



HPLC quantification of carotenoids in astringent and non-astringent persimmon peel and flesh

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ABSTRACT

High-performance liquid chromatography was used to assess carotenoid compositions in the peel and flesh of 25 cultivars from four persimmon types: pollination-constant astringent, pollination-constant non-astringent (PCNA), pollination-variant astringent (PVA), and pollination-variant non-astringent (PVNA). The carotenoid content was assessed across different cultivars, types, and tissue parts (peel and flesh). The comprehensive analysis demonstrated that peel showed consistently higher carotenoid concentrations than flesh, with β -carotene emerging as the most abundant compound, followed by β -cryptoxanthin and α -carotene. Notably, persimmons of the Jinhong and Maegamajiro (PCNA type) had the highest total carotenoid content, while the PVA type exhibited lower concentrations. Variations were more closely associated with cultivar differences than persimmon types. These findings enhance the current understanding of persimmon carotenoid profiles, nutritional profiles, and the influence of key factors on carotenoid content.

1. Introduction

Carotenoids are bioactive compounds that are crucial to human health due to their roles as antioxidants and vitamin A precursors (Bose et al., 2024; Gea-Botella, Moreno-Chamba, et al., 2021). Compounds such as lutein, zeaxanthin, β -cryptoxanthin, α -carotene, and β -carotene are associated with numerous health benefits, including enhanced vision, immune system support, and reduced chronic disease risk (González et al., 2021; Zaghdoudi et al., 2017). Carotenoids have also been shown to protect cells from oxidative damage by neutralizing free radicals (Shin et al., 2020). Elevated serum carotenoid levels are associated with lower all-cause and cardiovascular mortality risks (Zhu et al., 2023). Lutein and zeaxanthin specifically exhibit protective effects against cardiovascular diseases (Wang, Tang, et al., 2023). β -Cryptoxanthin and β -carotene, as provitamin A carotenoids, have been linked to a lower incidence of cardiovascular disease and hypertension (Zhu et al., 2023). Persimmons (*Diospyros kaki*), known as a rich source of carotenoids, are widely consumed owing to their nutritional and sensory qualities (Lee et al., 2001).

Persimmons are important fruit crops in East Asia that have garnered

attention as they are rich in nutrients and bioactive compounds. Their antioxidant properties, attributed primarily to carotenoids, phenolic compounds, and flavonoids, contribute to their popularity among consumers (Hossain et al., 2018). Beyond their nutritional benefits, persimmons exhibit a unique range of flavors and textures depending on their variety and ripening stage, enhancing their appeal among consumers and culinary versatility across various cultures (Jang et al., 2011).

Persimmon varieties are categorized based on pollination effects and variants. They can also be classified into astringent and non-astringent categories, based on the presence of soluble tannins (Dincel et al., 2019). This classification is critical as it affects the chemical composition, taste, and consumer preference for persimmons (Gea-Botella, Agulló, et al., 2021). Moreover, distinguishing between the peel and flesh of persimmons is essential, as these components differ in their phytochemical profiles and potential health benefits (Zhou et al., 2011).

Despite the growing interest in the carotenoid content of persimmons, comprehensive analyses comparing carotenoid profiles across different varieties and fruit parts remain limited (Lalou et al., 2021). Persimmon varieties are categorized into pollination-constant

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(independent of pollination effects) and pollination-variant (affected by pollination) types, and further classified into astringent and non-astringent categories based on their tannin content (Parfitt et al., 2015; Sato & Yamada, 2016). Previous studies have examined carotenoid content. A recent study has reported on the colorimetric analysis of total carotenoid content across four persimmon types (Novillo et al., 2016). However, comprehensive high-performance liquid chromatography (HPLC) profiling that encompasses both moisture levels and astringency types remains limited.

This study addresses this gap in knowledge by employing HPLC to profile carotenoids in persimmon peel and flesh. The primary objective

is to understand variations in carotenoid composition between persimmon peel and flesh across different pollination and astringency types.

2. Materials and methods

2.1. Plant materials

Four specific types of persimmon peel and flesh were supplied by the Pear Research Center, National Institute of Horticultural & Herbal Science, Rural Development Administration, Naju, Korea (Fig. 1). The 25

