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Antioxidant Activity and Mineral Content in Unripe Fruits of 10 Apple Cultivars Growing in the Northern Part of Korea

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Abstract: This study evaluated unripe fruits of ten apple cultivars removed during fruit thinning for their antioxidant activity and mineral content. The unripe fruits were collected from Chuncheon and Jeongseon located at Gangwon-Do of Korea. Antioxidant activities such as total phenolic content (TPC), total flavonoid content (TFC), DPPH (2,2-diphenyl-1-picrylhydrazyl) activity, FRAP (Ferric reducing antioxidant power), vitamin C and mineral contents were measured. In the unripe fruits obtained from Chuncheon and Jeongseon, TPC was in the range 8.97–81.4 and 7.11–42.15 mg GAE/g, TFC was in the range 9.38–33.81 and 6.83–19.24 mg QE/g, DPPH was in the range 27.17–82.58 and 29.73–73.24, FRAP was in the range 33.54–371.12 and 26.76–185.69 µM trolox /g, and Vitamin C was in the range 1.1–4.9 and 1.1–2.8 mg/AA/g, respectively. Among the cultivars tested, 'Hongro' and 'Honggeum' had consistently highest antioxidant activity, while 'Summer King,' 'Tsugaru,' and 'Arisoo' had the lowest value. 'Picnic' had the highest mineral contents expect P and K in Joengsoen, while 'Summer King' and 'Tsugaru' had the lowest value in both locations. These differences could be due to the genetic characteristics and/or their growth environments. These results could provide information to help with better utilization of thinned unripe fruits of apples in Korea.

Keywords: DPPH; *Malus domestica*; Korean apple; total phenolic content

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1. Introduction

Apples (*Malus x domestica* Borkh) have plenty of nutrients, including vitamin C, minerals, and bioactive substances [1,2]. Minerals such as potassium (K), phosphorus (P), magnesium (Mg), and Calcium (Ca) in the apple fruit are metabolized to produce nutrients that help maintain the human physiological processes [3–5]. The phytochemical contents in apples have high levels of antioxidant and anti-inflammatory activities, which help reduce age-associated diseases such as (CVD) cardiovascular disease, hypertension, diabetes, and stroke [6] and improve bone health [7,8]. Polyphenolic extracts of apples treat chronic diseases such as diabetes [9], CVD associated with obesity [10], and other inflammatory responses [7]. In addition to the above, apples have a highly appealing flavor and are considered one of the most important fruits worldwide.

In Korea, apples are the most widely cultivated fruit crop with high industrial processing. During apple cultivation, most excess fruits are removed after full bloom to promote fruit quality, encourage tree vigor, and return bloom [11,12]. Fruit thinning is essential for producing competitive apples in the market, but it increases the burden of personal expenses and agricultural waste [13]. Hence, efforts should be made to utilize thinned fruits. Unripe fruits contain bioactive compounds such as polyphenols, organic acids, monosaccharides, and starches [12]. Interestingly, unripe apples have been reported to contain higher antioxidant and phenolic content [14–17]. Unripe strawberries and oranges have been reported to have a higher mineral content than mature fruit [18,19]. Although there are no reports on the mineral content in unripe apples, they could likely be used for food preservation and for producing functional foods, cosmetic juices, cider, and drugs [16,20,21].

Moreover, in Korea, apple cultivation has been moved to the northern parts due to global warming, and attempts are now being made to select high-quality apple cultivars in this region. In doing so, the characteristics of unripe apples should also be considered, given their potential health benefits and other uses. The major characteristics of a fruit are affected by its genetic background and the environment where it is grown. However, there is limited information on the phytochemical, antioxidant, and mineral contents of unripe apples. In addition, the environmental and genotypic effects on these characteristics have also not been evaluated. Therefore, the primary purpose of this study is to evaluate unripe apples grown in two different regions in the northern part of Korea, provide information about their nutrient content supporting the utilization of unripe apple, and also help to select suitable apple cultivars in these regions.

