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Correlation of Polyphenols, Pectin Substances and Antioxidant Activity of Persimmon Fruits (Diospyros kaki L.) According to Ripening Stage and Processing Conditions

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Abstract: Persimmon (Diospyros kaki), a member of the Ebenaceae family, has been cultivated in Georgia since the 1950s, with an annual production of approximately 50,000 tons. Among the commercially important cultivars, the astringent 'Hachiya' and the non-astringent 'Hyakume' are predominant. Persimmon fruits are characterized by high nutritional value and are rich in bioactive compounds, particularly phenolic compounds and dietary fiber. The present study investigated the chemical composition and antioxidant capacity of 'Hachiya' and 'Hyakume' fruits at different stages of ripeness and after various pre-treatments (CO_2 exposure, freezing, and thermal treatment). The quantitative determination included sugars, total polyphenols, pectin substances, titratable acidity, vitamin C, carotenoids, cellulose, total nitrogen, and protein content. Significant variability in phenolic compounds and pectin fractions was observed during ripening and following pre-treatment. A correlation between pectin content and tannin-related astringency was established. During ripening, the formation of insoluble pectin–tannin complexes reduced the level of soluble tannins and consequently decreased bitterness. Antioxidant activity, evaluated by DPPH and ABTS assays, was strongly correlated with total polyphenol content. Overall, both ripening stage and pre-treatment significantly affect the chemical composition and antioxidant potential of persimmon fruits. Fruits at the technical ripeness stage represent a particularly rich source of polyphenolic compounds, especially tannins, which may largely determine the health-promoting properties of persimmon.

Keywords: Persimmon, Polyphenols, Pectin substances, Antioxidant activity, Ripening

Introduction

Persimmon (Diospyros kaki L.), a member of the Ebenaceae family, was originally cultivated in China and Japan and is now grown worldwide, with 90% of global production concentrated in China, Japan, and Korea. World persimmon production amounts to approximately five million tons annually. In Georgia, persimmons are widely

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distributed in the Black Sea coastal regions (Giordani, 2002; Denev & Yordanov, 2013; Ardzenadze et al., 2018). The fruit develops initially with a greenish color; as it matures, chlorophyll is degraded and carotenoids alter its color from yellow to orange. The color of the skin and consistency of the pulp serve as indicators of ripeness (Salvador et al., 2006).

Two primary types of persimmons are recognized: astringent and non-astringent. Astringent varieties have a high content of soluble tannins. Persimmon fruits are classified according to their tannin content: fruits containing 0.1–0.2% tannins are slightly astringent, those with less than 0.1% are virtually non-astringent, and fruits with more than 0.2% tannins are highly astringent. Astringency results from the binding of soluble tannins to salivary proteins, producing a rough sensation in the mouth.

Persimmon fruits are rich in a diverse array of nutrients and phytochemicals, including carbohydrates, vitamins, proanthocyanidins, flavonoid oligomers, tannins, phenolic acids, dietary fiber, and carotenoids, which contribute significantly to their flavor, color, and nutritional and medicinal value. Total phenolic content varies between cultivars and is characterized by high levels of dietary fiber, tannins, polyphenols, flavonoids, and carotenoids (Yoo et al., 2019; Suzuki et al., 2015; Del Bubba et al., 2009).

The accumulation of biologically active substances and the physiological activity of persimmon fruits depend on variety and time of harvest. The stage of maturity is a critical factor determining organoleptic and functional properties and significantly affects the concentration of phenolic compounds (Senter et al., 1991; Kondo et al., 2004; Baltacioğlu & Artik, 2013).

Dietary antioxidants, particularly phenolic compounds, play an important role in preventing oxidation of low-density lipoprotein cholesterol (LDL-C) and inhibiting the development of atherosclerosis. A diet rich in vegetables and fruits is associated with significantly reduced coronary artery mortality (Hertog et al., 1993; Paganga et al., 1999). Dietary fiber has been linked to the prevention and treatment of several conditions, including diverticular and coronary heart disease (Anderson et al., 1994; Grigelmo-Miguel et al., 1999).

There is substantial evidence that antioxidants in food sources play a critical role in maintaining health and preventing disease. Natural antioxidants have attracted increasing interest, as they can provide bioactive compounds capable of preventing many chronic diseases. Processing or industrialization through jams, jellies, juices, dried persimmons, spirits, and vinegars is one approach to increase accessibility to consumers and add value to the final product (González et al., 2015; Sharma et al., 2017; Ardzenadze et al., 2025).

