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A strategy for healthy eating habits of daily fruits revisited: A metabolomics study

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ABSTRACT

Many people peel fruits, commonly persimmon, grape, apple, and peach, before eating as table fruits. Differences of bioactive compounds between peels and pulps of daily fruits are widely known but limited to individual compound because understanding of differences in their global metabolites is lack. We employed ^{1}H NMR-based metabolomics to explore the global metabolite differences between their peels and pulps from the fruits, which included changes of diverse metabolites in persimmon after harvest ripening. Of diverse metabolites observed among the fruits tested, various health-beneficial metabolites were present in the peels rather than the pulps and their classes were dependent on the type of fruit: gallocatechin, epicatechin and epigallocatehin only in persimmon, apple, and peach, respectively; quercetin only in persimmon and apple; kaempferol only in persimmon; chlorogenic acid only in grape and peach; neochlorogenic acid only in apple and peach; *p*-coumaric acid only in grape; phloridzin and catechin only in apple. These metabolites in the peels of each fruits were strongly correlated with free radical-scavenging activity and delay of carbohydrate digestion. Therefore, intake of whole fruits, rather than removal of their peels, were recommended for potential improvement of healthy lifespan and human wellness. This study highlights the critical role of metabolomic studies in simultaneous determinations of diverse and intrinsic metabolites in different types of fruits and thus providing a strategy for healthy eating habits of daily fruits.

1. Introduction

Fruits are important natural resources that have abundant nutrients such as sugars, organic acids, and secondary metabolites (Konstantinos et al., 2021). The Food and Agriculture Organization (FAO) in 2021 proposed a healthy diet that includes at least 400 g of fruits and vegetables per day (FAO et al., 2021). Also, taking a healthy diet that includes sustainability considerations such as fruits and vegetables can contribute to reducing climate change costs. The world's current dietary patterns which consume too high animal source food is responsible for around 21–37% of total greenhouse gas emissions, which mainly drives climate change. Plant-based foods can reduce negative environmental impacts, including on land use, freshwater extraction, and biogeochemical flows (FAO et al., 2021).

According to personal preference, the peels of fruits above are often removed prior to intake and become food waste. However, the peels of most fruits are rich in dietary fibers, phenolic acids, and flavonoids, and have more effective free radical-scavenging activity than pulps (Kumar et al., 2017; Suleria et al., 2020). Due to their high-value components, the peels of fruits can also be a source of functional ingredients and utilized as ingredients in food, feed, and nutraceutical products instead of becoming food waste (Baiano, 2014).

Phytochemicals are metabolite group that exists in various plants and contribute to positive health benefits. Various phytochemicals have been found in fruits and grouped based on their function, chemical structure, and source (Tiwari and Cummins, 2013). These phytochemicals can prevent diseases caused by oxidative stress (Kaur and Kapoor, 2001). Phytochemicals, or secondary metabolites, derived from plants are not directly involved in the growth and development of organisms but have beneficial effects in the case of long-term impairment of organisms (Costa et al., 2012). Secondary metabolites can mainly be divided into terpenes, phenolics, and nitrogen-containing compounds

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(Costa et al., 2012), among phenolic compounds are most common. In plants, phenolic compounds are considered as stress metabolites induced by various environmental stimuli (Awad and de Jager, 2002). The term 'polyphenol' is not a strictly chemical term but is used widely to refer to flavonoids, tannins, phenolic acids, and their derivatives (Williamson, 2017). Phenolics are unevenly distributed in plants. Insoluble phenolics such as tannins and lignins are components of cell walls, while soluble phenolics, such as phenolic acids and flavonoids, are compartmentalized in plant cell vacuoles (Haminiuk et al., 2012; Naczk and Shahidi, 2004).

Recent studies have attempted to determine the interactions between secondary metabolites and cancer through collective technologies called machine learning algorithm (Veselkov et al., 2019). The term 'Omics' is used to express comprehensive measurements that produce large or big data sets and thus can be applied to machine learning technology. Metabolomics is a field of study that profiles all metabolites in organisms, providing systematic identification and quantitation (Idle and Gonzalez, 2007). Metabolomics can provide overall information about metabolic physiology in plants, which includes primary and secondary metabolite compositions (Kim et al., 2010). The metabolome is an assemblage of metabolites considerably affected by the genotype, cultivar, environmental or external stress, and developmental signals. In order to discover the interactions of diverse metabolites with various conditions, non-selective, specific and information-rich analytical approaches are required. Currently, analytical technologies of nuclear magnetic resonance (NMR) and mass spectroscopy (MS) are mainly being used in metabolomics. The recent applications of metabolomics in food safety, food authenticity and quality, and food traceability have been introduced, which included the analytical technologies for metabolomics (Li et al., 2021).

Metabolomic analysis of various fruits, such as citrus (Kim et al., 2021), persimmon (Veberic et al., 2010), and southern African indigenous fruits (Nkosi et al., 2022), has shown the strong dependence of bioactive compound compositions on varieties and cultivars. However, many studies have focused on variations of individual compounds in the given fruits, rather than in different types of fruits. Also, differences in diverse metabolic compositions between the pulps and peels of different types fruits has not yet been reported. In the present study, we investigated the global metabolite differences between the fruits of persimmon, grape, apple and peach and between their pulps and peels using ^{1}H NMR-based metabolomics, and the metabolic changes of an astringent persimmon after post-harvest ripening. We selected these fruits because people generally peel them before eating as table fruits, even though eating habits with peeling of the fruits likely depends on individuals and the types of fruits. In addition, we explored correlations of metabolite compositions with free radical-scavenging and antidiabetic activity, especially between pulps and peels, to establish healthy eating habits of daily fruits.

2. Materials and methods

2.1. Fruit samples

Five fruits of Daebong persimmon, an astringent cultivar (*Diospyros kaki cv.* Hachiya), sweet persimmon, a non-astringent cultivar (*Diospyros kaki* Thunb.), peach (*Prunus persica* Batsch), apple (*Malus pumila* Mill.), and grape (*Vitis coignetiae*) were chosen, because they are commonly peeled before eating. Daebong persimmon and sweet persimmon were harvested from Damyang-gun, Jeollabuk-do, South Korea, in November 2020 and October 2021, respectively. Peach was harvested from Yonginsi, Gyeonggi-do, South Korea, in August 2021. Apples was harvested from the orchard in Jangseong-gun, Jeollanam-do, South Korea, in November 2021. Sweet persimmon, apples, and peaches (n = 40 each) were collected at valid maturity from two different trees (n = 20 per tree). In contrast, 100 fruits (n = 50 per tree) of Daebong persimmon were harvested at maturity and divided into 2 groups. One group (n = 50) was postharvest-ripened, and the other (n = 50) was the control group. The postharvest ripening group of Daebong persimmon was kept in a well-ventilated and dry room at 16.5°C–20 °C until the fruits ripened. The postharvest ripening group of Daebong persimmon was kept in a well-ventilated and dry room between 16.5 °C and 20 °C until the fruits ripened. In addition, 20 grapes (n = 10 per tree) were collected in Gochang, Jeollabuk-do, South Korea, in August 2021.

