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Antioxidative Activities of Korean Apple Polyphenols

－ Research Note －

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Abstract

The antioxidative activity and the polyphenolic composition were examined in four different cultivars of apple (*Malus domestica*), 'Fuji', 'Tsugaru', 'Hongro' and 'Kogetsu', and their parts (peel, core, pulp and juice). The total phenolics, flavonoids and anthocyanins differed among the tested cultivars and parts. Peel parts had the highest total phenolics and anthocyanin content. Contributions of those phenolics to total antioxidative activity were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, and the linoleic acid oxidation assay. Concentration of phenolics contributes significantly to the total antioxidative activity of apples. Clearly, apple peels, especially from Hongros and Kogetsus, possess high levels of phenolic compounds and antioxidants. Therefore, apple peels may potentially function as a value-added ingredient.

Key words: apple polyphenol, flavonoids, antioxidative activity

INTRODUCTION

Apples are a popular fruit in Korea, accounting for 35% of total fruit production (1). As a significant part of the diet, apples are the top contributors of flavonoids in Finland and the United States (2,3) and third largest in the Dutch diet (4). Consumption of apples has been linked to the prevention of chronic diseases (5). Recent epidemiological studies have shown the inverse correlation between the consumption of apples, and/or related products, and many human chronic diseases. Most noticeably, apples can lower the risk of cardiovascular diseases, lung dysfunctions, and various cancers, particularly prostate, liver, colon and lung cancers (6-9). This biological impact of apple may be due largely to the presence of antioxidants (10), which are considered to be from phytochemicals, such as the polyphenolics commonly found in fruits and vegetables. The activity and concentration of antioxidants in fruits differ among cultivars and the specific part of the fruit (11,12). Total phenolic content was the highest in cranberries (527.2 mg/100 mg), followed by apples (296.3 mg/100 mg) and red grapes (201.0 mg/100 mg) and there was a direct relationship between total phenolic content and antioxidative activity in phytochemical extracts of different fruits (13,14). The total extractable phenolic content, previously investigated, ranges from 110 to 357 mg/100 g of fresh apples (15,16). The concentration of total phenolic compounds in the apple peels is much greater than the flesh with 75.7～103.2 mg/100 mg (17-19). Furthermore, antioxidative activity depends on the specific phenolics (14). Previous reports have focused on phenolic compounds and their antioxidative activities of Fuji cultivar only (20) while none report on the contents and antioxidant activities comparing cultivars and parts of apples consumed in Korea. In this study, the phenolic compound content and antioxidative activity were investigated in the top four consumed and produced cultivars ('Fuji', 'Tsugaru', 'Hongro' and 'Hongwoel') of apples (*Malus domestica*), and their different parts (peel, core, pulp and juice), in Korea.

MATERIALS AND METHODS

Materials

Four different apple cultivars, Fuji, Tsugaru, Hongro and Hongwoel, were purchased from a local market during harvest season. Peels were separated from 10 randomly chosen apples using a manual peeler with 0.5 cm thickness. The core part was cut with a manual corer. Flesh was extracted using a mechanical juicer (Warren Electrical Manufacturing Ltd., Hong Kong, China) and juice and pulp parts were separated. All solvents and chemicals used were of analytical grade. Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), linoleic acid, gallic acid, catechin and potassium chloride were obtained from Sigma Chemical Co. (St. Louis, MO,

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USA). Sodium carbonate, sodium nitrite and aluminum chloride were purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). 2-Thiobarbituric acid (TBA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Extraction

The phenolic compounds of apples were extracted by a previously described method (7). Briefly, 200 g of apple parts (peel, core, pulp or juice) was blended with 1000 g of chilled 80% acetone solution in a blender for 5 min, The sample was then homogenized for 3 min using an Ultra Turrax mixer (T25, IKA, Staufen, Germany). The slurry was filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum. The solids were scraped into 400 g of 80% chilled acetone and homogenized again for 3 min before refiltering. The filtrate was recovered and evaporated using a rotary evaporator at 45°C until a coin size remained. The extract was freeze-dried.