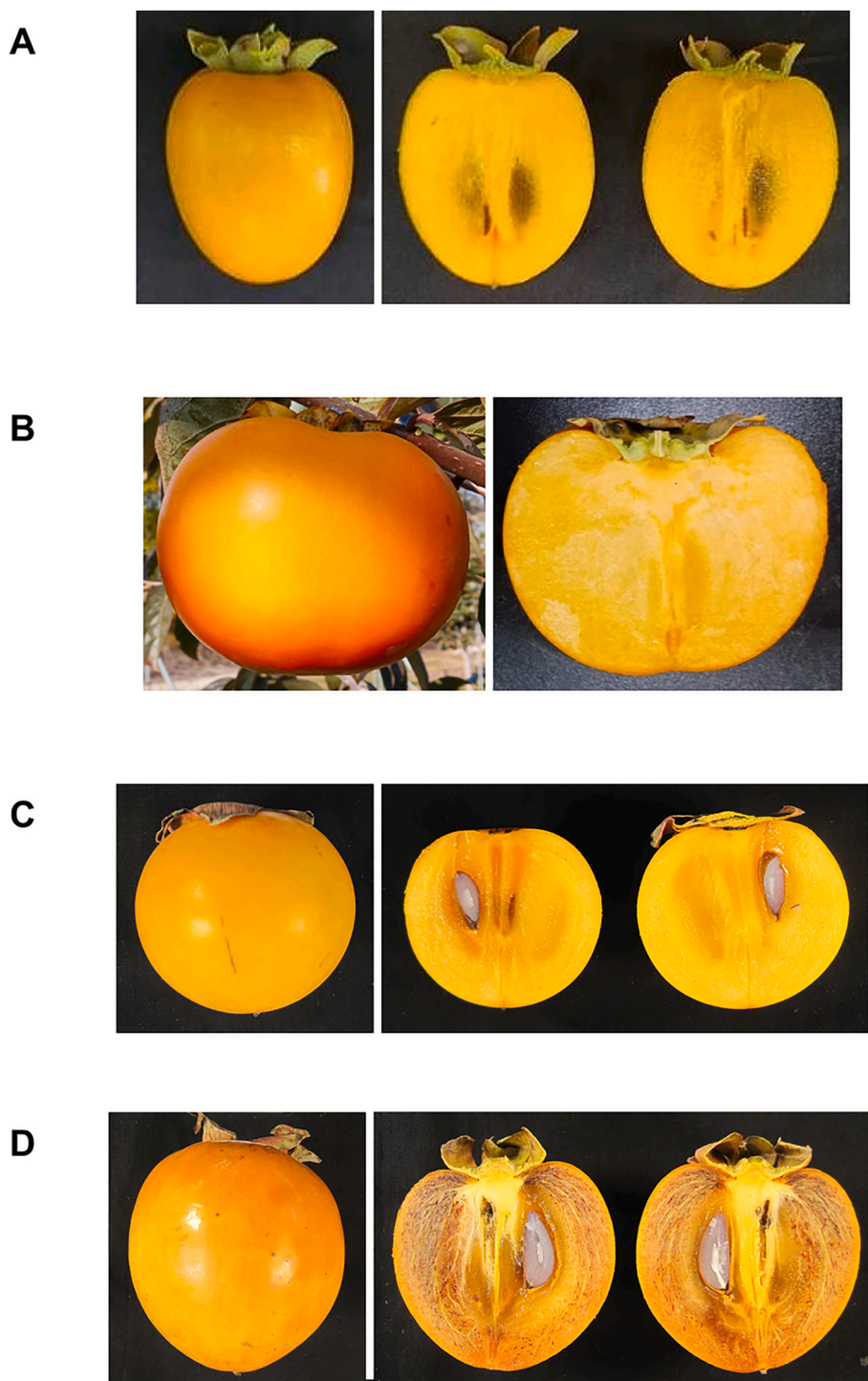


Fig. 1. Cross-section of Deshimaru (A); Jinhong (B); Sangjudungsi (C); and Kyara (D) cultivars showing their cortex.

persimmon cultivars used in this study included early- to late-maturing types harvested from late September to late November 2023; their specific harvest dates (s), weight (g), firmness (N), and soluble solids ($^{\circ}$ Brix) are presented in Table S1. Immediately after harvest, fruits from all cultivars were freeze-dried without a storage period. The four persimmon types were: pollination-constant astringent (PCA), pollination-constant non-astringent (PCNA), pollination-variant astringent (PVA), and pollination-variant non-astringent (PVNA). The corresponding cultivars for each type were as follows: PCA: Deshimaru; PCNA: Gampung, Ro-19, Romang, Bongwhang, Fuyu, Yeonsu, Allflesh, Wonmi, Maegamajiro, Jowan, Jinhong; PVA: Sangjudungsi, Spurihiratanenasi, Partner, Honeyman; PVNA: Kyara, Daeandangam, Migamjosaeng, Sacheonchalgam, Nishimurawase, Chosi, Kurogaki, RN252 (Fig. S1).

2.2. Instruments and reagents

HPLC was performed using the Agilent 1260 Infinity II Quat Pump system (Santa Clara, CA, USA), Quat with pump, autosampler, and Agilent variable wavelength (VW) detector (Santa Clara, CA, USA). HPLC-grade solvents, including methanol (MeOH), ethyl acetate, and water, were purchased from J. T. Baker (Radnor, PA, USA), while trifluoroacetic acid (TFA) was purchased from Thermo Fisher Scientific (Cleveland, OH, USA). Standard compounds, including lutein (1), zeaxanthin (2), β -cryptoxanthin (3), α -carotene (4), and β -carotene (5) (Fig. S2), were provided by the Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea.