All fruits were carefully and manually divided into pulp and peel in our laboratory immediately after harvesting, and seeds and endocarps, except for persimmons, were removed. We peeled all fruits using a fruit peeling slicer, producing the peels with unique thickness of approximately 0.2 mm. Each sample was freeze-dried for a week. The dried samples were ground by mortar and pestle under liquid nitrogen, transferred into a plastic tube using a spatula, and stored separately in a deep freezer at – 80 °C until extraction.

2.2. Extraction of fruit extracts for ^{1}H NMR spectroscopic analysis

Briefly, 50 mg of freeze-dried samples were dissolved in a mixture of 1 mL of 70% deuterated methanol (MeOD) and 30% deuterium water (D_2O) solution in a 2 mL tube. The mixture was sonicated at room temperature for 20 min to extract fruit metabolites and then centrifuged at 13,000 rpm at 4 °C for 15 min. The supernatants were collected and used for ^{1}H NMR spectroscopic analysis.

2.3. ^{1}H NMR spectroscopic analysis of pulps and peels extracts

Briefly, 550 μL supernatant of each MeOD extract of the pulps and peels was transferred into a 5 mm NMR tube. MeOD in the supernatant provided a field frequency lock, and the glucose was used as a chemical shift reference (^{1}H, δ 5.23). ^{1}H NMR spectra were acquired using a Brucker Advance 700 spectrometer (Brucker Biospin, Rheinstetten, Germany) equipped with a cryogenic triple-resonance probe and a Brucker automatic injector, operating at a 700.40 MHz ^{1}H frequency and 298 K. Signal assignment for representative samples was facilitated by two-dimensional (2D) total correlation spectroscopy (TOCSY) and heteronuclear single-quantum correlation (HSQC). In addition to ^{1}H for TOCSY, ^{13}C was applied for the HSQC experiments.

2.4. NMR data processing and multivariate statistical analysis

All NMR spectra were manually adjusted using TOPSPIN (Version 4.0.7 Brucker Biospin, Rheinstetten, Germany) for phase and baseline distortions. After transformed to American Standard Code for Information Interchange (ASCII) format, the spectra were calibrated to glucose (^{1}H, 5.23 ppm) and was aligned using the *i*coshift method (Savorani et al., 2010) in MATLAB (R2010b, Mathworks Inc., Natick, MA, USA). The regions corresponding to methanol and residual water were removed prior to normalization and spectrum alignment. Normalization is a method of scaling each spectrum to the same virtual concentration in order to avoid the dilution effect of extracts and the effect of metabolites (Dieterle et al., 2006). After normalization, data were imported to SIMCA-P version 17.0 (Umetrics, Umea, Sweden) for principal component analysis (PCA), an unsupervised pattern recognition method. Also, orthogonal projection to latent structures-discriminant analysis (OPLS-DA), a supervised pattern recognition method was used to elicit maximum information about discriminant compounds from sample data (Bylesjö et al., 2006). Using MATLAB with scripts developed at Imperial College London (Cloarec et al., 2005), OPLS loading and score plots were generated with a color-coded correlation coefficient for each piece of data to obtain highly interpretable models.

2.5. Total phenolic and total flavonoid content

To quantify the total phenolic content (TPC) and total flavonoid content (TFC), 10 mg freeze-dried sample was dissolved in 1 mL of 70%

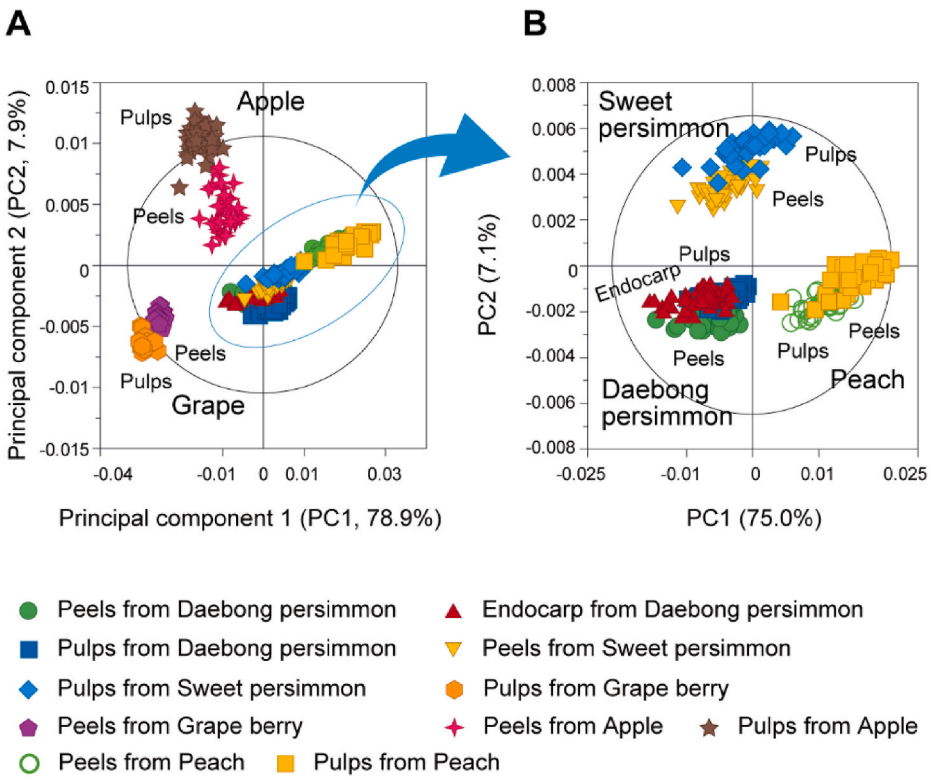

Fig. 1. Principal component analysis (PCA) generated with ^{1}H NMR spectra of peel and pulp extracts collected from five different fruits, demonstrating clear metabolic differentiations between the fruits and between their pulps and peels.

methanol (MeOH) in a 1.5 mL tube. Next, the mixture was sonicated to extract fruit metabolites at room temperature for 20 min and then centrifuged at 13,000 rpm at 4 °C for 15 min. The supernatants were collected and used for assay.