Although persimmon fruit contains significant amounts of biologically active compounds, difficulties often arise in the production of products with high organoleptic characteristics. It is therefore necessary to study the variability in polyphenol and pectin content in relation to ripening and varietal characteristics to facilitate rational selection of raw materials. Various technological processing methods are needed to produce pastes, purées, and glucose–fructose extracts with selected physicochemical and organoleptic properties.

Astringency can be effectively reduced by soaking in warm water, which promotes binding and precipitation of tannins with endogenous macromolecules. CO_2 treatment inhibits aerobic respiration and forces anaerobic respiration, leading to acetaldehyde accumulation, insoluble complex formation, and elimination of astringency. Furthermore, astringency can also be reduced by freezing and thawing, even though acetaldehyde accumulates in only small quantities under these conditions (Ding et al., 2025; Ozdemir et al., 2020; Han et al., 2022).

The aim of this study was to investigate changes in the composition of phenolic and pectin substances in two persimmon varieties grown in Adjara (Black Sea region of Georgia) during on-tree ripening and after harvesting under different processing conditions.

Materials and Methods

Plant Material and Sample Collection

For the purposes of this study, persimmon (Diospyros kaki L.) ‘Hachiya’ and ‘Hyakume’ varieties were collected in the Khelvachauri municipality (Akhalsheni) of the Adjara region, Georgia, in the 2025 harvest year. Selection criteria included a yellow-green hue at the early stage of ripening, a pronounced yellow-orange hue at the harvest (technical ripeness) stage, and a dense texture. To study variation in phenolic compound content at different ripening stages, analytical samples were taken from the same trees every 15 days. For processing experiments,

'Hachiya' fruits were sorted by size (170–200 g), color uniformity, and absence of surface defects. Fruits were divided into four batches of 20 fruits each and subjected to: (1) untreated harvest-stage control; (2) CO_2 atmosphere (88%) for 60 h; (3) hot water treatment at 45°C for 12 h; (4) freezing at −20°C for 20 h. After processing, juice and pomace were obtained by direct pressing.

Chemicals

The following reagents were used: Folin–Ciocalteu's phenol reagent (Sigma-Aldrich, BCCH3316), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), vanillin (Sigma-Aldrich, V1104-100G), chlorogenic acid (Sigma-Aldrich, Lot#SLBJ3632V), caffeic acid (ROTH, Art. Nr. 5869.3), (+)-catechin hydrate (Biosynth, FC30661), β-carotene (Sigma-Aldrich, C4582), n-hexane (Merck, CAS-No: 110-54-3), acetic acid (Merck, CAS-No: 64-19-7), methanol (Roth, Art.-Nr. 8388.6).

Standard Methods of Chemical Analysis

Dry matter was determined by the thermogravimetric method; water-soluble dry matter using a digital refractometer (Carl Zeiss, Germany); total titratable acidity and pH using an automatic titrator (Mettler-Toledo AG, Switzerland) with 0.1 N NaOH titration against malic acid. Sugars and pectin fractions were determined according to AOAC Official Methods (Association of Official Analytical Chemists, 2005; John Bean Technologies Corporation, 2011).

Determination of Polyphenolic Substances

Tannins in persimmon fruit are present in both free (soluble) and bound (insoluble) forms, determined by oxidation with potassium permanganate in the presence of indigo carmine. A 25.0 g analytical sample was weighed, transferred to a 250 mL volumetric flask, extracted with 200 mL water at 80°C for 30 min, made up to 250 mL, filtered, and the free tannin percentage (Extract I) was determined. For bound tannins, 10 mL of 1% NaOH was added to the residue, extraction was performed under reflux, and the extract was neutralized with 1% H_2SO_4 and made up to 250 mL (Extract II). In both cases, 10 mL of extract was titrated with 0.1 N $KMnO_4$ in the presence of indigo carmine until the blue color turned amber, using the conversion coefficient 0.004157.

Total Phenolic Content

Total phenolic content was determined by the Folin–Ciocalteu method: 1 mL Folin–Ciocalteu reagent was added to 1 mL extract, followed after 3 min by 1 mL of 10% Na_2CO_3, and the volume was made up to 10 mL. Absorbance was measured at 750 nm after 90 min. Results were expressed as chlorogenic acid equivalents (mg/g sample) (Datuashvili et al., 2025; Surmanidze et al., 2024).