2.3. Preparation of sample and standard solutions

Each 200 mg portion of freeze-dried powder was dissolved in a solvent mixture of chloroform, methanol, and tetrahydrofuran (3:6.5:0.5) to prepare a solution with a final concentration of 50 mg/mL. The mixture was then extracted for 30 min at room temperature using an ultrasonic bath. The extracted sample was filtered through a 0.22 μ m polytetrafluoroethylene (PTFE) membrane filter to obtain the test solution. The standard substance was weighed accurately and dissolved in a solvent mixture to prepare the standard stock solution. The mixture was completely dissolved using an ultrasonic bath, followed by filtration through a 0.22 μ m PTFE membrane filter. The stock solution was diluted when necessary to prepare the working standard solution for subsequent analysis.

2.4. HPLC/UV conditions

HPLC analysis was conducted using an Agilent 1260 Infinity II Quat Pump equipped with a VW detector and a YMC carotenoid column (4.6 \times 250 mm, 3 μ m). The column oven temperature was maintained at 30 $^{\circ}$ C. The injection volume was set at 10 μ L, and the mobile phase consisted of a gradient system with solvent A (95 % MeOH) and solvent B (ethyl acetate), with a flow rate of 1.0 mL/min. The mobile phase gradient was as follows: 0 min, 25 % B; 13 min, 25 % B; 23 min, 46 % B; 37 min, 60 % B; 40 min, 95 % B; 45 min, 95 % B; 48 min, 25 % B; and 60 min, 25 % B.

2.5. Calibration curve

Stock solutions of standard compounds were serially diluted to five different concentrations to construct calibration curves. The calibration curve linearity was determined based on the correlation coefficient (r^2), and compound levels were quantified from extracted samples. The calibration functions for the five compounds were derived from the peak area (Y), concentration (X, μ g/mL), and mean ($n = 3$) \pm standard deviation values (Table 1).

Table 1

Calibration curve equation for lutein (1), zeaxanthin (2), β -cryptoxanthin (3), α -carotene (4), and β -carotene (5).

Compound	t_R^a	Calibration equation ^b	Correlation factor, r^{2c}
1	10.9	$Y = 110.65 \times - 41.892$	0.9992
2	13.7	$Y = 80.382 \times - 151.2$	0.9955
3	22.8	$Y = 7.283 \times - 0.7482$	0.9986
4	26.6	$Y = 94.601 \times + 71.971$	0.9964
5	29.5	$Y = 31.084 \times + 1.0788$	0.9999

^a t_R = retention time.

^b Y = peak area, X = concentration of the standard (μ g/mL).

^c r^2 = correlation coefficient for five calibration data points.

2.6. Statistical analysis

Results were reported as mean \pm standard deviation values and were derived from three independent trials (triplicates). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons (Table 2) using GraphPad Prism (version 8.0.2) statistical software (GraphPad Software, Boston, MA 02110, USA). To evaluate trends in carotenoid content between peel and flesh tissues across 25 persimmon cultivars, correlation coefficient analysis, and principal component analysis were performed for heatmap generation and cluster analysis, respectively, using MetaboAnalyst (version 6.0) (Pang et al., 2024).

3. Results

3.1. Carotenoid content in persimmon peel and flesh

The quantitative analysis highlighted notable differences in the carotenoid content between persimmon peel and flesh. The data, presented in Figs. S3, S4, and 2, emphasize the contrasting carotenoid profiles between the peel and flesh of each persimmon variety. Among the four persimmon types (Fig. 2A), Jinhong cultivar (275.9 μ g/g DW) and Maegamajiro cultivar (263.7 μ g/g DW) persimmons of the PCNA type exhibited the highest overall carotenoid concentrations in their peel, with Maegamajiro cultivar also showing a high carotenoid content in its flesh (82.1 μ g/g DW) (Table S2). The Migamjosaeng cultivar exhibited the lowest total carotenoid content (22.3 μ g/g DW) (Table S2). Zeaxanthin was notably abundant only in the flesh of the Wonmi, Maegamajiro, and Deshimaru cultivars. While zeaxanthin was present at trace levels in the flesh of most other cultivars, the lowest total carotenoid content (2.8 μ g/g DW) among the five analyzed carotenoids was observed in the Tailihong cultivar (Table S3).

3.2. Variations among different persimmon types

In persimmons of the PCA type (Fig. 2), represented by the

Table 2

One-way ANOVA results for carotenoid content in persimmon peel and flesh samples.