The TPC was determined using the modified Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965). Briefly, 10 μL of each sample extract was added to a 96 well microplate, and then a mixture of 100 μL of deionized water and 20 μL of Folin-Ciocalteu reagent was added to the microplate and left to stand at room temperature for 5 min. Next, 70 μL of 7% sodium carbonate was mixed and left to stand in the dark at room temperature for 60 min. Absorbance was measured at 765 nm using a spectrometer (BioTek, Winooski, VT, USA). The TPC was calculated by a calibration curve of 0–500 μg/mL of gallic acid standard solution and expressed as milligram gallic acid equivalent (GAE) per 1 g of dry weight (GAE mg/g). Data were reported as the mean ± standard deviation (SD) in triplicate.

The TFC was measured using the colormetric method (Zhishen et al., 1999) with some modifications. Briefly, 20 μL of each sample extract was mixed with 100 μL of deionized water in a 96-well microplate, and 20 μL of 1.25% $NaNO_2$ was added and left to stand at room temperature for 10 min. Next, 20 μL of 2.5% $AlCl_3$ was added, followed by 40 mL of 1 M NaOH. After mixing well, absorbance was measured at 510 nm. The TFC was calculated by a calibration curve of 0–500 μg/mL of catechin standard solution and expressed as a milligram catechin equivalent (CE) per 1 g of dry weight (CE mg/g). Data were reported as the mean ± SD in triplicate.

2.6. Antioxidant activities

Samples for free radical-scavenging assays were prepared in the same way as for quantifying TPC and TFC assays. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical–scavenging effect of the samples was measured as described by Yen and Chen (Yen and Chen, 1995) with slight modifications. Briefly, 10 μL of each sample and 0.1 mM DPPH ethanolic solution were added to 96-well microplate, mixed well, and left to stand in the dark at room temperature for 30 min. Absorbance was measured at 515 nm, and gallic acid was used as the standard substance. The percentage of DPPH free radical-scavenging activity was calculated as follows:

$$\text{Radical scavenging activity } (\%) = \left[1 - \left(\frac{A_1}{A_0}\right)\right] * 100, \quad (1)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of each sample. Data were reported as the mean ± SD in triplicate.

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical–scavenging activity of the samples was measured as described by Re et al. (1999) with some modifications. ABTS was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate in potassium phosphate buffer (pH 7.54). The ABTS stock mixture was allowed to stand in the dark at room temperature for 16–24 h and then diluted with triple-distilled water before use. For free radical-scavenging assay, 10 μL of each sample was added to 190 μL of ABTS solution in a 96-well microplate, and absorbance was measured at 734 nm. Gallic acid was used as the standard substance. The percentage of ABTS free radical-scavenging activity was calculated using Equation (1). Data were reported as the mean ± SD in triplicate.

2.7. α-Amylase inhibitory activity

Samples for assays of α-amylase inhibitory activity assays were prepared in the same way as for antioxidant activity assays. α-Amylase inhibitory activity was determined as described in the *Worthington Enzyme Manual* (Worthington, 1993) with some modifications. Briefly, 10 μL of each sample and 150 μL of 20 mM sodium phosphate buffer containing 3U/mL of α-amylase was incubated in a 2 mL tube at 25 °C for 10 min. After preincubation, 150 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) buffer was added, and the mixture was again incubated at 25 °C for 10 min. The reaction was stopped by

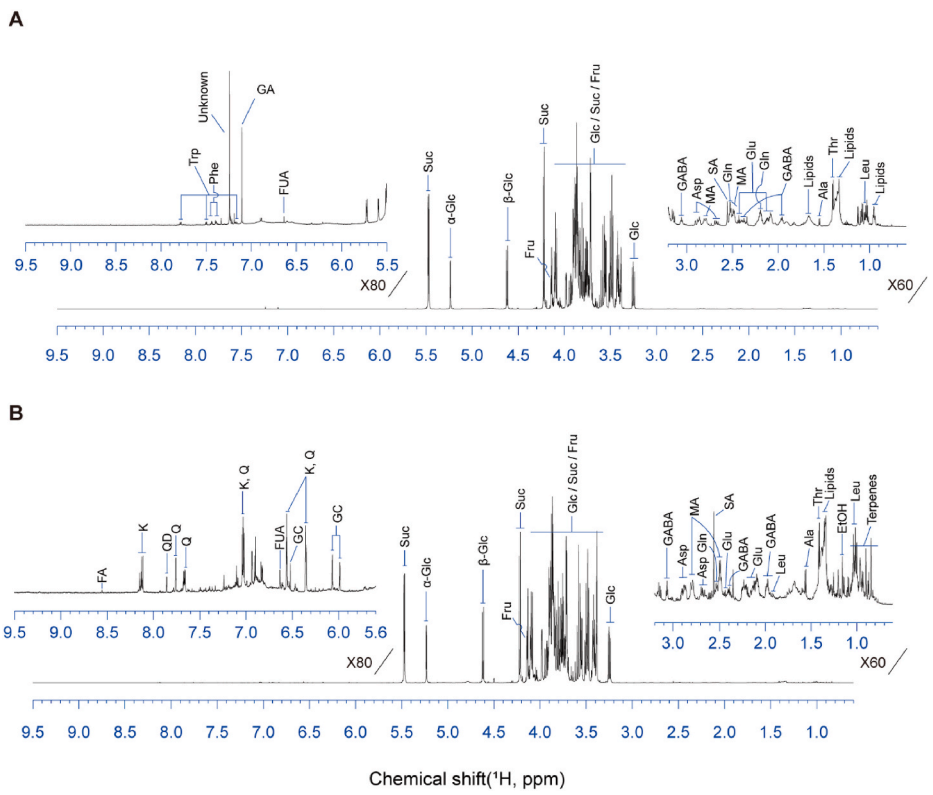

Fig. 2. Representative ^{1}H NMR spectra of pulps (A) and peels (B) collected from Daebong persimmon. Leu, leucine; Thr, threonine; Ala, alanine; Arg, arginine; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamic acid; MA, malic acid; SA, succinic acid; Asp, aspartic acid; EtOH, ethanol; Glc, glucose; Suc, sucrose; Fru, fructose; FUA, fumaric acid; GA, gallic acid; Phe, phenylalanine; Trp, tryptophan; GC, gallocatechin; K, kaempferol; Q, quercetin; QD, quercetin derivatives; FA, formic acid.

adding 300 μL of dinitrosalicylic acid (DNS) color reagent, and the tube was boiled for 10 min and then cooled on ice to room temperature. Next, the mixture was diluted with ultrapure water in a 1:2 ratio (50 μL: 100 μL) in a 96-well microplate, and absorbance was measured at 540 nm. Acarbose was used as the standard substance. The α-Amylase inhibitory activity was expressed as the percentage inhibition and was calculated as follows:

$$\alpha - \text{Amylase inhibitory acticity } (\%) = \left[\frac{A_0 - (A_1 - A_2)}{A_0}\right] * 100, \quad (2)$$

where A_0 is the absorbance of control and A_1 is the absorbance of each sample. A_2 is the absorbance of the reagent without α-Amylase (negative control). Data were reported as the mean ± SD in triplicate.

