; Supplementary Table S6). This observation aligns with studies in other plant systems. For example, *C. exaltatus* shows that specific flavonoids and phenolic acids contribute significantly to antioxidant capacity (Choi, Lee, et al., 2023). Similarly, in *Sambucus pendula*, isolated compounds from potent antioxidant fractions, such as (+)-isolariciresinol and (+)-cycloolivil, significantly enhance DPPH and ABTS radical-scavenging activities and exhibit anti-inflammatory effects (Kang et al., 2024). These findings underscore the major role of specific phenolic or flavonoid constituents in the overall antioxidant potential of plant tissues, including the peel of 'Jeramon.'

Among citrus fruits in the Rutaceae family, lemon exhibits high antioxidant capacity, with ABTS radical scavenging activities of 92.8% when dried and 94.9% when fresh, a property influenced by genetic background (Kim, 2021). While ascorbic acid is traditionally recognized as a major antioxidant contributor in citrus, phenolic compounds, especially flavonoids, also play a crucial role. The relative contribution of these compound classes may vary between studies due to differences in extraction methods and composition profiles (Papoutsis et al., 2016). In the case of 'Jeramon,' understanding the specific contributions of TPC and TFC components will be essential for optimizing its antioxidant potential.

4.6. Correlation coefficient analysis

Although rutin (2) exhibited the highest intrinsic antioxidant activity, correlation analysis between extract composition and overall antioxidant capacity suggested that eriocitrin (1) contributed more substantially to the extract's antioxidant effect. This apparent discrepancy indicates that the antioxidant property of the extract cannot be explained solely by the potency of individual compounds. Instead, the observed activity likely results from the combined influence of constituent abundance, solubility, and synergistic interactions (Caesar & Cech, 2019). Therefore, the antioxidant capacity of the extract should be regarded as a cumulative outcome of complementary effects rather than the independent action of single compounds.

Eriocitrin (1) thus appears to play a key role in the antioxidant activity, particularly as evaluated by the ABTS assay. The discrepancy between DPPH and ABTS results may be attributed to differences in their reaction mechanisms: ABTS radicals are soluble in both aqueous and organic media, allowing interactions with a wider range of antioxidants, whereas DPPH radicals are confined to organic solvents and primarily react with lipophilic antioxidants. Consequently, the ABTS assay is often considered a more comprehensive indicator of total antioxidant capacity, and the strong correlation with eriocitrin (1) further supports its prominent contribution (Sadeer et al., 2020). Nevertheless, the antioxidant potential of the extracts is unlikely to depend solely on a single compound, but rather on the combined and possibly synergistic actions of multiple phenolic and other bioactive constituents. Importantly, the DPPH assay still provides valuable complementary information, as it remains a well-established and standardized method particularly sensitive to lipophilic compounds. The strong correlation observed between DPPH and ABTS IC₅₀ values ($p < 0.001$) further demonstrates that both assays consistently reflect the overall antioxidant potential, justifying their use in parallel (Fig. 7).

5. Conclusions

The peel extracts of 'Jeramon,' a newly developed Korean lemon cultivar, is a promising source of natural antioxidants. Among the different ripening stages, YJP contained the highest total concentration of these key flavonoids, establishing it as the most promising sample for extraction and utilization. Rather than merely profiling its phytochemicals, this study highlights an antioxidant mechanism driven by key flavonoids, primarily eriocitrin and hesperidin, combining direct radical scavenging with indirect modulation of the metalloenzyme CA4. *In silico* analysis demonstrated strong binding affinities between flavonoids such as hesperidin and CA4, suggesting that 'Jeramon' peel may help mitigate oxidative stress by stabilizing the local pH microenvironment. While these findings establish the distinct biochemical behavior and functional potential of 'Jeramon' peel, a limitation of the present study is its primary reliance on *in vitro* and *in silico* models. Therefore, future research should focus on *in vivo* studies and clinical trials to validate the physiological efficacy of this mechanism. Ultimately, 'Jeramon' represents a metabolically stable, high-value lemon cultivar. The resulting peel powders hold strong potential for specific practical applications, such as antioxidant-enriched beverage formulations, natural food preservatives,

and active ingredients in dietary supplements.

CRediT authorship contribution statement

Chang-Dae Lee: Writing – original draft, Validation, Investigation, Formal analysis. **Taesoo Kim:** Validation, Data curation. **Sungwook Chae:** Project administration, Data curation. **Sanghyun Lee:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2026.148948>.

Data availability

Data will be made available on request.

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