	Sample part	Significance		
		Compound	F	p
One-way ANOVA ^a	Peel	lutein	7,159	< 0.0001
		zeaxanthin	11,808	< 0.0001
		β -cryptoxanthin	1,439	< 0.0001
		α -carotene	6,944	< 0.0001
		β -carotene	4,070	< 0.0001
	Flesh	lutein	–	–
		zeaxanthin	398,555	< 0.0001
		β -cryptoxanthin	345.5	< 0.0001
		α -carotene	40,950	< 0.0001
		β -carotene	296.3	< 0.0001

^a Mean separation by Tukey's multiple range test at $p < 0.05$.

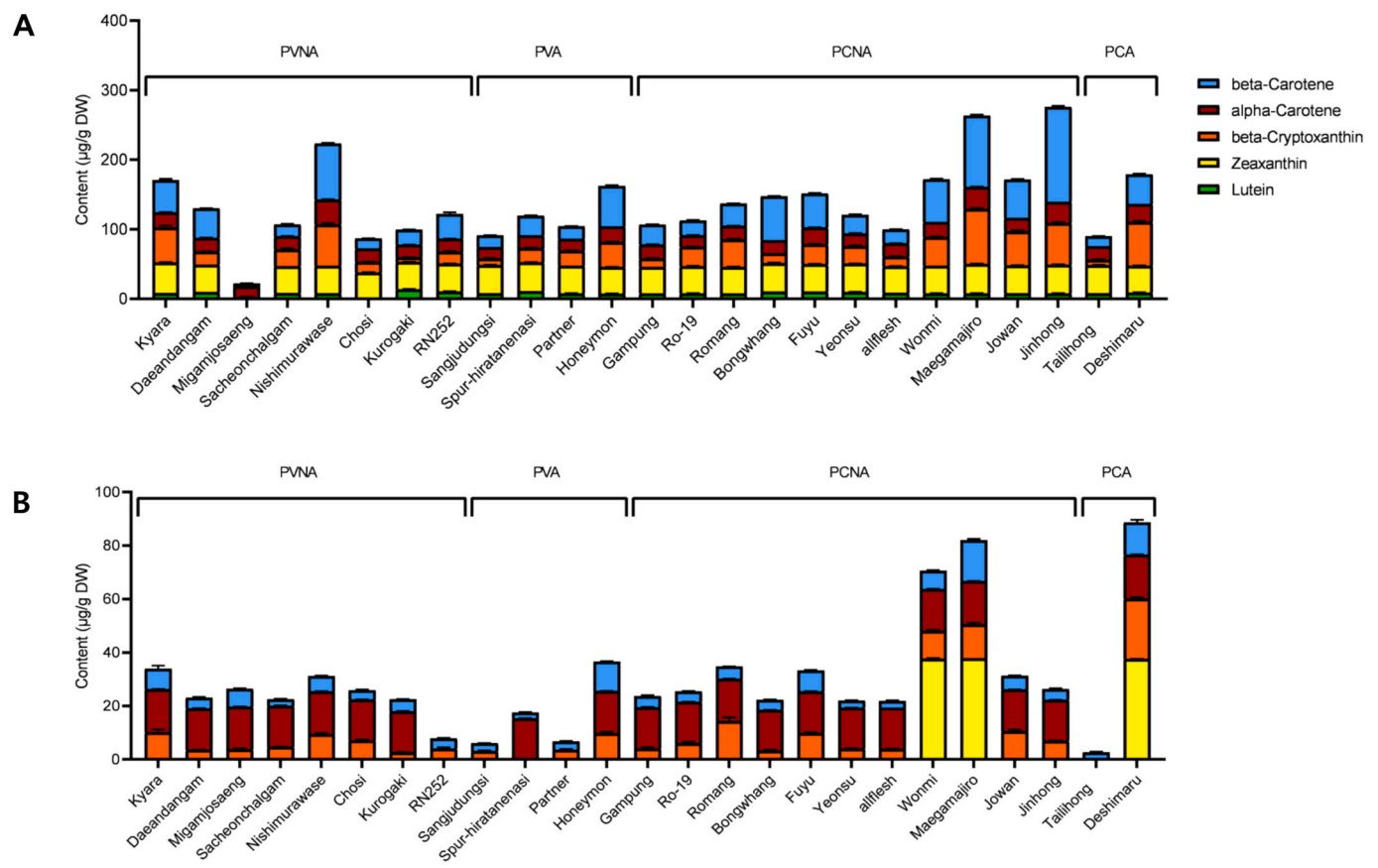


Fig. 2. Carotenoid content in persimmon peel (A) and flesh (B) of different cultivars. Each bar represents mean \pm SD values.