2.8. Statistical analysis

All experimental results were expressed as the mean ± SD. Student's t-test was performed for paired samples to analyze differences, and significances for the differences was considered at $P < 0.05$, using SPSS Statistics version 26 (IBM Corporation, Armonk, NY, USA). Correlations between antioxidant activity and individual metabolite were visualized using the corrplot package in R version 3.6.2.

3. Results and discussion

3.1. Metabolic differentiations between five fruits and between their pulps and peels

Fig. 1 shows the PCA score plot derived from the ^{1}H NMR spectra of pulps and peels collected from Daebong persimmon, sweet persimmon, peach, apple, and grape pulps and peels. The plot showed large metabolic discriminations between the fruits and between clear differentiations between their pulps and peels (Fig. 1A). Daebong persimmon, sweet persimmon, and peach pulps and peels (Fig. 1B), which were partially overlapped, were further differentiated in corresponding OPLS-DA models (Fig. 3). Moreover, each type of all fruits was collected from two different trees to investigate the natural variations of the metabolites, but any differences between two trees were not found (data not shown). To find out which metabolites were responsible for these metabolic differentiations, diverse metabolites in the pulps and peels of all five fruits were identified through 2D NMR experiments, and then OPLS-DA models were employed for pairwise comparison between pulps and peels.

3.2. Metabolite identification of pulps and peels of five fruits by ^{1}H NMR spectroscopy

Representative one-dimensional (1D) ^{1}H NMR spectra of the Daebong persimmon pulp and peel are given in Fig. 2. Metabolites were identified or assigned by 2D NMR experiments, as shown in Fig. S1. Diverse metabolites and their amounts in the Daebong persimmon pulp and peel were observed in the metabolite profile on the 1D ^{1}H NMR spectra. As expected, sugar compounds, including sucrose, glucose, and fructose, were dominant in both pulp and peel. Moreover, amino acids (leucine, threonine, alanine, aspartic acid, phenylalanine, tryptophan, and arginine), organic acids (γ-aminobutyrate, glutamine, glutamic acid, malic acid, succinic acid, fumaric acid, gallic acid, and formic acid), lipids, flavonoids (quercetin and its derivatives, kaempferol, and gallocatechin), terpenoids, and tannis were observed. In particular, flavonoid compounds were observed only in the peel, while gallic acid was found only in the Daebong persimmon pulp. These results are consistent with the previous studies that showed that the peels of these fruits contain high amounts of phenolic compounds (Saidani et al., 2017).

Diverse metabolite profiles on the representative 1D ^{1}H NMR spectra of sweet persimmon, peach, apple, and grape for diverse metabolite profiles are provided in Figs. S2, S3, S5, and S7, respectively. The 2D NMR results for identification of the secondary metabolites in peach, apple, and grape are also shown in Figs. S4, S6, and S8, respectively. Sucrose, glucose, and fructose were dominant in the pulps and peels of