Determination of total phenolic content

The total phenolic contents of the apple samples were measured using a modified colorimetric Folin-Ciocalteu method (21). A volume of 0.5 mL of deionized water and 0.125 mL of a known dilution of the extract were added to a test tube. Folin-Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. Then, 1.25 mL of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 3 mL with deionized water. The color developed for 90 min, and the absorbance was read at 760 nm using a spectrophotometer (DU650, Beckman, Minneapolis, MN, USA). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as milligrams of gallic acid equivalents per 100 g of fresh apple component for the triplicate extracts.

Determination of flavonoid content

The flavonoid contents of the apple samples were measured using a modified colorimetric method (22). A volume of 0.25 mL of a known dilution of extract was added to a test tube containing 1.25 mL of distilled water. To the mixture, 0.075 mL of 5% sodium nitrite solution was added and allowed to stand for 5 min. Then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 mL of 1 N NaOH was added, and the mixture was diluted with 0.275 mL distilled water. The absorbance of the mixture at 510 nm was measured immediately using a spectrophotometer and compared to a standard curve of catechin concentrations. The flavonoid content was expressed as mg catechin equivalents/100 g for the triplicate extracts.

Determination of anthocyanin content

Monomeric anthocyanin content of the apple was measured using a spectrophotometric pH differential protocol (23). The apple acetone extracts were mixed thoroughly with 0.025 M potassium chloride pH 1 buffer in 1:3 or 1:8 ratio of extract : buffer. The absorbance of the mixture was then measured at 515 and 700 nm against the distilled water blank. The apple peel extracts were then combined similarly with sodium acetate buffer pH 4.5, and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin content was calculated as follows: Total monomeric anthocyanins (mg of cyaniding-3-glucoside equivalents/100 g fresh weight) = $A \times MW \times 100 / (\varepsilon \times C)$. A = [(A510 nm − A700 nm) pH 1.0 − (A510 nm − A700 nm) pH 4.5] with a cyanidine-3-glucoside molar extinction coefficient of 29600.

DPPH radical scavenging activity

The antioxidative activities of apples and standard compounds were evaluated according to Brand-Williams et al. (24) with some modifications. All extracts were diluted 1:5 with 95% methanol. An aliquot of 100 mL of diluted samples or standard compounds was added to 2.9 mL of DPPH solution (0.1 mM in methanol) and a 517 nm absorbance was read at 0 min followed by 10 min intervals until the reactions reached a plateau. The DPPH methanolic solution was used as a blank to correct absorbance at the end of the reaction. The radical scavenging activity of the samples was calculated according to the following formula: DPPH radical scavenging activity (%) = {1 − (absorbance of sample/ absorbance of control)} × 100.

ABTS radical anion scavenging activity

The method used by Lee et al. (25) was slightly modified in this experiment. 2,2'-Azinobis(2-amidinopropane) dihydrochloride (AAPH), 1.0 mM, was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution (100 mM potassium phosphate buffer (pH 7.4) containing 150 mM NaCl). The mixture was heated in a water bath at 68°C for 13 min. The concentration of the resulting blue-green ABTS solution was adjusted to an absorbance of 0.3 at 734 nm. The sample solution of 20 mL was added to 980 mL of the resulting blue-green ABTS radical solution. The mixture, protected from light, was incubated in a water bath at 37°C for 10 min. The decrease of absorbance at 734 nm was measured at the endpoint of 10 min. A control consisted of 20 mL of 50% methanol and 980 mL of ABTS solution. The stable ABTS radical scavenging activity of apple phenolic extracts and selected pure chemical compounds

was expressed as μg catechin equivalent/g in 10 min, respectively. The radical stock solution was prepared fresh daily.

Antioxidative activity against linolate oxidation

The TBA assay was performed according to the modified method of Tanaka et al. (26). The 10% linoleic acid emulsion was incubated at 37°C for 24 hr. The incubated samples (200 L) was mixed with 650 L of a mixture of 5.2% (w/v) sodium dodecyl sulfate, 0.8% (w/v) BHT in acetic acid, 0.8% (w/v) TBA, and 150 L of 20% (w/v) acetate buffer (pH 3.5) and heated at 100°C for 1 hr. After cooling, the mixture was centrifuged at 3000 rpm for 10 min. Absorbance of the supernatant was measured at 532 nm. The level of TBA reactive substances (TBARS) by autoxidation of linoleic acid was calculated as the amount of malondialdehyde (mg MDA/mL).