Total Phenolic Acid Content

Total phenolic acids were determined spectrophotometrically: 250 µL of extract was mixed with 250 µL of 0.1% ethanolic HCl and 4.55 mL of 2% HCl, vortexed, incubated for 15 min, and absorbance was measured at 320 nm. Results were expressed as caffeic acid equivalents (mg/g sample) (Abashidze et al., 2024).

Total Flavonoid Content

Flavonoids were determined by the $AlCl_3$ spectrophotometric method. One mL of extract was mixed with 5 mL H_2O and 0.3 mL of 5% $NaNO_2$ (5 min incubation), followed by 0.3 mL of 10% $AlCl_3$ (6 min), and 2 mL of 1N NaOH. Absorbance was measured at 510 nm and results were expressed as quercetin equivalents (mg/g sample) (Abashidze et al., 2024; Datuashvili et al., 2025).

Catechin and Leucoanthocyanin Determination

Catechins were determined using the vanillin reagent method; 3 mL vanillin reagent was added to 1 mL extract and absorbance measured at 500 nm after 3 min, with results recalculated from a (+)-catechin calibration curve (Surmanidze et al., 2024). For leucoanthocyanins, 8 mL leucoanthocyanidin reagent was added to 1 mL extract; a parallel unheated sample served as blank while the heated sample (40 min) was measured at 550 nm, expressed as cyanidin-3-O-glucoside equivalents (mg/g dry weight) (Datuashvili et al., 2025).

Antioxidant Activity

DPPH radical-scavenging activity was measured at 517 nm after 30 min dark incubation. The half-maximal inhibitory concentration (IC_{50}) was determined by four-parameter logistic regression and expressed as mg dry sample per 50% inhibition (Brand-Williams et al., 1995; Surmanidze et al., 2024). $ABTS^{+\bullet}$ decolorization activity was measured at 734 nm after 6 min, with the working solution adjusted to an absorbance of 0.70 ± 0.02; IC_{50} was determined by the same method (Re et al., 1999).

Statistical Analysis

All analyses were performed in triplicate. Data are presented as mean ± standard deviation. Statistical significance was evaluated at $p \leq 0.05$ using Microsoft Excel.

Results and Discussion

Tannin Content During Ripening

The dynamics of soluble, bound (insoluble), and total tannin content during on-tree ripening are presented in Figures 1 and 2 for the 'Hachiya' and 'Hyakume' varieties, respectively. In both cultivars, a pronounced and progressive decline in total tannin content was observed throughout the ripening period (October–December 2025), consistent with the well-documented deastringency process in persimmon (Del Bubba et al., 2009; Denev & Yordanov, 2013).