2. Materials and Methods

2.1. Plant Material

Fruit of ten apple cultivars (‘Arisoo', 'Fuji', 'Gamhong', 'GreenBall', 'Honggeum', 'Hongro', 'Picnic', 'Shinano Gold', 'Summer King' and 'Tsugaru'**)** were collected from the research orchards of Chuncheon and Jeongseon in the Gangwon Province at the end of May 2022 located at 37°95′ N, 127°77′ E, and 37°43′ N, 128°66′ E, respectively. Three apple trees from the replication row were randomly selected, and 20 apples of each cultivar were collected from the top, middle, and within the crown each of tree. The collected apples were brought to the plant breeding laboratory Gangneung-Wonju National University.

2.2. Sample Preparation and Extraction

Apples were sliced and deseeded. Apple slices containing the peel and flesh were dried, at 60 °C, for 72 h in an oven. The dried slices were ground into powder using a mortar and pestle. Apple powder (0.01 g) of each cultivar was taken into a 10 mL test tube and mixed by vortexing for a minute. Methanolic extracts apples were prepared using a Bransonic ultrasonic bath (model CPX3800H-E, made in Connecticut, U.S.), at 60 °C, for 30 min. The homogenized solution was centrifuged at 4500 rpm for 20 min, and the supernatant was collected. The remaining residue was reprocessed as described above. The apple methanolic extract was stored, at −20 °C, until further analysis.

2.3. Assay for Antioxidant Activity

2.3.1. Total Phenolic Content

The total phenolic content (TPC) was determined using the method of [22]. The methanol extracts sample 0.5 mL (1 mg/mL) were mixed with 2.5 mL Folin–Ciocalteu reagent diluted tenfold, vortexed, and allowed to stand for three minutes. Afterward, 0.75 mL of sodium bicarbonate (20%) and 1.25 mL of distilled water were added, mixed thoroughly, and allowed to stand in the dark, at room temperature, for one hour. The absorbance was measured at 765 nm using a spectrophotometer (Model, NEO UV-5490, NEOGEN). Gallic acid (0–100 µg) was used as the standard and the TPC was expressed as mg gallic acid equivalent per gram dry weight (GAE mg/g DW).

2.3.2. Total Flavonoid Content

The total flavonoid content (TFC) was determined using the method of [23] with slight modification. Then, 0.5 mL (1 mg/mL), methanol 1.5 mL, 10% aluminum chloride (0.1 mL), and 1M sodium acetate (0.1 mL) were added to the sample extract and mixed thoroughly by vortexing. After allowing the mix to stand for 30 min in the dark at room temperature, absorbance was measured at 415 nm. Quercetin (0–250 µg) was used as the standard to construct the calibration curve. TFC was expressed as mg quercetin per gram dry weight (QE mg/g DW).

2.3.3. DPPH Free Radical Scavenging Activity

The free radical scavenging activity was measured using DPPH as described by Brand-Williams et al. [24]. Briefly, 100 µL of the sample extract was in a test tube, to which was added 3 mL of 0.004% DPPH solution, and the contents were mixed by vortexing. After allowing the mixed solution in the dark for 30 min, at room temperature, absorbance was read at 415 nm using a spectrophotometer. Using DPPH absorbance as a control, free radical scavenging activity was calculated as follows:

$$\text{DPPH activity inhibition (\%)} = [(\text{A Control} - \text{A sample})/\text{A Control})] \times 100$$

2.3.4. Ferric Reducing Antioxidant Power (FRAP)

FRAP was assessed to determine the reduction of ferric to ferrous ions as described by Benzie and Strain [25]. The FRAP solution was prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (diluted in 40 mM HCl), and 20 mM $FeCl_3$ at a ratio of 10:1:1. FRAP solution (3 mL) was added to the sample extract (100 µL) and mixed by vortexing. After incubating the mix, at 37 °C, for 4 min, absorbance was read at 593 nm. The standard curve trolox (0–100 µg) was constructed, and FRAP was expressed as µM trolox/g DW.