Statistical analysis

All data were reported as mean with standard deviation of three replicates. The results were compared by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Content of phenolic compounds

Apples are commonly eaten in the world. Phytochemicals such as polyphenolics have been found to be the major source of antioxidants in apples (13). The total phenolics contents of the peel, core, pulp and juice of the four apple varieties were determined in this study (Fig. 1). Of the peels, the total phenolic contents from Hongros were the highest ($p<0.05$) with 294.75±2.51 mg of gallic acid equivalents/100 g of peels, followed by 254.19±8.21 for Fuji, 252.14±3.11 for Kogetsu, and 177.22±4.50 for Tsugaru. The total phenolic content of the core of Hongro (232.98±3.273 mg of gallic acid equivalents/100 g of core) was the highest and those of Kogetsu (174.41±8.54) and Tsugaru (164.55±4.40) apples were similar ($p>0.05$). The pulp presented 201.65±9.37 for Kogetsu, 192.69±6.07 for Hongro, 38.69±2.18 for Fuji and 126.80±2.79 mg of gallic acid equivalents/100 g for Tsugaru. The pulp of Kogetsu had the highest total phenolic content. The juice from Hongro had the highest phenolic content with 95.53±4.27 mg of gallic acid equivalents/100 g. The juice of Fuji had a lower phenolic content (22.26±0.38 mg of gallic acid equivalents/100 g) than the juice from Tsugaru (36.54±1.07 mg of gallic acid equivalents/100 g) and Kogetsu apples (43.13±1.68 mg of gallic acid equivalents/100 g) ($p<0.05$). The total extractable phenolic content is reported to range from 110 to 350 mg/100 g of fresh apple (15, 16). The total phenolic content tended to be highest in the peel, followed by the core, pulp and juice for all four apple varieties and many reports also revealed that the apple peel possesses high contents of phenolic compounds than flesh (5,7,11,13). The peels are often discarded as a waste product from apple processing manufacturers, perishing a valuable source of nutrition.

The soluble flavonoids of the apple components were measured (Fig. 2). The peels of Hongro apples had the highest flavonoid contents (277.98±6.87 mg of catechin equivalents/100 g). The flavonoid contents of Kogetsu and Fuji apple peels (220.94±5.27 and 206.94±7.87 mg of catechin equivalents/100 g, respectively) were not significantly different ($p>0.05$) yet higher than the contents of the peels from Sugaru apples (142.50±3.92) ($p<0.05$). The flavonoid contents of the core of apples were, with decreasing order, 188.00±2.15, 128.01±1.21, 114.50±2.37, and 75.64±1.71 mg of catechin equivalents/100 g for Hongro, Kogetsu, Tsugaru and Fuji, respectively. The Kogetsu pulp possessed significantly more flavonoids (227.87±3.00 mg of catechin equivalents/100 g) than the other varieties ($p<0.05$), even more than peel parts of Kogetsus ($p>0.05$). The juice contained 68.59±

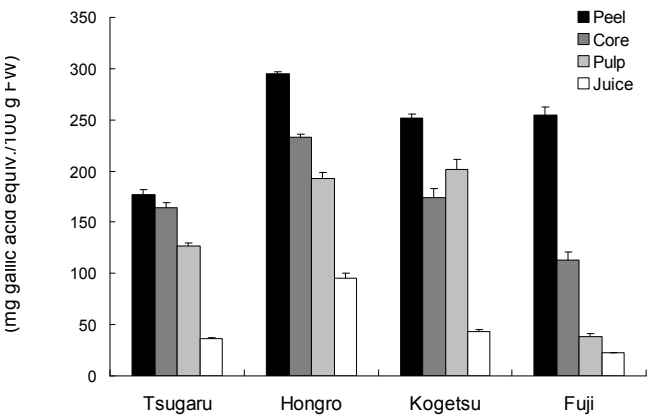

Fig. 1. Total phenolic content of different portions from four varieties of apples (mean±SD, n=3).