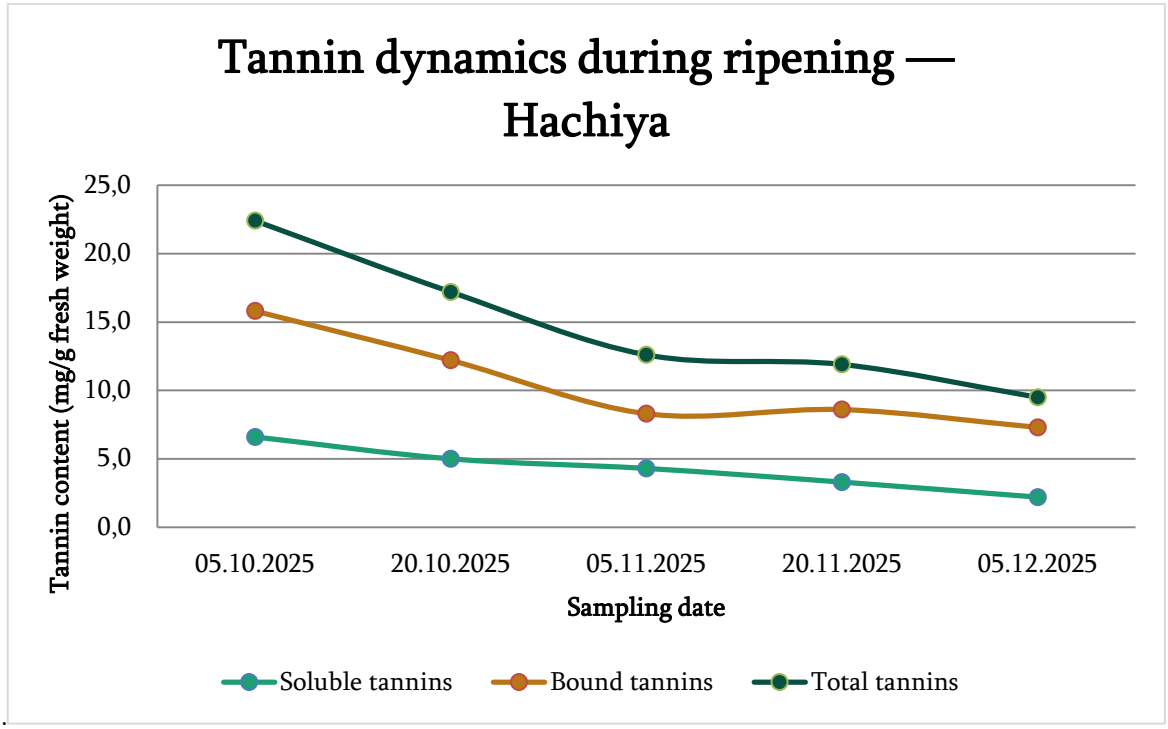

Figure 1. Tannin dynamics during ripening — 'Hachiya' cultivar (mg/g fresh weight)

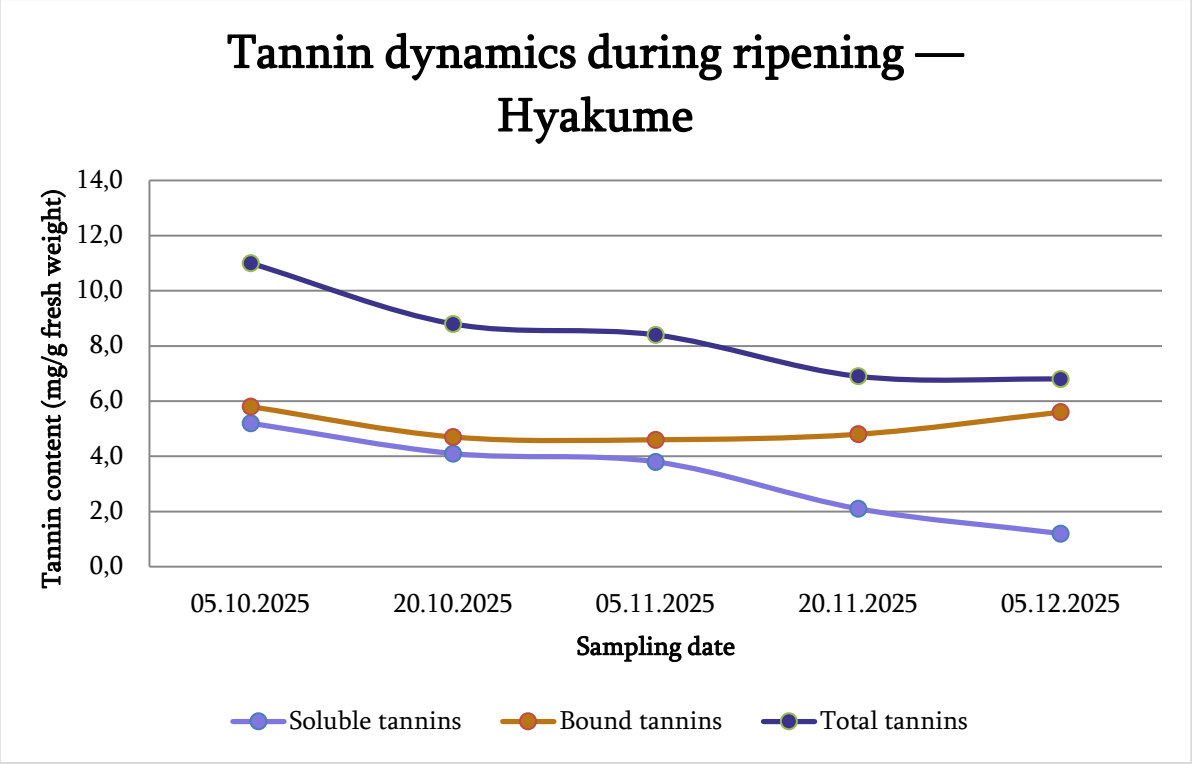

Figure 2. Tannin dynamics during ripening — 'Hyakume' cultivar (mg/g fresh weight)

At the early (unripe) stage, soluble tannins predominated and were present at their highest concentrations, conferring strong astringency to the fruit. As ripening progressed, the content of soluble tannins decreased markedly — by approximately 80% relative to initial values — while bound (insoluble) tannin fractions also declined, though less steeply, by up to 50%. This transition reflects the polymerization and complexation of tannins with endogenous proteins and polysaccharides, particularly pectin, rendering them insoluble and organoleptically inactive (Denev & Yordanov, 2013).