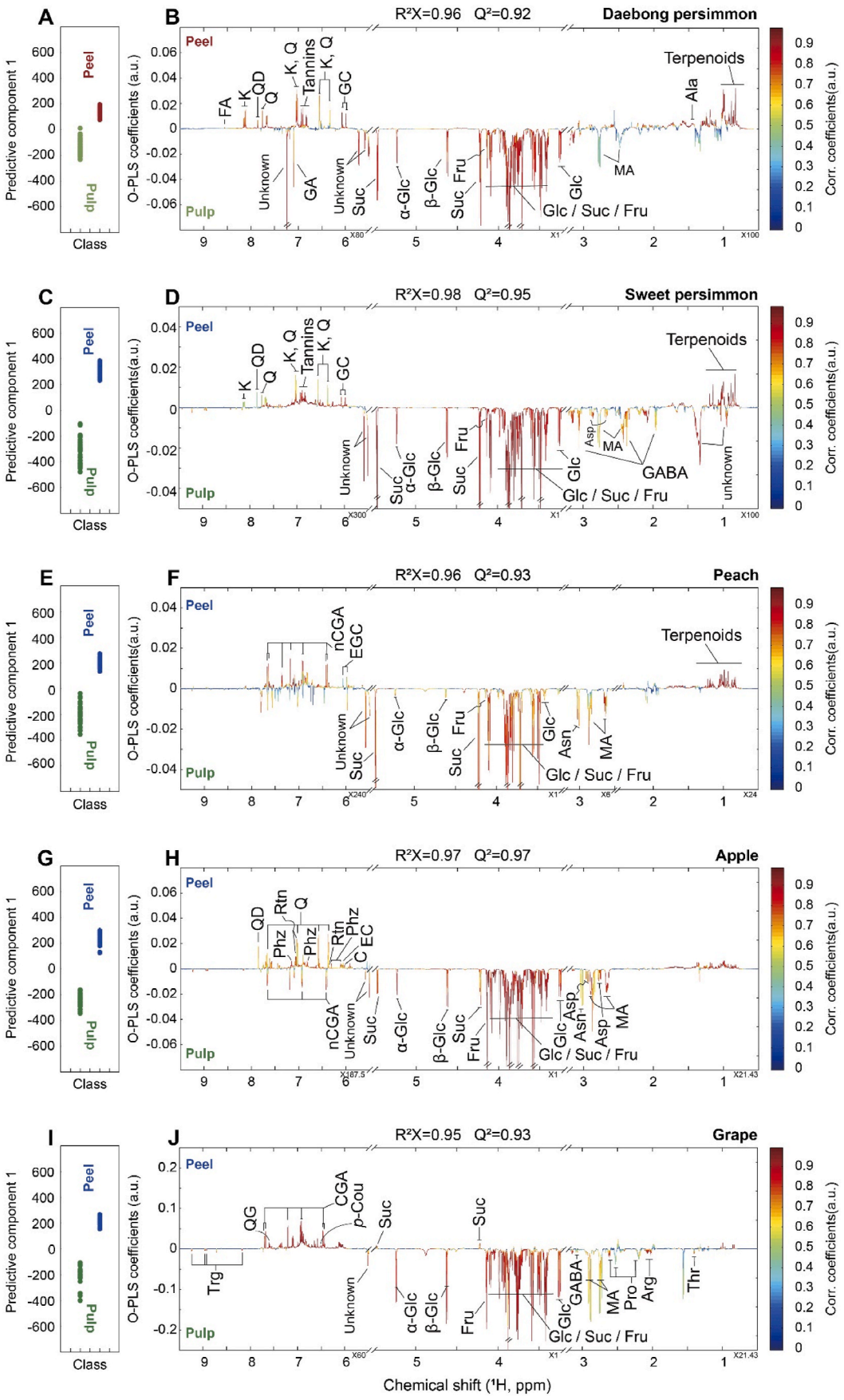

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Fig. 3. OPLS-DA score (A, C, E, G, and I) and loading (B, D, F, H, and J) plot generated with ^{1}H NMR spectra of pulps and peels of Daebong persimmon (A and B), sweet persimmon (C and D), peach (E and F), apple (G and H), and grape (I and J) for comprehensive identification of metabolites that were different between pulps and peels. Ala, alanine; Arg, arginine; Asp, aspartic acid; C, catechin; CGA, chlorogenic acid; nCGA, neochlorogenic acid; EC, epicatechin; EGC, Epigallocatechin; FA, formic acid; Fru, fructose; GA, gallic acid; GABA, gamma-amynobutyric acid; Glc, glucose; Gln, glutamine; K, kaempferol; MA, malic acid; Phz, phloridzin; Pro, proline; Q, quercetin; QD, quercetin derivatives; QG, quercetin glucoside; *p*-Cou, *p*-coumaric acid; Rtn, rutin; Suc, sucrose; Thr, threonine; Trg, trigonelline. The upper sections in OPLS-DA loading plots represent relatively higher amounts of metabolites in the peels of each fruits, compared to their pulps (B, D, F, H, and J), whereas the lower sections indicate relatively lower amounts of metabolites in the peels.

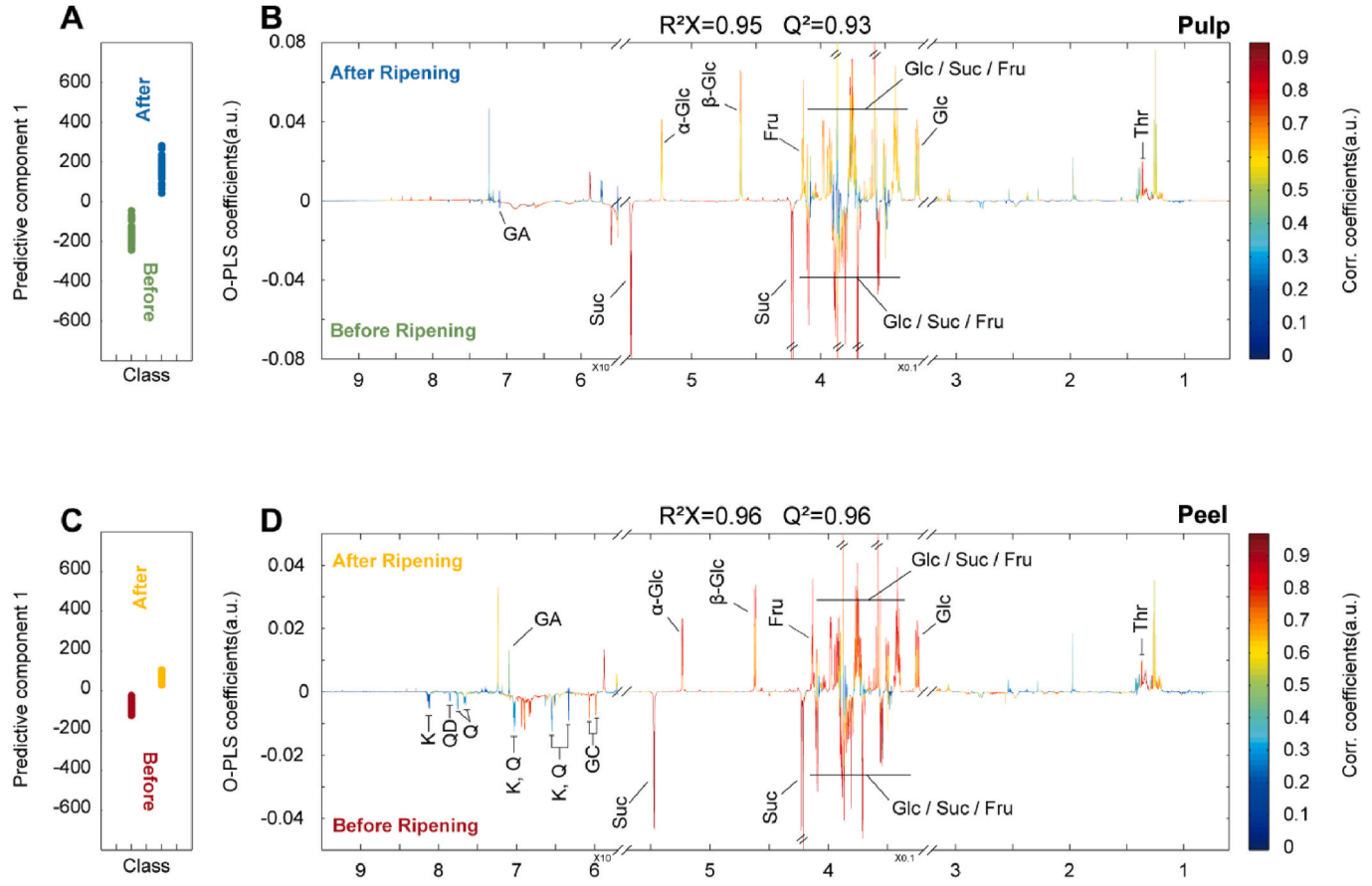

Fig. 4. OPLS-DA score (A and C) and loading (B and D) plot generated with ^{1}H NMR spectra of Daebong persimmon pulps and peels for comprehensive identifications of metabolites that were different between before and after postharvest ripening.

all five fruits. However, we also found distinct and intrinsic secondary metabolites in the fruits. For example, quercetin and its derivatives were found in Daebong persimmon, sweet persimmon, and apple peels (Figs. 2, S2, and S5); kaempferol and gallocatechin were observed only in Daebong persimmon and sweet persimmon peels (Figs. 2 and S2); chlorogenic acid was observed only in grape and peach (Figs. S3 and S7); neochlorogenic acid was present only in apple and peach (Figs. S3 and S5); *p*-coumaric acid and quercetin glucoside were observed in grape (Figs. S7 and S8); epicatechin, catechin, rutin, and phloridzin were found only in apple (Fig. S5); and epigallocatechin was observed only in peach (Fig. S3).