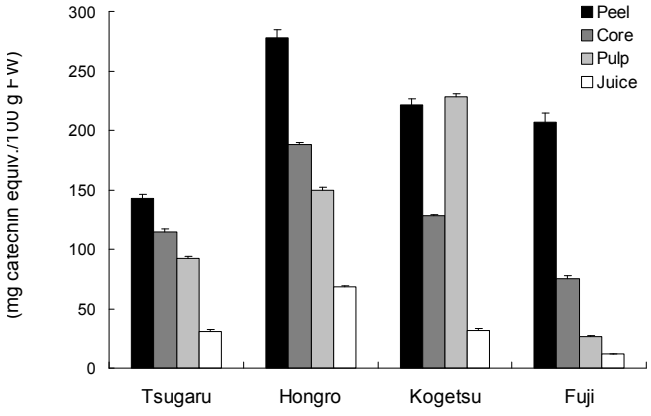

Fig. 2. Flavonoid content of different portions from four varieties of apples (mean±SD, n=3).

Table 1. Monomeric anthocyanin content of different portions from apples

Apple portions	Monomeric anthocyanin (mg cyanidin 3-glucoside equiv./100 g FW)			
	Tsugaru	Hongro	Kogetsu	Fuji
Peel	2.37±0.03[1)]	4.95±0.07	6.31±0.05	3.35±0.09
Core	ND[2)]	0.36±0.00	0.27±0.00	ND
Pulp	ND	ND	ND	ND
Juice	ND	ND	ND	ND

[1)]Mean±SD, n=3. [2)]No data

0.90 (Hongro), 32.01±1.18 (Kogetsu), 30.53±1.87 (Tsugaru), and 12.27±0.08 (Fuji) mg of catechin equivalents/100 g of juice. The flavonoid content of the peel of Hongro was the highest. Within all varieties, the peels tended to contain the most flavonoids, followed by the core, pulp, and juice. The flavonoid contents of the peels were statistically higher than the other three parts from each variety ($p<0.05$). Only Kogetsu apples had higher flavonoid content in pulp than in the peel, regardless of no significant differences ($p<0.05$). The total phenolic and flavonoid contents in the peels of apples are much greater than in the flesh (5,17-19).

The anthocyanins in the apple peels were analyzed (Table 1). The anthocyanin was not detected from the pulp or juice parts of any varieties. The anthocyanin content of Kogetsu apple peels was the highest, with 6.31 ±0.05 mg of cyanidin 3-glucoside equivalents/100 g ($p<0.05$), followed by Hongro (4.95±0.07), Fuji (3.35 ±0.09) and Tsugaru (2.37±0.03). Cores from Hongro and Kogetsu contained a low amount of anthocyanins. The anthocyanin content of Kogetsu peels was significantly higher than the contents of the peels of the other varieties ($p<0.05$). The measured anthocyanin content of the apple peels was related to their appearance; the red color of apple peels is due to the presence of cyaniding-3-galactoside (11). The Hongro and Kogetsu apples had a deep red color for all surfaces and contained the most anthocyanins. The Fuji apples were deep red but with green patches, and the Tsugaru apples were green. Fuji and Tsugaru varieties had far less anthocyanins than the Kogetsu and Hongro.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the peels was greater than that of pulp, core or juice parts for all varieties (Fig. 3). Hongro peels possessed the greatest activity with 92.90±0.13% scavenging activity for DPPH radicals. Peels of Kogetsu, Fuji, and Tsugaru apples had the highest DPPH radical scavenging activity of 90.71± 1.25%, 89.23±0.51%, and 73.82±0.08%, respectively. The core of Hongro and Kogetsu apples had similar activities (92.63±0.08% and 92.79±0.15%) compared to peel parts of the same varieties; however, the cores of Tsugaru and Fuji (58.19±0.15% and 58.60±0.25%, respectively) were significantly lower than their peel parts. The DPPH radical scavenging activity of the pulp parts from Hongro (92.63±0.11%) and Kogetsu (92.79±0.12 %) were statistically higher ($p<0.05$) than those of Tsugaru and Fuji. There was no difference between the values for Hongro and Kogetsu pulps ($p>0.05$). The juice parts had significantly lower activities than those from other parts. The DPPH radical scavenging activity of Hongro apples was higher than that of the other varieties. The antioxidative activity was highest from the peels in all varieties, followed by the core, pulp and juice, but not significantly ($p>0.05$). The apple flesh contains catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid, and chlorogenic acid; the apple peel possesses epicatechin, phloridzin, chlorogenic aicd, epicatechin dimmer (procyanidin B2) and quercetin glycosides (27). Lu and Foo (27) showed that procyanidins and quercetin glycosides were the highest in antioxidative acivity against linolate oxidation and the DPPH method. Ariga et al. (28) also reported that the procyanidins were more effective in scavenging free radicals than catechin, which is more prevalent in flesh. The measured anthocyanin content of the apple peels was related to their appearance.