The 'Hachiya' cultivar exhibited consistently higher total tannin concentrations compared to 'Hyakume' across all sampling dates, in agreement with the classification of persimmon cultivars based on tannin content and astringency. These findings confirm that ripening stage is a primary determinant of persimmon astringency and must be carefully considered when selecting fruit for processing

Pectin Fractions During Ripening

The quantitative dynamics of soluble pectin, protopectin, and total pectin content during the ripening process are presented in Figures 3 and 4. For both varieties, a consistent decline in total pectin was observed from October to December, amounting to approximately 68% in 'Hachiya' (from 2.79 to 0.90 g/100g) and approximately 21% in 'Hyakume' (from 7.05 to 5.60 g/100g). Notably, 'Hyakume' contained substantially higher pectin concentrations at all ripening stages, which may be associated with structural differences between astringent and non-astringent cultivars in cell wall composition.

At the technical ripeness stage (early October), protopectin clearly exceeded soluble pectin in 'Hachiya' (2.32 vs. 0.47 g/100g), reflecting the predominance of the water-insoluble, high-methoxyl pectin fraction typical of unripe fruit. As ripening advanced, protopectin underwent hydrolytic degradation, progressively releasing soluble pectin chains. By physiological maturity, the soluble fraction accounted for a greater relative proportion of total pectin, consistent with fruit softening.

Of particular relevance is the relationship between pectin and tannin dynamics. The formation of insoluble pectin–tannin complexes during ripening is considered one of the principal mechanisms responsible for natural deastringency: soluble tannins are sequestered by pectin macromolecules, forming insoluble aggregates that no longer interact with salivary proteins (Del Bubba et al., 2009; Denev & Yordanov, 2013). The parallel decline of

both soluble tannins (Figures 1 and 2) and total pectin (Figures 3 and 4) over the ripening period supports this mechanistic interpretation. It underlines the functional interdependence of these two phytochemical classes.