3.3. Multivariate statistical analysis of ^{1}H NMR spectra of five fruits

To identify the metabolites that were significantly different between the pulps and peels of the five fruits, OPLS-DA models were generated (Fig. 3). A high goodness of fit (R^2X) and predictability (Q^2) were observed in all OPLS-DA models for pairwise comparison between pulps and peels from $R^2X = 0.95$–0.98 and $Q^2 = 0.92$–0.97. Sucrose, glucose, and fructose levels were higher in all pulps than in peels, but sucrose levels only in grape were higher in the peel than in the pulp (Figs. 3B, D, 3F, 3H, and 3J). Gallocatechin, kaempferol, quercetin, quercetin derivative, tannin and terpenoid levels were higher in the Daebong and sweet persimmon peels than in their pulps (Figs. 3B and D). However, gallic acid levels were higher in the Daebong persimmon pulp than in the peel. In fact, gallic acid was found only in the Daebong persimmon pulp among the fruits tested (Fig. 2).

In the comparison between pulps and peels of peach, higher levels of neochlorogenic acid were found in the peel, compared to the pulp (Fig. 3F). Considering that most secondary metabolites were observed only in the peels rather than in the pulps, it was interesting that the secondary metabolites in peach (epigallocatechin, neochlorogenic acid, and chlorogenic acid) were observed in both pulps and peels (Fig. S3). These metabolic characteristics of peach might lead to high levels of neochlorogenic acid in its peel and chlorogenic acid in its pulp (Fig. 3F).

Phloridzin, quercetin, quercetin derivatives, rutin, catechin, and epicatechin levels were significantly higher in the apple peel than in the pulp. Also, apple pulp was characterized by higher levels of malic acid, asparagine, and aspartic acid, compared to the peel (Fig. 3H). In grape, the amounts of most secondary metabolites, including *p*-coumaric acid, quercetin glucoside, and chlorogenic acid, were found to be higher in the peel than in the pulp (Fig. 3J), while threonine, arginine, proline,

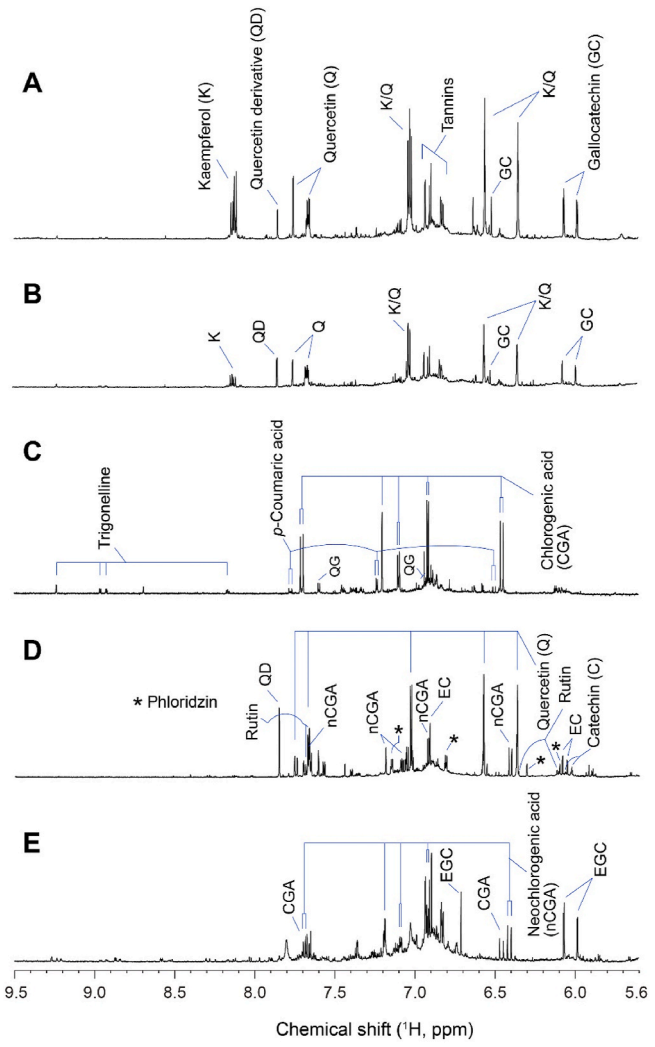

Fig. 5. ^{1}H NMR spectra of the secondary metabolites highlighted in peel of Daebong persimmon (A), sweet persimmon (B), grape (C), apple (D), and peach (E). These spectra were plotted after their normalization methanol, and the amounts of secondary metabolites were thus comparable among the fruits. K, kaempferol; Q, Quercetin; QD, quercetin derivatives; QG, quercetin glucoside; CGA, chlorogenic acid; nCGA, neochlorogenic acid; C, catechin; GC, gallocatechin; EC, epicatechin; EGC, epigallocatechin.

malic acid and GABA were higher in the grape pulp than in the peel.

3.4. Metabolite changes in Daebong persimmon during postharvest ripening

Persimmon can be divided into two types, astringent and non-astringent. In general, astringent persimmon is consumed after postharvest ripening. Daebong persimmon has an astringent taste even at maturity because of the high amounts of soluble tannin (Shin et al., 2014). This astringency can be naturally reduced during postharvest ripening or by some treatment that condenses or polymerizes soluble tannin into insoluble forms (Taira et al., 1997). Other phytochemical compounds in persimmon can also be affected by the type of postharvest ripening technology (Persic et al., 2019). In the current study, we easily observed global metabolite changes in Daebong persimmon pulp and peel before and after postharvest ripening (Fig. 4). Threonine, glucose, and fructose levels increased in the pulp and peel after postharvest ripening, while sucrose levels were decreased (Figs. 4B and D). These changes in the soluble sugars levels likely reflect the hydrolysis of sucrose to glucose and fructose by invertase in the pulp and peel (Koch, 2004). In addition to the increase in glucose, fructose and threonine levels, and the decrease in sucrose levels in the Daebong persimmon peel after postharvest ripening, gallocatechin, kaempferol, quercetin, quercetin derivative, and tainnins levels in the peel significantly decreased after postharvest ripening (Fig. 4D). Interestingly, gallic acid levels found only in the Daebong persimmon pulp were less changed after postharvest ripening. However, the amounts of gallic acid largely increased in the Daebong persimmon peel after postharvest ripening (Fig. 4D). It is commonly known that tannase hydrolyzes tannins and produces gallic acid as a byproduct (Mahendran et al., 2006).