2.3.5. Estimation of Vitamin C Levels

Vitamin C levels were measured by spectrophotometry [26]. Briefly, 0.05 g of apple powder was homogenized with 15% meta-phosphoric acid and −10% acetic acid in a 10 mL test tube. The homogenate was filtered and centrifuged at 4000 rpm for 15 min, and the supernatant was collected. The supernatant (4 mL) was added to 3% of bromine water (0.23 mL) and thiourea solution (0.13 mL) to remove excess bromine. Subsequently, 1 mL of 2, 4, DNP was added to form osazone. All samples and standard solutions were kept in a thermostatic bath for three hours. They were then cooled in an ice bath for 30 min and treated with 5 mL of 85% H_2SO_4. Absorbance was read at 521 nm, and the standard absorbic acid graph was constructed, ranging from 0–100 µg/g, and the result vitamin C concentration was expressed as ascorbic acid mg per gram (AA mg/g DW).

2.3.6. Mineral Composition

The mineral elements in the apple powder were determined by McCleary and Murphy [27] using inductively coupled plasma atomic emission spectrometry and expressed as mg /100 g DW.

2.4. Statistical Analysis

All assays were performed in triplicate (n = 3), and the results have been presented as mean and standard deviation. Two-way ANOVA was performed, and means were separated using Duncan multiple range tests (DMRT) at $p < 0.05$, using IBM, SPPS Version 25 (IBM, New York, NY, USA). Pearson's correlation coefficients were calculated to analyze the correlation between the phenolic and antioxidant activities.

3. Results

Significant differences were noted between cultivars, locations, and their interactions for all variables tested based on the analysis of variance (Table 1). The mean effect of interaction between locations and cultivars significantly affected the antioxidant activity and mineral contents. This finding show that growing location and genetic background have profound effect on antioxidant activity and mineral contents of unripe apple fruits.

Table 1. Mean analysis of antioxidant activity and mineral contents of unripe apple fruits based on cultivars in the locations.

	Source of Variation			
Variable	**Cultivar**	**Location**	**C×L**	**Error**
TPC	1946.6 **	10,137.920 **	327.851 **	2.46
TFC	152.295 **	506.109 **	56.504 **	1.09
DPPH	1278.980 **	4528.186 **	232.064 **	2.7
FRAP	57,216.78 **	581,496.8 **	20,025.1 **	10.89
VC	3.315 **	9.064 **	1.566 **	0.02
P	4.513 **	0.275 **	1.663 **	0.02
K	31.732 **	6.292 **	20.8 **	0.19
Ca	19.558 **	1.233 **	3.2 **	0.019
Mg	7.528 **	1.077 **	1.1 **	0.008

** indicates significance at $p < 0.05$. The values for TPC, TFC, FRAP, Vitamin C, and mineral elements have been expressed as mg GAE/g dry weight, mg QE/g dry weight, μM trolox/g dry weight, AA mg/g dry weight, and g/100 g dry weight, respectively. TPC: Total phenolic content; TFC: Total flavonoid content; FRAP: Ferric reducing antioxidant power; DPPH: 2,2-diphenyl-1-picrylhdrazyl.

3.1. Total Phenolic and Flavonoid Contents

Table 2 presents the TPC and TFC of unripe apple cultivars from the Jeongseon and Chuncheon locations. The TPC varied substantially among the cultivars and locations ranging from 7.1–81.4 mg GAE/g DW. 'Hongro' had the highest TPC followed by 'Honggeum', and 'Gamhong', while 'Summer King', 'Tsugaru', and 'Arisoo' had the lowest across the locations. The TFC also varied considerably among the different cultivars and locations, ranging 6.83–33.81 mg QE/g DW. 'Hongro' and 'Honggeum' cultivars had the highest TFC in Jeongseon, whereas in Chuncheon 'Hongro' had highest TFC followed by 'Honggeum', while 'Summer King', 'Tsugaru', and 'Arisoo' had the lowest in both locations. The main variation might be due to the genetic background, and growing condition. Among the locations, high concentration of TPC and TFC were obtained in Chuncheon expect Arisoo. This may be due to environmental factors such as temperature, solar radiation, sunshine duration and management practice.

Table 2. Total phenolic and flavonoid contents in unripe apple fruits from two locations.