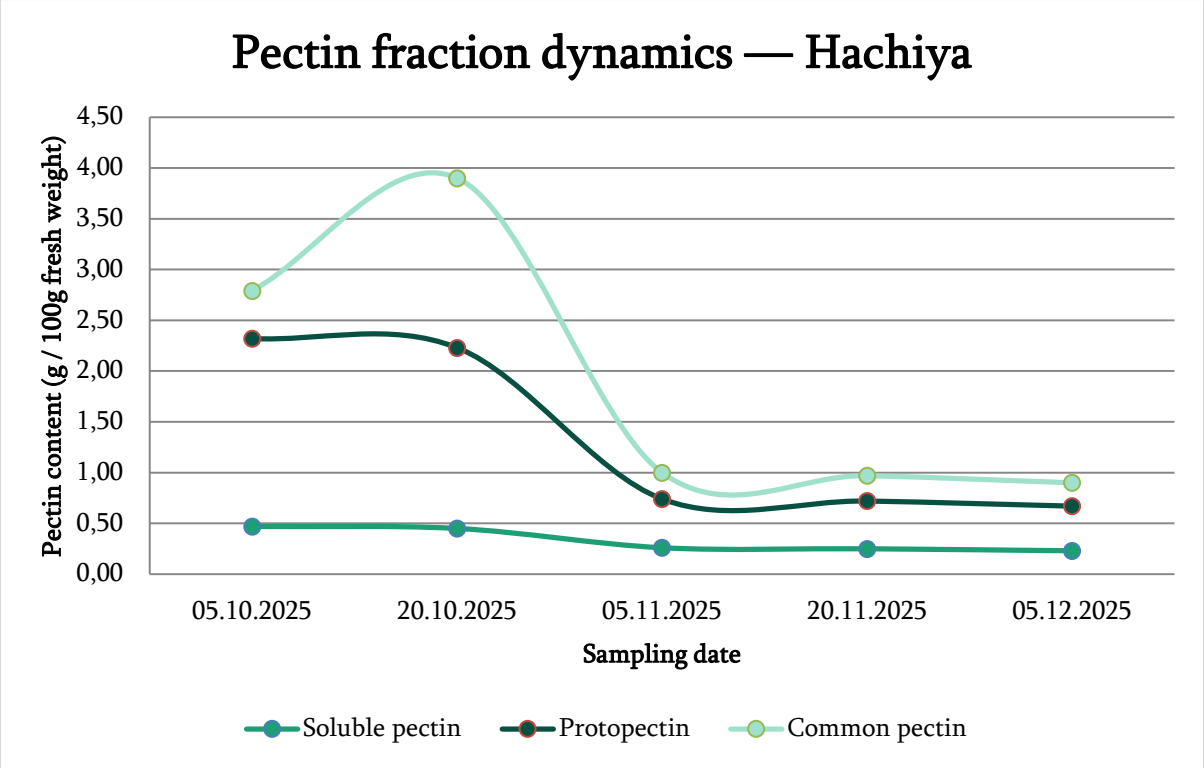

Figure 3. Pectin fraction dynamics during ripening — 'Hachiya' (g/100g fresh weight, mean ± SD)

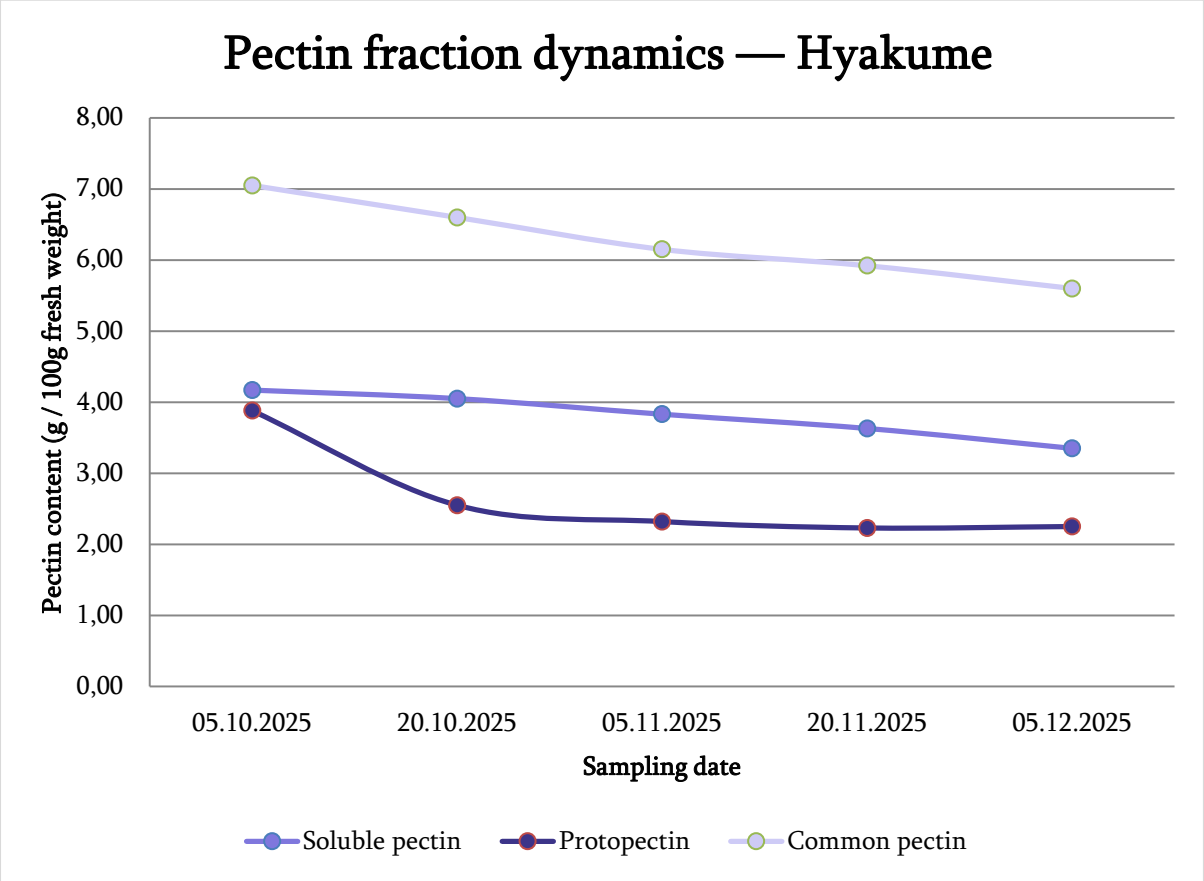

Figure 4. Pectin fraction dynamics during ripening — 'Hyakume' (g/100g fresh weight, mean ± SD)