3.5. Quantitative comparisons of the secondary metabolites between the fruits

Normalization of ^{1}H NMR spectrum is a crucial step in their data processing in metabolomic studies in order to account for variations in the overall concentrations of diverse metabolites in the samples and to make all metabolites comparable to each other among the same kinds of samples, which results from mainly dilution of samples, or different amounts of samples (Kohl et al., 2012). However, due to possible misleading of normalization by total integral area of NMR spectra collected from different types of fruits that have their intrinsic metabolites, normalization to methanol used as a solvent was applied in the current study to compare the relative concentrations of the intrinsic secondary metabolites in the five fruits. Fig. 5 highlights the 1D ^{1}H NMR spectra of the secondary metabolites in the peels from all five fruits, obtained by methanol normalization. Furthermore, to compare the relative amounts of these secondary metabolites and their statistical differences, we calculated their integral areas following methanol normalization (Figs. 6E-J). Results showed large differences in the amounts of the secondary metabolites between the pulps, peels, and endocarps of the five fruits, indicating that their amounts in the pulps and endocarps were negligible, except for neochlorogenic acid in apple and chlorogenic acid in peach. Also, the amounts of chlorogenic acid in grape peels were 10 times more than in peach peels.

3.6. Correlation between secondary metabolites and antioxidant capacity and α-amylase inhibition

Antioxidant compound-rich diets can prevent free radical-induced cellular damage (Nkosi et al., 2022). In general, most metabolites with antioxidant activity are composed of phenolic groups that act as electron donors from the hydroxyl moieties to the oxidizing radical species, inhibit the oxidation of other molecules, and prevent cellular damage (Carvalho et al., 2021). In addition, synergistic and additive actions between phenolic compounds and other compounds present in fruits must be considered (Hossain and Rahman, 2011). In the current studies, the peels of all five fruits showed significantly strong ABTS and DPPH free radical-scavenging activity, compared to the pulps (Fig. 6B). This free radical-scavenging activity might likely be correlated with the amounts of total phenolic (TPC) and flavonoid (TFC) compounds. Similar to previous studies (Jang et al., 2011; Maulidiani et al., 2018), we found a higher TPC in the Daebong persimmon pulp and peel than in sweet persimmon (Fig. 6A). These differences in the TPC and TFC between Daebong and sweet persimmon likely caused the stronger scavenging activity of Daebong persimmon toward ABTS and DPPH free radicals, compared to sweet persimmon. Interestingly, the TPC and TFC in Daebong persimmon were largely higher in the pulp than in the peel. These characteristics might lead to DPPH free radical-scavenging activity of the pulp, compared to the peel (Fig. 6B). However, ABTS free radical-scavenging activity was similar between the Daebong persimmon pulp and peel.

α-Amylases are hydrolytic enzymes that catalyze the hydrolysis of starch (Konstantinos et al., 2021). Inhibition of this enzyme activity slows down starch digestion, and decreases the blood sugar absorption rate (Barrett and Udani, 2011). α-Amylases inhibitors developed in plant