	Jeongseon		Chuncheon	
Cultivar	**TPC**	**TFC**	**TPC**	**TFC**
Arisoo	9.2 ± 1.6^{d}	16.47 ± 0.5^{b}	14.28 ± 0.9^{g}	11.38 ± 0.3^{f}
Fuji	20.77 ± 2.4^{c}	15.16 ± 0.6^{b}	45.75 ± 0.4^{d}	21.34 ± 0.4^{d}
Gamhong	29.92 ± 0.3^{b}	10.19 ± 0.6^{c}	69.33 ± 1.8^{b}	18.51 ± 1.1^{e}
Green Ball	14.73 ± 1.4^{d}	11.56 ± 1.5^{c}	60.26 ± 1.3^{c}	24.54 ± 0.7^{c}
Honggeum	30.92 ± 0.4^{b}	18.83 ± 0.9^{a}	69.57 ± 1.3^{b}	26.78 ± 2.2^{b}
Hongro	42.18 ± 2^{a}	19.24 ± 0.9^{a}	81.46 ± 2.6^{a}	33.81 ± 0.2^{a}
Picnic	20.80 ± 1.7^{c}	15.16 ± 0.4^{b}	59.39 ± 0.8^{c}	20.91 ± 0.5^{d}
Shinano Gold	12.96 ± 1^{d}	14.17 ± 1.7^{b}	29.87 ± 0.5^{f}	$19.78 \pm 1.14^{d,e}$
Summer King	7.1 ± 0.8^{e}	6.8 ± 0.7^{d}	8.9 ± 0.6^{h}	9.3 ± 1.47^{g}
Tsugaru	7.5 ± 0.3^{e}	12.58 ± 0.8^{c}	33.62 ± 1^{e}	13.18 ± 0.7^{f}

Different letters indicate the significant differences between the mean at $p < 0.05$. TPC and TFC are expressed as mg GAE/g dry weight and QE/g dry weight, respectively. TPC: Total phenolic content; TFC: Total flavonoid content.

3.2. Antioxidant Activity

Table 3 summarizes the antioxidant activities of unripe apple fruits determined by the DPPH, FRAP, and vitamin C assays. The percent DPPH inhibition ranged from 27.17 to 82.58, with significant differences between locations as well as cultivars. The highest DPPH inhibition was seen in 'Hongro', followed by 'Honggeum', while the lowest value was in

'Summer King', 'Tsugaru', and 'Arisoo'. The lower inhibition percentage was observed in Jeongseon. This might be due to environmental factors and management practice.

Table 3. Variation of antioxidant activities in unripe fruits of 10 apple cultivars from the two locations.

	Jeongseon			Chuncheon		
Cultivar	**DPPH**	**FRAP**	**Vitamin C**	**DPPH**	**FRAP**	**Vitamin C**
Arisoo	30.68 ± 1.8 ^{d,e}	41.99 ± 0.4 ^{f}	1.6 ± 0.1 ^{e}	40.58 ± 0.6 ^{g}	115.73 ± 0.5 ^{h}	0.8 ± 0.07 ^{f}
Fuji	33.65 ± 0.5 ^{d}	50.41 ± 2.03 ^{e}	1.7 ± 0.02 ^{c}	55.58 ± 0.4 ^{d}	340.01 ± 1.6 ^{c}	3 ± 0.3 ^{c}
Gamhong	28.87 ± 1.5 ^{e}	23.68 ± 0.2 ^{g}	1.2 ± 0.01 ^{f}	66.98 ± 0.4 ^{b,c}	276.81 ± 3.5 ^{e}	2.2 ± 0.03 ^{d,e}
Green Ball	30.23 ± 4.4 ^{de}	54.30 ± 1.9 ^{e}	1.6 ± 0.04 ^{d,e}	66.2 ± 0.3 ^{c}	323.64 ± 3.5 ^{d}	2.3 ± 0.01 ^{d}
Honggeum	60.02 ± 2.1 ^{b}	185.69 ± 4.3 ^{a}	1.9 ± 0.02 ^{b}	68.53 ± 1.6 ^{b}	371.12 ± 2.9 ^{b}	4.9 ± 0.1 ^{a}
Hongro	73.24 ± 0.7 ^{a}	158.63 ± 3.3 ^{b}	2.8 ± 0.03 ^{a}	82.58 ± 0.04 ^{a}	562 ± 5.5 ^{a}	3.3 ± 0.06 ^{b}
Picnic	48.92 ± 1.1 ^{c}	107.93 ± 4.4 ^{c}	1.7 ± 0.05 ^{c,d,e}	66.56 ± 2.3 ^{c}	326.39 ± 3.4 ^{d}	1.9 ± 0.07 ^{e}
Shinano Gold	33.71 ± 2.7 ^{d}	65.80 ± 3.5 ^{d}	1.8 ± 0.01 ^{c}	51.18 ± 0.9 ^{e}	220.74 ± 4.8 ^{f}	2.4 ± 0.1 ^{d}
Summer King	29.73 ± 1.2 ^{e}	26.76 ± 0.5 ^{g}	1.1 ± 0.12 ^{f}	27.17 ± 0.1 ^{h}	33.54 ± 1.4 ^{i}	1.1 ± 0.07 ^{f}
Tsugaru	29.79 ± 1.8 ^{e}	36.8 ± 2.2 ^{f}	1.7 ± 0.05 ^{c,d,e}	46.53 ± 1.2 ^{f}	150.89 ± 0.0 ^{g}	3.06 ± 0.4 ^{c}