Deshimaru cultivar, the total carotenoid content was markedly higher in the peel (179.4 $\mu\text{g/g DW}$) compared to the flesh (88.7 $\mu\text{g/g DW}$). Persimmons of the PCNA type displayed considerable variability in carotenoid content among cultivars. Peel carotenoid levels ranged from 99.7 $\mu\text{g/g DW}$ (Allflesh cultivar) to 275.9 $\mu\text{g/g DW}$ (Jinhong cultivar). Notably, β -carotene was the predominant carotenoid found in most

PCNA peel, with Jinhong exhibiting the highest concentration of β -carotene (136.4 $\mu\text{g/g DW}$). The carotenoid content in the flesh of PCNA varieties was generally lower than in the peel, ranging from 21.9 $\mu\text{g/g DW}$ (Allflesh cultivar) to 82.1 $\mu\text{g/g DW}$ (Maegamajiro cultivar). β -Cryptoxanthin consistently emerged as the major carotenoid in PCNA flesh, with concentrations ranging from 15 to 16 $\mu\text{g/g DW}$ across

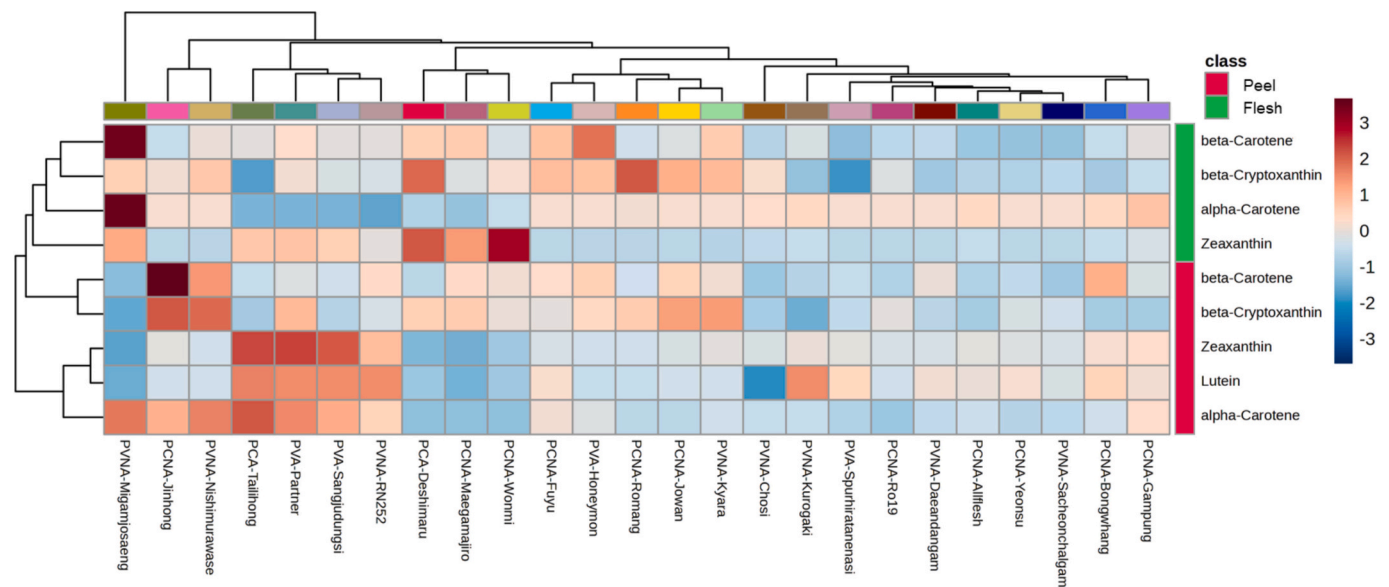


Fig. 3. Heatmap analyses of Pearson's correlation coefficient (r) for the targeted carotenoids in persimmon peel and flesh across different cultivars. Red and blue colors indicate positive and negative normalized responses between the targeted carotenoids content. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cultivars.

Persimmons of the PVA type showed intermediate carotenoid levels compared to PCA and PCNA types (Table S4). The peel carotenoid content ranged from 91.2 $\mu\text{g/g}$ DW (Sangjudungsi cultivar) to 162.5 $\mu\text{g/g}$ DW (Honeymon cultivar). The flesh of PVA varieties contained notably lower total carotenoid levels, ranging from 6.0 $\mu\text{g/g}$ DW (Sangjudungsi cultivar) to 36.6 $\mu\text{g/g}$ DW (Honeymon cultivar). PVNA-type persimmons exhibited diverse carotenoid profiles (Table S5). Peel carotenoid content varied widely, from 87.1 $\mu\text{g/g}$ DW (Chosi cultivar) to 223.4 $\mu\text{g/g}$ DW (Nishimurawase cultivar). Flesh carotenoid levels in PVNA types were generally higher than in PVA types, ranging from 7.9 $\mu\text{g/g}$ DW (RN252 cultivar) to 35.0 $\mu\text{g/g}$ DW (Kyara cultivar).