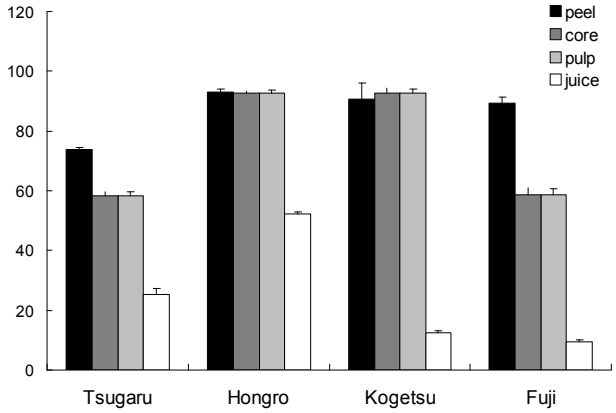

Fig. 3. DPPH radical scavenging activity of different portions from four varieties of apples (mean±SD, n=3).

ABTS radical scavenging activity

The ABTS radical scavenging activity of the peels was greater than that of pulp, core or juice parts for all varieties (Fig. 4). Hongro peels possessed the greatest activity, with 171.79±9.27 μg catechin equivalent/g. The peels

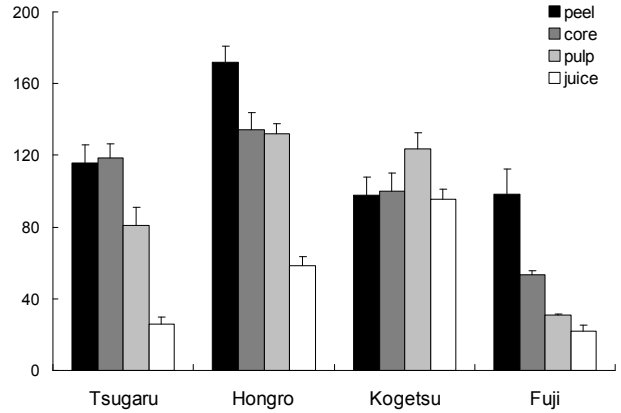

Fig. 4. ABTS radical scavenging activity of different portions from four varieties of apples (mean±SD, n=3).

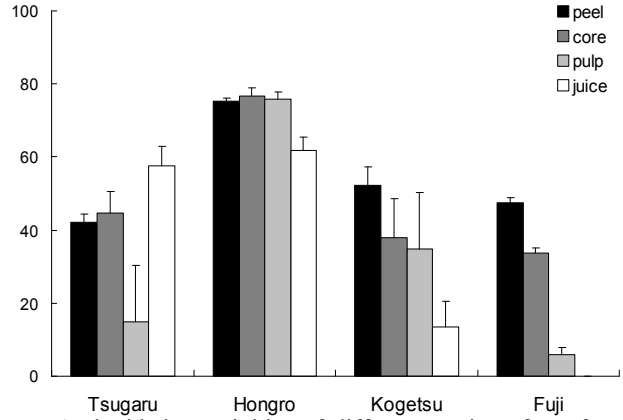

Fig. 5. Antioxidative activities of different portions from four varieties of apples in the linolate peroxidation system (mean±SD, n=3).