Different letters indicate the significant differences among the mean values at $p < 0.05$. FRAP: Ferric reducing antioxidant power; DPPH: 2,2-diphenyl-1-picrylhdrazyl;Vitamin C are expressed as inhibition percentage (%), μM trolox /g dry weight, mg/AA/g dry weight, respectively.

The FRAP assay determined the reducing capacity of antioxidants by measuring the reduction of ferric to ferrous ions. The FRAP values for the samples ranged from 26.76–567.5 (μM Trolox/g) in both locations. In both locations, the highest FRAP was seen in 'Hongro' (158 and 567 μM trolox/g) and 'Honggeum' (185 and 323 μM trolox/g) apples, while the 'Summer King' (26.76 and 33.54 μM trolox/g) had the lowest FRAP. These findings indicate that the phytochemical content and antioxidant activity of apples are influenced by grown area. Significant differences were observed in the levels of vitamin C, a major antioxidant. 'Honggeum' and 'Hongro' had high concentrations of vitamin C in both locations. Overall, the 'Hongro' followed by 'Honggeum' had consistently highest antioxidant activity, while 'Summer King' fared poorly in comparison across the locations.

3.3. Correlation between Phytochemical, Antioxidant Activity and Mineral Contents

Table 4 presents Pearson's correlation between the phytochemical content and antioxidant activity of apples. The phytochemical contents (TPC and TFC) showed a positive correlation with antioxidant activity (DPPH, FRAP, and Vitamin C), respectively (Table 4). This indicates that TPC and TFC highly contribute to antioxidant activity. Mineral contents were highly correlated with each other, but there was no correlation with phytochemical contents and antioxidant activates except P with FRAP.

Table 4. Correlation between phytochemical, antioxidant activity and mineral contents of unripe apple fruit.

	TPC	**TFC**	**DPPH**	**FRAP**	**VC**	**P**	**K**	**C**	**Mg**
TPC									
TFC	0.831 **								
DPPH	0.895 **	0.851 **							
FRAP	0.916 **	0.901 **	0.890 **						
VC	0.710 **	0.589 **	0.683 **	0.712 **					
P	0.357	0.288	0.326	0.468 *	0.182				
K	0.220	0.232	0.220	0.301	0.064	0.847 **			
C	0.222	0.187	0.270	0.358	−0.008	0.719 **	0.596 **		
Mg	0.077	0.066	0.176	0.244	−0.083	0.741 **	0.605 **	0.956 **	

**, *: significance level at $p < 0.05$ and $p < 0.01$, respectively. TPC: Total phenolic content; TFC: Total flavonoid content; FRAP: Ferric reducing antioxidant power; DPPH: 2,2-diphenyl-1-picrylhdrazyl; P: Phosphorus; K: Potassium C: Calcium; Mg: Magnesium.

3.4. Mineral Composition

Significant differences were found in the macronutrient levels in unripe apple fruits from two locations. Potassium was the most prevalent element among the macronutrients studied, with concentrations ranging from 134.17 mg/100 g ('Summer King' in Chuncheon) and 240.7 mg/100 g ('Green Ball' in Jeongseon). In Jeongseon, Green ball' (240.7 mg/100 g) had the highest K levels, while Tsugaru' (135.4 mg/100 g) had the lowest K levels, whereas in Chuncheon, Picnic' (218.3 mg/100 g) and 'Green Ball' (217.4218.3 mg/100 g) had the highest K levels, while Summer King' (134.17 mg/100 g) had the lowest K levels. The phosphorus, calcium, and magnesium concentrations, depending on the cultivars and locations, were in the range 37.4–75.5 mg/100 g, 21.3–100.7 mg/100 g, and 18.6–66.9 mg/100 g, respectively (Table 5).