3.3. Heatmap responses of the targeted carotenoid contents in persimmon peel and flesh across cultivars

A normalized heatmap matrix evaluated carotenoid content trends in 25 persimmon cultivars, distinguishing between peel and flesh tissues (Fig. 3). The analysis revealed differential carotenoid profiles across cultivars and four persimmon types. The normalized heatmap further demonstrated that the peel generally exhibited higher concentrations of β -carotene and lutein compared to the flesh, while α -carotene and zeaxanthin contents varied significantly depending on the cultivar. Moreover, clustering analysis revealed close grouping of the PCA and PCNA cultivars, reflecting similar carotenoid profiles, while PVA and PVNA types formed relatively distinct clusters. These findings indicate that carotenoid biosynthesis and accumulation are influenced by tissue types, and genetic and environmental variability of individual cultivars (Fig. 3).

3.4. Principal component analysis of carotenoid profiles across persimmon cultivars and tissue types

A principal component analysis score plot was applied to evaluate variations in carotenoid profiles across persimmon peel and flesh among different cultivar types. The principal component analysis score plot explained 48.8 % and 28.8 % of the total variance for principal components (PC) 1 and 2, respectively, in the peel (Fig. 4A), and 67.9 % and 26.6 % for PCs 1 and 2, respectively, in the flesh (Fig. 4B).

The principal component analysis plot reveals overlapping

distributions among cultivar types (PCA, PCNA, PVA, and PVNA) in both peel and flesh, indicating no strong distinction between carotenoid profiles of cultivar types. While specific cultivars, particularly in the flesh, reveal some clustering tendencies, the overall separation remains unclear.

3.5. Correlation between astringency and carotenoid levels

Peel consistently exhibited higher carotenoid levels than flesh across all persimmon types, a finding consistent with previous research (Yaqub et al., 2016). This distribution probably reflects the protective role of carotenoids against UV radiation and oxidative stress in fruit peel (Gea-Botella, Moreno-Chamba, et al., 2021). β -Cryptoxanthin and α -carotene emerged as the predominant carotenoids across most varieties, particularly in the flesh, which is consistent with the findings of previous studies on persimmon carotenoid composition. The elevation in the levels of these compounds is attributable to their antioxidant and pro-vitamin A activities (Hosseinienejad et al., 2022). Notably, PCNA types, especially Jinhong and Maegamajiro cultivars, showed the highest overall carotenoid content. This finding has potential implications for breeding programs targeting enhanced nutritional value in persimmons. The observed variability in carotenoid profiles across persimmon types and cultivars underscores the role of genetic factors and pollination characteristics in carotenoid biosynthesis. This diversity provides opportunities for selective breeding to optimize carotenoid content and composition in persimmon fruits (Yaqub et al., 2016).

4. Discussion

Saponification is commonly employed in carotenoid analysis to remove interfering lipids, fatty acid esters, and chlorophyll pigments, thereby simplifying chromatograms and improving quantification (Generalić Mekinić et al., 2023). However, it can also induce carotenoid degradation through isomerization, oxidation, and decomposition under alkaline and oxidative conditions, particularly affecting sensitive xanthophylls such as lutein and zeaxanthin, and may result in the formation of artefacts (Jing et al., 2023; Neves et al., 2024; Rodriguez-Amaya & Kimura, 2004). Instead, carotenoids were extracted directly with chloroform, MeOH, and tetrahydrofuran, which effectively prevent emulsion formation, often associated with other solvent systems. According to De