Phenolic Compound Profile at Commercial Harvest

Table 1. Phenolic compound composition of 'Hachiya' and 'Hyakume' persimmon fruits at commercial harvest stage (mg/g fresh weight)

Cultivar	Total phenols	Phenolic acids	Total flavonoids	Catechins	Leucoanthocyanins
Hachiya	8.210	0.144	6.162	1.227	3.990
Hyakume	5.770	0.108	3.986	0.623	1.964

The concentrations of individual phenolic fractions in 'Hachiya' and 'Hyakume' fruits at the commercial harvest stage are presented in Table 1. The 'Hachiya' variety exhibited significantly higher total phenolic content (8.210 mg/g) compared to 'Hyakume' (5.770 mg/g), reflecting the characteristic chemical distinction between astringent and non-astringent cultivars. Similar differences have been reported by other authors (Suzuki et al., 2005).

In both varieties, total flavonoids constituted the dominant phenolic fraction — 6.162 mg/g in 'Hachiya' and 3.986 mg/g in 'Hyakume' — followed by leucoanthocyanins (proanthocyanidins): 3.990 and 1.964 mg/g, respectively. Catechin concentrations were also notably higher in 'Hachiya' (1.227 vs. 0.623 mg/g). Phenolic acids represented the smallest fraction in both varieties (0.144 and 0.108 mg/g), consistent with the literature describing persimmon polyphenols as predominantly flavanolic in character (Jiménez-Sánchez et al., 2015; Yoo et al., 2019). These data were used as baseline indicators for evaluating subsequent technological processing variants.

Physicochemical Characteristics at the Optimal Harvest Stage

Based on the tannin and pectin dynamics described above, 20 November 2025 was identified as the optimal harvest window corresponding to the technical (commercial) ripeness stage, most suitable for production of glucose–fructose syrups and phenolic-rich extracts. Physico-chemical parameters of both varieties at this stage are presented in Table 2.

Table 2. Physicochemical characteristics of 'Hachiya' and 'Hyakume' persimmon fruits at the optimal harvest stage (20 November 2025)

Indicator	Hachiya	Hyakume
Dry matter, %	19.4 ± 0.58	15.88 ± 0.57
pH	6.03 ± 0.20	6.0 ± 0.18
Titratable acidity, %	0.10 ± 0.003	0.11 ± 0.003
Invert sugar, %	15.88 ± 0.53	11.9 ± 0.35
Sucrose, %	1.12 ± 0.03	0.9 ± 0.03
Ascorbic acid, mg/100g	30.23 ± 0.90	35.0 ± 1.05
Carotenoids, mg/100g	32.25 ± 0.96	29.47 ± 0.88

Dry matter content was 19.4 ± 0.58% for 'Hachiya' and 15.88 ± 0.57% for 'Hyakume'. Both varieties showed very low titratable acidity (0.10–0.11%), indicative of a mild, sweet flavor profile favorable for syrup production. Invert sugar content was substantially higher in 'Hachiya' (15.88 ± 0.53%) than in 'Hyakume' (11.9 ± 0.35%), with glucose and fructose together accounting for 92–93% of total sugars in both cultivars, making this fraction well-suited for glucose–fructose syrup recovery without prior hydrolysis. Ascorbic acid content was slightly higher in 'Hyakume' (35.0 ± 1.05 mg/100g), while carotenoid concentrations were higher in 'Hachiya' (32.25 vs. 29.47 mg/100g). The near-neutral pH (approximately 6.0 for both varieties) confirms the suitability of this harvest stage for processing into stable, high-quality purées and extracts.

Effect of Processing Conditions on Phenolic Content and Antioxidant Activity

The influence of four processing methods on total phenolic content (TPC) and antioxidant activity (DPPH and ABTS IC_{50}) of 'Hachiya' fruit is summarized in Table 3. Results are presented separately for the juice and pomace fractions. A strongly negative correlation between TPC and IC_{50} was confirmed across all treatment variants: the lower the IC_{50}, the stronger the radical-scavenging capacity, consistent with published data (Park et al., 2008; Jang et al., 2010; Jang et al., 2011).