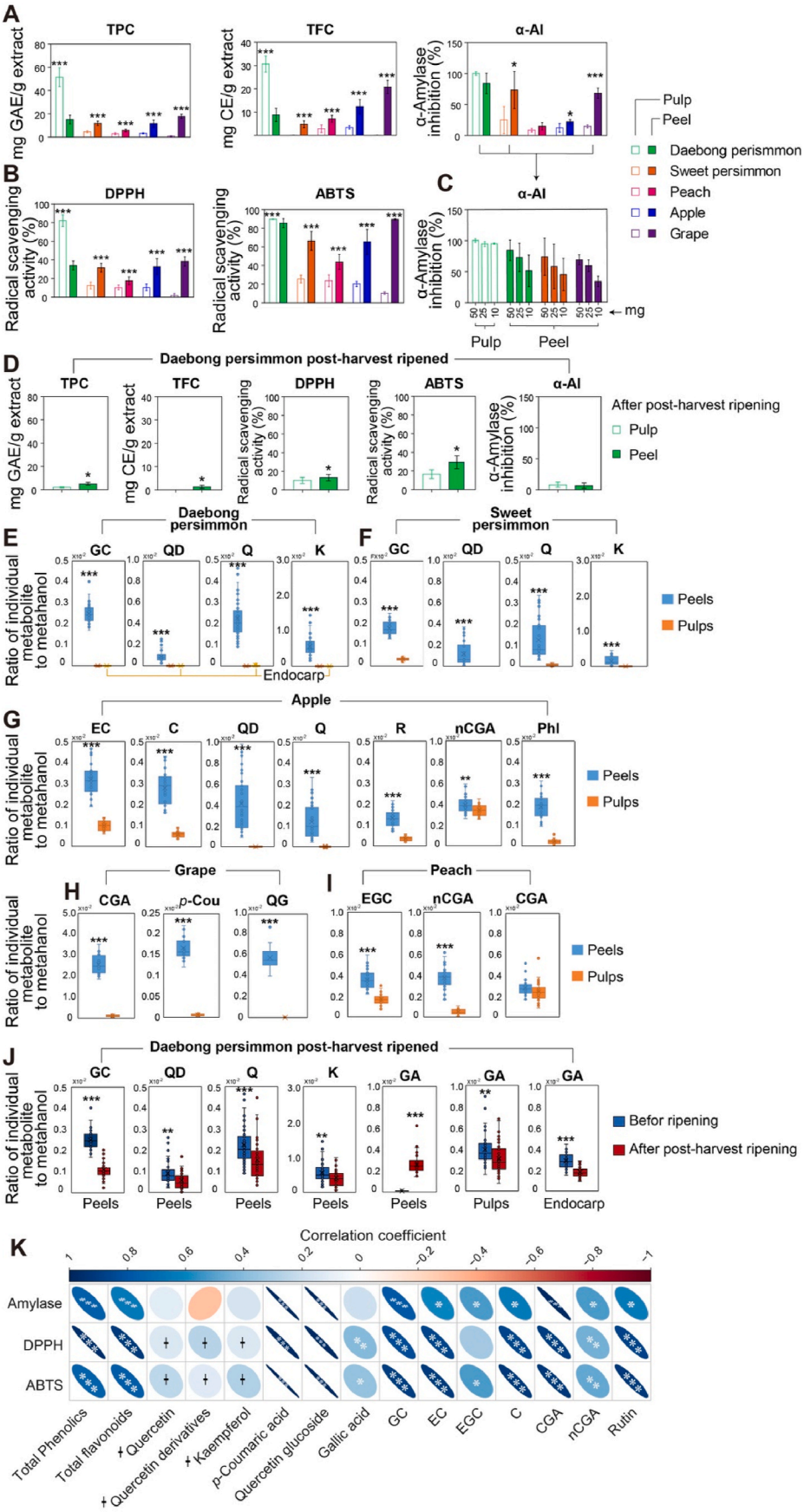

Fig. 6. Total phenolics contents (TPC), total flavonoid contents (TFC), scavenging activities against DPPH and ABTS free radicals, and α-amylase inhibitory activity (α-AI) in the pulps and peels from five fruits (A–C) and from Daebong persimmon after postharvest ripening (D). The panel C denotes α-amylase inhibitory activity dependent on concentrations of the Daebong persimmon pulps and Daebong persimmon, sweet persimmon, and grape peels. The panels E, F, G, H, and I demonstrate the differences in the secondary metabolite levels between pulps and peels of Daebong persimmon, sweet persimmon, apple, grape, and peach, respectively, which were calculated by the integral area of ^{1}H NMR peaks corresponding to the metabolites and expressed as ratio of metabolites to methanol. The panel J shows the changes in the secondary metabolite levels in the pulp, peel, and endocarp of Daebong persimmon before and after postharvest ripening. The panel K shows correlations of secondary metabolites with antioxidant and α-amylase inhibitory activities. Direction and strength of the correlations in the panel B are visualized with an oval shape and a color gradient. †Quercetin, quercetin derivatives and kaempferol were significantly correlated with ABTS and DPPH free radical-scavenging activity in correlation analysis within a single fruit, for example, using only the dataset from the Daebong persimmon pulp and peel ($P < 0.05$). GC, gallocatechin; EC, epicatechin; EGC, epigallocatechin; CGA, chlorogenic acid; nCGA, neochlorogenic acid; Phz, phloridzin; QG, quercetin glucoside; *p*-Cou, *p*-coumaric acid. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to strengthen their defense against predators (Barrett and Udani, 2011). Secondary metabolites, such as anthocyanins, flavonoids, and phenolic acids, in fruits can inhibit α-amylases activity (Konstantinos et al., 2021). In this study, the patterns of α-amylase inhibitory activity in fruits were similar to the free radical-scavenging activity observed (Fig. 6A). In particular, α-amylase inhibitory activity in the Daebong persimmon pulp was found to strong even with a low amount of pulp (Fig. 6C). Recently, flavonoids from *Lycium barbarum*, a traditional medicinal and edible plant in China, have showed the antidiabetic activity in high-fat diet/streptozotocin-induced type 2 diabetes mice, through improving glucose and lipid metabolism, blocking pro-inflammatory cytokines, and regulating gut microbiota (Yang et al., 2022). Therefore, diverse phytochemicals or secondary metabolites in the fruits used for the current study might contribute to the antidiabetic activity.

Phytochemicals, including primary and secondary metabolites, in fruits can affect the quality of fruits. For example, organic acids can contribute to the organoleptic properties of fruits, such as flavor, color, and aroma (Flores et al., 2012). Organic acids can also affect the cellular pH and anthocyanin structure and change the visual color of fruits (Perkins-Veazie and Collins, 2001). In particular, most secondary metabolites in the five fruits in this study, which included quercetin, kaempferol, rutin, chlorogenic acid, neochlorogenic acid, catechin, epicatechin, epigallocatechin, phloridzin, and gallic acid, have an extensive range of human health benefits (Tian et al., 2021; Zhen et al., 2016).

The correlation of the secondary metabolites in the pulps and peels with antioxidant and a-amylase inhibitory activities was highlighted by Pearson's correlation analysis (Fig. 6K). As expected, we found a strong association of the TPC and TFC with DPPH and ATBS free radical-scavenging activity, and with α-amylase inhibitory activity (α-AI). Also, *p*-coumaric acid, quercetin glucoside, gallocatechin, epicatechin, catechin, chlorogenic acid, neochlorogenic acid, and rutin showed the strong correlations with antioxidant and α-AI activities. However, quercetin, quercetin derivatives, and kaempferol were significantly correlated with ABTS and DPPH free radical-scavenging activity only in the dataset of the Daebong persimmon pulp and peel ($P < 0.05$). Also, correlation between gallic acid and antioxidant activity in fruit extracts was observed, but gallic acid were not correlated with α-amylase inhibitory activity, indicating that gallic acid might play limited role in α-amylase inhibitory activity. Gallic acid shows various biological activities, especially antioxidant activity (Phonsatta et al., 2017). In the current study, ATBS free radical-scavenging activity was still observed in the Daebong persimmon pulp and peel even after postharvest ripening (Fig. 6D), even though the TPC and TFC in the pulp largely decreased (Fig. 6J). Also, there was less decrease and large increase in the gallic acid levels in the pulp and the peel, respectively, after postharvest ripening (Fig. 6J). Therefore, gallic acid may partly contribute to the antioxidant activity. Quercetin, quercetin derivatives, and kaempferol were not correlated with ABTS in the whole dataset collected from Daebong persimmon, sweet persimmon, and apple, but were correlated with ABTS ($P = 0.04$, $P = 0.01$, and $P = 0.02$, respectively) in the dataset with sweet persimmon and apple excluded. The same results were observed in the correlation of these compounds with DPPH. Therefore, a biological correlation study between health-beneficial effects and individual metabolites in extracts containing diverse metabolites should be carefully considered, which might be dependent on the kinds of datasets. Thus, a precise way to assess the evidence of the biological roles of individual metabolites in extracts needs to be developed. Nevertheless, chlorogenic acid, *p*-coumaric acid, and quercetin glucoside observed in the peels of grape and peach might contribute to strong α-amylase inhibitory and antioxidant activities, highlighting different roles of the phytochemicals in health-beneficial effects because the contributions of other phytochemicals in the fruits to α-amylase inhibitory activities were lack. Indeed, even high amounts of bioactive compounds in the peels of the fruits and their potential health benefits, pesticide residues likely limit intake of whole fruits. However, various ways to reduce the level of pesticides in foods have been introduced, which included washing with water or soaking in solutions of salt and some chemicals (Bajwa and Sandhu, 2014).

4. Conclusion

We chose five fruits (Daebong persimmon, sweet persimmon, peach, apple, and grape) to explore their diverse metabolite compositions and metabolic differences between their peels and pulps, through ^{1}H NMR-based metabolomic approach. Of the results, simultaneous determinations of distinct secondary metabolites in each fruit and their associations with antioxidant capacity and α-amylase inhibitory activity were highlighted. Moreover, large productions of gallic acid, a strong bioactive compound, in the peel of astringent persimmon during postharvest ripening were observed for the first time in the current study. Since the secondary metabolites of fruits were mostly dominant in their peels, intake of whole fruits was recommended, rather than removal of peels, to give maximum health benefits.

CRediT authorship contribution statement

June Song: Writing – original draft, Methodology, Formal analysis, Investigation. **Jaesik Jeong:** Formal analysis, Statistical analysis, Validation, Software. **Eun-Hee Kim:** Metabolite profiling, Metabolite Assignment, Validation. **Young-Shick Hong:** Writing – review & editing, Funding acquisition, Conceptualization, Supervision, Resources.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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