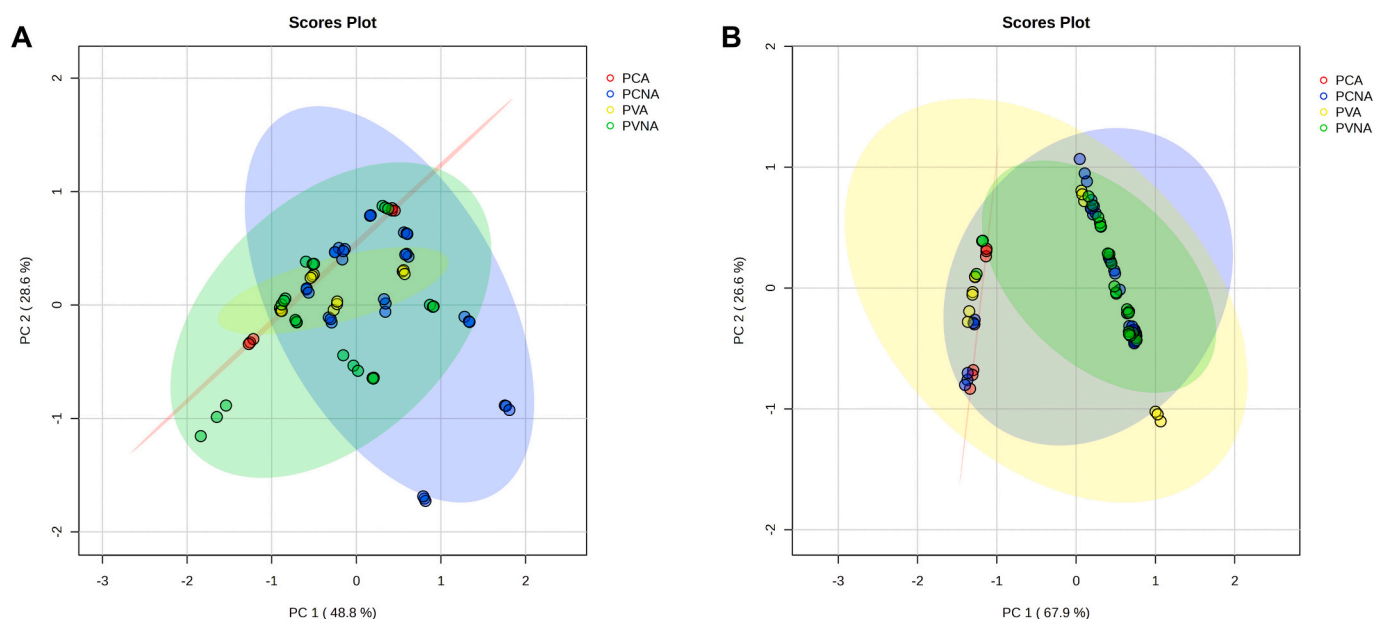


Fig. 4. Principal component analysis score plot of targeted carotenoids in persimmon peel and flesh across different cultivars: Analysis of peel (A) and flesh (B). The plot illustrates the distribution of carotenoid content by cultivar type.

Ritter and Purcell (1981), the primary purpose of saponification is to remove interfering lipophilic components such as neutral fats, fatty acids, and esters, thereby simplifying the chromatogram (Fisher & Rouseff, 1986). However, the optimized chromatography method in this study was provided for the comparison of cultivars.

Carotenoid levels in persimmons vary remarkably based on cultivar, variety, and environmental factors (Hosseinienejad et al., 2022; Zhou et al., 2011). In this study, non-astringent persimmons exhibited elevated β -carotene levels, consistent with earlier findings that linked increased β -carotene and reduced zeaxanthin levels to reflective foil exposure (Bae et al., 2022; Smrke et al., 2019). Such results underscore the effects of environmental factors such as light exposure on carotenoid biosynthesis. Additionally, higher concentrations of carotenoids in peel compared to flesh align with prior studies highlighting their protective role in outer fruit layers, particularly against oxidative stress and UV radiation (Gea-Botella, Moreno-Chamba, et al., 2021).

Although carotenoid compositions varied among samples, these differences were not statistically significant, as shown by principal component analysis and Pearson's correlation heatmap analysis. The clustering patterns in the principal component analysis plot indicated a considerable overlap among samples, suggesting that variations in individual carotenoid levels did not lead to distinct grouping by cultivar or astringency type. The heatmap further revealed positive correlations between specific carotenoids, suggesting that their biosynthesis and accumulation are influenced by independent factors unique to particular cultivars rather than shared metabolic pathways.

The results also highlight the critical role of cultivar selection in optimizing carotenoid content. Variations in biosynthetic pathways, influenced by genetic and environmental factors, present opportunities for selective breeding to enhance nutritional value (Wang, Suo, et al., 2023). Beyond their biological importance in plants, carotenoids have immense industrial value. Carotenoids have applications across multiple industries, serving as natural colorants in food and beverages (Lourenço-Lopes et al., 2022), and as nutritional supplements that support cardiovascular health and reduce chronic disease risk (Fiedor & Burda, 2014; Focsan et al., 2021). Additionally, carotenoids are also used in cosmetics and pharmaceuticals, where they contribute to skin health, UV protection, and anti-aging benefits (Baswan et al., 2021; Mendes-Silva et al., 2020). Recent advancements in sustainable production methods, including algae cultivation and biotechnological synthesis, underscore the growing significance of carotenoids across industries (Dansou et al., 2023).

The composition of carotenoids, including key compounds such as β -cryptoxanthin, lutein, zeaxanthin, and β -carotene, varies across cultivars and contributes significantly to their nutritional value (Novillo et al., 2013). Notably, β -cryptoxanthin is recognized as the predominant carotenoid in persimmons, frequently exceeding β -carotene levels across cultivars and tissue types (Zhou et al., 2011). Reported concentrations range from 60.91 to 940.61 $\mu\text{g}/100\text{ g FW}$ in flesh and up to 8.36 $\text{mg}/100\text{ g FW}$ in peel, far surpassing typical β -carotene levels, which often remain below 12 $\mu\text{g}/100\text{ g FW}$ (Zhou et al., 2011). This dominance, alongside its role as a potent provitamin A compound with high bioavailability, highlights β -cryptoxanthin's critical contribution to both the color and nutritional value of persimmons. β -Cryptoxanthin is generally considered better than β -carotene in terms of bioavailability and safety, especially when consumed as part of a diet rich in fruits like persimmons (Olmedilla-Alonso et al., 2020). Both contribute to vitamin A status and antioxidant protection, but β -cryptoxanthin's superior absorption and potential health benefits make it particularly valuable (Gebregziabher et al., 2023). The discrepancy found in these results can be attributed to the different methods employed (Oliver et al., 1998); primarily, saponification was not conducted in the present study.