Table 3. Effect of processing conditions on phenolic content and antioxidant activity of 'Hachiya' persimmon fruit

Processing method	Fraction	TPC (mg/g)	DPPH IC_{50} (mg)	ABTS IC_{50} (mg)
Fruit — harvest stage (control)	Fruit	8.21	1.52	1.26
Direct pressing	Juice	2.04	5.79	4.75
	Pomace	5.17	2.25	1.98
Warm water treatment 45°C/12h	Juice	3.73	5.08	4.15
	Pomace	3.80	4.75	3.28
CO_2 treatment 88%/60h	Juice	1.30	13.04	10.27
	Pomace	7.04	1.17	0.87
Freezing −18°C/20h	Juice	4.62	5.86	5.00
	Pomace	3.42	3.01	2.36

The unprocessed fruit at the harvest stage showed the highest TPC (8.21 mg/g) and the strongest antioxidant activity (DPPH IC_{50} = 1.52 mg; ABTS IC_{50} = 1.26 mg), serving as the reference level for all processing comparisons. Direct pressing resulted in pronounced partitioning of phenolics: the pomace (TPC 5.17 mg/g; DPPH IC_{50} = 2.25 mg) retained substantially more phenolics than the juice (TPC 2.04 mg/g; DPPH IC_{50} = 5.79 mg), confirming that high-molecular-weight tannins and proanthocyanidins remain preferentially associated with the cell wall matrix.

Warm water treatment (45°C/12 h) led to a relative equalization of TPC between juice (3.73 mg/g) and pomace (3.80 mg/g) fractions, both showing intermediate antioxidant activity (DPPH IC_{50} = 5.08 and 4.75 mg, respectively). This equalization likely reflects thermal degradation of high-molecular-weight tannins and their partial extraction into the aqueous phase.

CO_2 treatment (88%/60 h) produced the most divergent fractionation. The CO_2-treated juice exhibited the lowest TPC of all variants (1.30 mg/g) and the weakest antioxidant activity (DPPH IC_{50} = 13.04 mg; ABTS IC_{50} = 10.27 mg). In contrast, the CO_2-treated pomace retained the highest TPC of all processed fractions (7.04 mg/g) with the strongest antioxidant capacity among processed samples (DPPH IC_{50} = 1.17 mg; ABTS IC_{50} = 0.87 mg), approaching that of the control fruit. This redistribution reflects the CO_2 deastringency mechanism: anaerobic conditions promote acetaldehyde accumulation, driving polymerization of soluble tannins into insoluble complexes retained in the solid phase (Ding et al., 2025; Ozdemir et al., 2020; Han et al., 2022).

Freezing (−18°C/20 h) yielded an intermediate pattern, with TPC of 4.62 mg/g (juice) and 3.42 mg/g (pomace), and DPPH IC_{50} values of 5.86 and 3.01 mg, respectively. Collectively, these results demonstrate that processing type determines not only the quantitative distribution of phenolic compounds between fractions, but also has a significant impact on their antioxidant properties. The CO_2-treated pomace is identified as the most phenolic-rich and antioxidant-potent fraction, with direct implications for valorization of persimmon processing by-products.

Conclusion

The present study provides information on the chemical composition of fruits of two persimmon varieties grown in Georgia, examining qualitative and quantitative variation in phenolic compound content at different stages of physiological ripening and under different processing methods (freezing, warm water treatment, and CO_2 treatment). The stage of ripening, as well as the type of technological intervention, essentially determines both the fractional distribution of phenolic compounds and antioxidant activity, ultimately affecting the functional and bioactive value of the product. Fruits at the stage of commercial harvest are a richer source of polyphenolic compounds, in particular soluble tannins. Such fruits can be used for the extraction of tannins, which have a wide range of biological activity. Fruits processed by various methods can be used for the preparation of pastes and glucose–fructose extracts. The CO_2-treated pomace in particular represents a high-value fraction with exceptional polyphenol retention and antioxidant potential, warranting further investigation for functional food and nutraceutical applications.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in the EPSTEM journal belongs to the authors. No ethics committee approval was required for this study, as it involved laboratory analysis of plant material only.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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