of Kogetsu, Tsugaru and Fuji apples had ABTS radical scavenging activity of 97.66±10.40, 115.48±10.62 and 98.51±13.97 μg catechin equivalent/g, respectively, showing no significant difference. The core of Hongro presented highest activity with 134.44 μg catechin equivalent/g. Tsugaru and Kogetsu apples had similar activities (118.37±7.97 and 100.26±9.64 μg catechin equivalent/g, respectively) but the cores of Fuji (53.14±2.45 μg catechin equivalent/g) were significantly lower than cores from other varieties. The ABTS radical scavenging activity of the pulp parts from Hongro (131.91±5.98 μg catechin equivalent/g) and Kogetsu (123.81±8.71 μg catechin equivalent/g) were statistically higher ($p<0.05$) than those of Tsugaru and Fuji pulp. There was no difference between the values for Hongro and Kogetsu ($p>0.05$). The juice parts had significantly lower ABTS radical scavenging activity than other parts for all varieties (95.59±5.29 (Kogetsu), 58.31 ±5.29 (Hongro), 25.84 ±4.01 (Tsugaru), and 22.19±3.09 (Fuji) μg catechin equivalent/g). The ABTS radical scavenging activity of juice from Kogetsu apples was significantly higher than that of the other varieties ($p<0.05$). Hongro was significantly high in ABTS radical scavenging activity compared to other varieties. There were no differences in ABTS radical scavenging activity among Kogetsu and Tsugaru ($p>0.05$). The ABTS radical scavenging activity was highest from the peels in all varieties, followed by the core, pulp and juice.

Antioxidative activity against linolate oxidation

The inhibition against linolate oxidation from Hongro was highest among the four varieties. Peels were more effective in preventing oxidation of linolate than that of pulp, core or juice parts for all varieties (Fig. 5). Hongro peel had the greatest activity, with 73.33±0.78% inhibition. The peels of Kogetsu, Fuji and Tsugaru apples had inhibitory effects against oxidation of linolate of 52.20±5.11, 47.48±1.46, and 42.19±2.23% inhibition, respectively. The core of Hongro presented highest activity with 76.80±2.08% and cores from Tsugaru were the second highest with 44.64±6.13%, followed by Kogetsu and Fuji apples with activities of 37.82±10.88 and 33.82±1.39%, respectively. The inhibitory effect against oxidation of linolate of the pulp part from Hongro was statistically higher ($p<0.05$) than other varieties. The juice parts had a significantly lower inhibitory effect than other parts for all varieties (61.70±3.85% (Hongro), 13.57±7.04% (Kogetsu), 37.55±5.47% (Tsugaru), and 0.00±0.00% (Fuji), respectively. The inhibitory effect of juice from Hongro apples was significantly higher than that of the other varieties ($p<0.05$). There were no differences in the inhibitory effect against oxidation of linolate among parts from Hongro ($p>0.05$). The oxidation of linolate was most effectively prevented by peels from Hongro. Wang et al. (29) and Vinson et al. (4) ranked apples ninth out of 12 and eighth out of 20, respectively, for their inhibitory effect. Apples exhibited high antioxidative activity in all tests investigated, and the value varied between apple varieties and their parts (27,30). The peels of the apple varieties under investigation exhibited high antioxidative activity compared to the core, pulp and juice ($p<0.05$). There was also a positive relationship between the phenolic content of apples and their antioxidative activity (16).

Our results show that apple peels may have health benefits for consumers who need antioxidants to prevent chronic diseases. Apple peels are a waste product during manufacturer processing. Clearly, they possess high levels of polyphenolic compounds and antioxidants and should be treated as a valuable resource. We believe that apple peels, especially from Hongro and Kogetsu, presented potential as a functional material for value-added ingredient due to their high source of antioxidants.

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