Freeze-drying does affect HPLC analysis results for carotenoids in persimmon, but the impact is relatively minor compared to other drying methods. It may cause a small reduction in total carotenoid recovery and potentially alter the profile of individual carotenoids (Seregelyj et al.,

2022; Kayacan et al., 2020). Powdering with liquid nitrogen is preferable when the most accurate quantification of carotenoids is required, as it minimizes degradation and loss prior to HPLC analysis. For most practical purposes, freeze-drying is acceptable, but for rigorous comparative or quantitative studies, liquid nitrogen powdering is recommended.

A previous study of 10 persimmon cultivars across four types revealed that differences in carotenoid accumulation, flesh firmness, and acetaldehyde production during ripening were cultivar-specific and independent of the astringency type (Novillo et al., 2016). Certain results of previous studies align with those of the present study, where the carotenoid content varied more by cultivar rather than astringency type. Additionally, the effects of astringency removal treatments such as CO_2 -destringency and high-pressure processing on carotenoid content are cultivar-dependent (Plaza et al., 2012). Furthermore, PCA and PCNA persimmons exhibit distinct metabolic profiles, potentially affecting carotenoid content, although further research is necessary to clarify the genetic factors involved in their synthesis. This complex relationship between astringency type and carotenoid composition needs to be examined through further studies to understand their interactions and implications. The biosynthesis of carotenoids involves enzymes such as phytoene synthase and lycopene β -cyclase, which are unrelated to the pathways producing tannins (Mora et al., 2022; Ye et al., 2022; Zhou et al., 2022). Although astringency has not been linked to carotenoid biosynthesis in persimmons, recent insights into tannin metabolism offer a possible basis for mechanistic speculation. Astringency is primarily caused by high levels of soluble condensed tannins, or proanthocyanidins, localized within tannin cell vacuoles (Amorim et al., 2023). These compounds are flavonoid-derived phenolics produced via the phenylpropanoid pathway, which may share regulatory nodes with the carotenoid biosynthetic pathway, especially in the context of ripening-related signals such as ABA and ethylene (Zhou et al., 2022). Notably, Tessmer et al. (2016) reported that soluble tannins are initially abundant in astringent cultivars and gradually become insolubilized during fruit maturation. Non-astringent cultivars, in contrast, exhibit faster tannin insolubilization, even in early developmental stages. This difference in soluble tannin dynamics may alter cellular oxidative states or developmental signaling cues that in turn affect carotenoid accumulation. Based on these observations, this study hypothesizes that high levels of soluble tannins in immature or astringent cultivars may modulate carotenoid biosynthesis indirectly through redox balance or hormonal cross-talk. However, the absence of a clear correlation between astringency type and carotenoid content in our results suggests that cultivar-specific regulatory mechanisms may override such effects. Future studies incorporating quantification of equimolar soluble tannins and transcriptomic analysis of carotenoid- and tannin-related genes would help clarify this relationship.

Moreover, by-products from persimmon juicing processes have been shown to retain significant carotenoid levels, with β -cryptoxanthin emerging as the predominant compound (Gea-Botella, Agulló, et al., 2021). However, variations in reported concentrations may occur because of differences in methodologies, including solvent extraction techniques.

5. Conclusions

This study evaluated the carotenoid content in various persimmon cultivars grouped by astringency using HPLC. Carotenoids were generally more concentrated in the peel than flesh. β -Carotene emerged as the predominant compound, followed by α -carotene and β -cryptoxanthin. Among PCNA persimmons, the Jinhong and Maegamajiro cultivars exhibited the highest total carotenoid content, while PVA types showed lower concentrations. Carotenoid accumulation patterns appeared to be more strongly influenced by cultivar differences than by astringency. This study is the first to quantify carotenoids in different persimmon cultivars using HPLC rather than conventional colorimetric methods,

offering improved specificity and accuracy. Although the analysis was performed using unsaponified extracts to avoid potential carotenoid degradation, the absence of a direct comparison with saponified counterparts limits the ability to definitely conclude that saponification is unnecessary. Future studies should include parallel analysis of saponified and unsaponified samples to fully assess recovery, isomerization, and ester profiles. Despite this limitation, the findings contribute valuable insights into carotenoid distribution in persimmons, with potential applications in food science, nutrition, horticulture, and breeding programs.

CRediT authorship contribution statement

Chang-Dae Lee: Investigation. **Hak-Dong Lee:** Investigation. **Gyeong-Bok Ma:** Resources, Project administration. **Byulhana Lee:** Writing – original draft, Resources, Conceptualization. **Sanghyun Lee:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that influenced the findings 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.2025.145880>.

Data availability

Data will be made available on request.

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