Table 5. Mineral contents in unripe apple fruits from two locations.

	Jeongseon				Chuncheon			
Cultivar	**Phosphorus** ^{z}	**Potassium**	**Calcium**	**Magnesium**	**Phosphorus**	**Potassium**	**Calcium**	**Magnesium**
Arisoo	67.6 ± 0.2^{c}	212.1 ± 1.4^{c}	63.4 ± 0.1^{b}	45.4 ± 0.2^{c}	58.1 ± 1.4^{e}	178.7 ± 1.4^{d}	42.8 ± 0.1^{d}	33.1 ± 0.7^{d}
Fuji	54.9 ± 0.3^{e}	161.6 ± 1.8^{g}	49.2 ± 0.4^{e}	44.3 ± 0.1^{c}	$60.5 \pm 0.6^{d,e}$	162.0 ± 2.9^{e}	47.5 ± 0.1^{c}	37.5 ± 1.2^{c}
Gamhong	$55.5 \pm 0.1^{d,e}$	168.9 ± 0.3^{f}	44.2 ± 0.4^{f}	42.6 ± 0.4^{d}	$63.5 \pm 0.2^{b,c}$	198.3 ± 3.9^{c}	57.6 ± 0.2^{b}	45.9 ± 0.3^{b}
Green Ball	72.0 ± 0.3^{b}	240.7 ± 0.2^{a}	61.1 ± 0.7^{c}	49.4 ± 0.9^{b}	64.4 ± 0.2^{b}	217.4 ± 4.2^{a}	$45.5 \pm 0.8^{c,d}$	37.4 ± 0.2^{c}
Honggeum	54.3 ± 0.3^{e}	180.4 ± 5.4^{e}	31.7 ± 0.8^{g}	29.3 ± 0.5^{e}	$61.2 \pm 0.5^{c,d}$	190.4 ± 3.8^{c}	46.9 ± 0.9^{c}	36.6 ± 0.1^{c}
Hongro	52.8 ± 3.1^{e}	183.1 ± 0.4^{e}	28.2 ± 0.6^{h}	27.7 ± 0.4^{f}	64.1 ± 0.4^{b}	208.1 ± 3.2^{b}	$44.7 \pm 0.9^{c,d}$	32.3 ± 0.1^{d}
Picnic	58.3 ± 0.2^{d}	200.5 ± 0.2^{d}	80.0 ± 0.0^{a}	58.1 ± 0.1^{a}	71.7 ± 0.3^{a}	218.3 ± 3.0^{a}	100.7 ± 2.6^{a}	66.9 ± 0.4^{a}
Shinano Gold	75.5 ± 0.2^{a}	231.2 ± 0.6^{b}	51.6 ± 0.2^{d}	49.0 ± 0.6^{b}	$62.3 \pm 1.9^{b,c,d}$	178.7 ± 1.3^{d}	46.8 ± 0.1^{c}	36.3 ± 0.2^{c}
Summer King	48.8 ± 0.0^{f}	201.1 ± 0.0^{d}	29.3 ± 0.3^{h}	26.9 ± 0.0^{f}	37.4 ± 0.0^{g}	134.1 ± 2.1^{f}	21.3 ± 0.4^{f}	18.6 ± 0.0^{f}
Tsugaru	40.3 ± 0.1^{g}	135.4 ± 0.2^{h}	22.4 ± 0.1^{i}	26.7 ± 0.2^{f}	50.6 ± 0.5^{f}	171.4 ± 3.2^{d}	35.9 ± 0.5^{e}	28.2 ± 0.3^{e}

Mineral contents are expressed as mg/100 g dry weight; ^{z} Different letters indicate the significant differences between the mean at $p < 0.05$.

4. Discussion

This study evaluated unripe fruits of apple cultivars grown in two regions in the northern part of Korea for their phenolic content, antioxidant activity, and mineral compositions. We obtained highly significant correlations between the antioxidant activity and phytochemical contents of unripe apple fruits, consistent with previous studies [3,28]. This correlation could be due to the proximity of the carboxylate and hydroxyl groups of polyphenols. However, the mineral content of the unripe apple fruits showed no significant relationship with their antioxidant and phytochemical contents, which is in line with a report on bananas [29]. These results strongly suggest that phytochemicals found in unripe apple fruits are potent free radical scavengers, whereas minerals do not contribute to antioxidant activity. In addition, different mechanisms are involved in the accumulation of phytochemicals and minerals in unripe fruits. Significant differences were found in the antioxidant activity, phytochemical content, and mineral contents among apple cultivars grown from different locations. Varying levels of antioxidants, phytochemicals, and minerals have been reported in apple cultivars [3,17,30], and climate is believed to be an important factor affecting their accumulation [5].

Most cultivars grown in Chuncheon had higher levels of minerals, phytochemicals, and antioxidant activity than those grown in Jeongseon. This variation may be due to different growth temperature and sunshine duration. The monthly mean temperatures in Jeongseon and Chuncheon during the fruit-bearing season are 14.7 °C and 15.2 °C, respectively. The sunshine duration in Jeongseon was much shorter compared to Chuncheon. Temperature is reported to be critical for the synthesis of secondary metabolites. Heat stress caused by high temperatures results in the production of strawberries with higher levels of phytochemicals, flavonols, anthocyanins, and antioxidants [31,32]. Shamloo et al. [33] also reported an increase in the total phenolic content of wheat genotypes grown at higher temperatures. Similarly, higher sunshine exposure enhances flavonoid and anthocyanin levels.

On the other hand, a drop in temperature is associated with lower water consumption and nutrient uptake, leading to a decrease in photosynthesis and carbon reallocation, negatively affecting mineral accumulation in fruits. Hence, compared to Jeongseon, Chuncheon, with a higher temperature during the fruit-bearing stage, provides more optimal conditions for the production of apples with higher levels of antioxidants, phytochemicals, and minerals.

Given that apple cultivation was managed similarly at both locations in this study, the differences in the levels of phytochemicals and minerals in unripe apple fruits could be a result of genetic variations. Among the tested cultivars, 'Hongro' and 'Honggeum' had high phytochemicals and antioxidants, while 'Summer King,' 'Tsugaru' and 'Arisoo' typically had lower levels regardless of the locations. Their genome background of the 'Hongro' and 'Honggeum' are 'Spur Earliblaze' x 'Supr Golden Delicious' and 'Shensu' x 'Hongro', respectively. Studies reported by Kim et al., 2020, also indicate that 'Honggeum' has high phenolic content. Among the tested cultivars, 'Picnic' had the highest mineral contents except for P and K in Joengsoen, while 'Summer King' and 'Tsugaru' had the lowest in both locations.

Our results provide information that will help in developing high phytochemical, antioxidant activity, and mineral content containing apple cultivars and breeding new apple varieties in Korea. Our study also shows that newly introduced apple cultivars such as 'Honggeum' have high phytochemical content and antioxidant activity compared to 'Fuji'. Similarly, 'Picnic' has a higher mineral content than 'Fuji'. 'Fuji' is among the most popular apples worldwide, and unripe 'Fuji' fruits are a rich source of natural products and could be applied to the production of health benefits antioxidants [15]. Our results suggest that the unripe fruits of some apple cultivars ('Fuji', 'Gamhong', 'Picnic', 'Shinano Gold' and 'Green Ball') tested in this study may have also significant health benefits and commercial applications. Hence, further research is required to understand their health-promoting effects and utilize them to improve human health in Korea.

5. Conclusions

In this study, significant differences were seen in the antioxidant activity and mineral composition of unripe apple fruits depending on cultivars and locations. 'Hongro' and 'Honggeum' were consistently found to have the highest phytochemical content and antioxidant activity, while 'Picnic' had the highest mineral contents except for P and K in Jeongseon. Our findings provide information to help with better utilization of thinned unripe apples and select the most suitable cultivars in the northern part of Korea.

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