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Antioxidant compounds and activities of pedicel and sepals from twelve varieties of colored cherry tomatoes

Yena Kim[1], Youngjae Shin[1], and Young-Jun Kim[2,*]

[1]*Department of Food Engineering, Dankook University*
[2]*Department of Food Science and Technology, Seoul National University of Science and Technology*

Abstract This study analyzed the antioxidant contents and activities of the pedicel and sepals from 12 colored cherry tomatoes ('Green Joy', 'TY Item', 'Dotori Red TY', 'TY Sispen', 'KT Orange TY', 'White Joy TY', 'Dotori Norang TY', 'Beta Tiny', 'Blacklin', 'KT Red TY', 'KT Norang TY', 'Black Joy 200') for their potential use as bioactive ingredients. 'Green Joy' had a significantly higher content of total flavonoids (92.55±3.20 mg CE/100 g FW), total phenolics (261.94±8.32 mg GAE/100 g FW), and total antioxidant activity than the other varieties. The main polyphenols were rutin, chlorogenic acid, and methyl gallate. For all 12 samples, the total flavonoids content was highly correlated with the total phenolics content and the total antioxidant activities. Non-edible parts of cherry tomato have high potential as functional food materials because they contain similar or more antioxidants and antioxidant properties than the pulp of tomato and cherry tomato or other fruits.

Keywords: cherry tomato, flavonoid, polyphenol, antioxidant, DPPH

Introduction

The proportion of food ingredients based on fresh fruits and vegetables is gradually increasing in response to demands from increasingly health-conscious consumers (Cho et al., 2007). Quality is important in this dietary pattern. Tomatoes are widely consumed year-round, and the consumption of tomatoes as a health functional food is increasing every year because of their preference for ingestion, cooking, and processing (Jun et al., 2013).

In Korea, tomato is one of the most representative fruits grown throughout the country due to the favorable climate (Kim et al., 2011a). Like regular tomato, cherry tomato contains about 90% moisture, 0.5-21% citric acid, 0.07-0.09% free amino acids, and 1.1% free sugars, along with vitamins A and C. It is also an important dietary source of carotenoids, especially lycopene and β-carotene (Kotikova et al., 2011). Lycopene stands out among carotenoids, indicating the red color of tomatoes (Matinz-Valverde et al., 2002). The red cherry tomato is the most commonly consumed, but the fruit varies in color, size, and shape depending on the variety, with new tomato varieties that range in color, from yellow through to orange, red, green, purple, and black (Ryu et al., 2019).

The popularity of the cherry tomato is due to its unique taste and numerous nutritional and health benefits, such as improving the strength of blood vessels and lowering blood pressure (Kim et al., 2011b). Cherry tomato is typically consumed fresh, during which the fruit pedicel and sepals are discarded as non-edible parts. In addition, consumers who purchase cherry tomatoes are increasingly embracing cherry tomatoes without a top (Choi et al., 2013).

A number of prior studies have been conducted on the antioxidant constituents and activities of discarded non-edible parts of fruits and vegetables. For example, a comparative analysis between the pomegranate non-edible (peels and seeds) and edible part (pulp) showed that the content of functional ingredients and antioxidant capabilities were higher in the peels and seeds than in the pulp (Jin, 2011). An independent study revealed that the total polyphenols and flavonoids were, respectively, 1.6-2.3 and 19.9-74.5 times higher in the non-edible part (peels) of mango than in the pulp, with antioxidant effects similar to vitamin C (Kim, 2017). In other work, the leaves and peels (non-edible parts) of kohlrabi exhibited high antioxidant activities, highlighting their potential use as a source of natural antioxidants (Pak et al., 2014).

The results of such studies show that typically the non-edible parts that are being discarded contain more antioxidants and antioxidant activity than the edible parts that are consumed directly or indirectly as food. Therefore, the cherry tomato's pedicel and sepals (non-edible part) could be expected to contain more antioxidants and antioxidant activities than the flesh (edible part). However, there is no prior research on the antioxidants of the non-edible part of cherry tomato. Accordingly, in this study, the non-edible parts (pedicle and sepals) from 12 varieties of colored cherry tomatoes were selected for a comparative analysis of their antioxidant compounds, antioxidant activities and the correlation between the contents of the total flavonoids and total phenolics with the antioxidant activities.

*Corresponding author: Young-Jun Kim, Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 01811, Korea
Tel: +82-2-970-6734
Fax: +82-2-970-6460
E-mail: kimyj@seoultech.ac.kr
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Materials and Methods

Materials

Twelve cherry tomato cultivars were harvested between July and August in 2020. 'Dotori Red TY', 'Black Joy 200', 'Dotori Norang TY', 'Green Joy' from Jayeonteo, Inc. (Goyang, Gyeonggi-do, Korea), 'KT Red TY', 'Blacklin', 'KT Orange TY', 'KT Norang TY', 'White Joy TY' from Haemi Farm Corporation (Seosan, Chungcheongnam-do, Korea), 'TY Item', 'TY Sispen' from Wansugmiin tomato farm (Wanju, Jeollabuk-do, Korea), and 'Beta Tiny' from Kkumkkuneun Farm (Buyeo, Chungcheongnam-do, Korea) were collected, respectively. After obtaining the cherry tomato at the commercially ripe stage, the pedicel and sepals were separated from the fruit. Only the pedicel and sepals were analyzed in this study. The separated pedicel and sepals were quickly frozen in liquid nitrogen at −196°C and stored in the freezer at −60°C before extraction.

Preparation of extracts

All pedicel and sepals were extracted with 80% acetone at 10 times the weight of the sample. Acetone (80%) was added to 25 g of the frozen sample, and the mixture homogenized twice for 3 min and once for 2 min using a blender (JB 3060, Braun Co., Kronberg, Germany). The homogenate was vacuum-filtered through Whatman #2 filter paper (Whatman International Ltd., Kent, England). The filtrate was concentrated using an Eyela N-1000 vacuum rotary evaporator (Tokyo, Japan) at 45°C and then stored at −20°C before analysis (Hwang et al., 2020).

Total flavonoids assay

The total flavonoids content of the sample was measured according to the colorimetric assay (Yang et al., 2019). Briefly, 0.3 mL of 5% $NaNO_2$ was added to 1 mL of the extract. The mixture was vortexed and left to react at room temperature for 5 min. Afterward, 0.3 mL of 10% $AlCl_3$ was added and vortexed, then incubated at room temperature for 6 min. After adding 2 mL of 1 N NaOH and 2.4 mL of distilled water, the mixture was adjusted to a total volume of 10 mL and vortexed. Absorbance was measured at 510 nm using a spectrophotometer (Optizen POP, Mecasys Co., Ltd., Daejeon, Korea). The calibration curve was prepared using (+)-catechin as the standard. The total flavonoids content was expressed as mg of catechin equivalents (CE)/100 g fresh weight (FW).

Total phenolics assay

The total phenolics content of the sample was measured according to the Folin-Ciocalteu colorimetric assay (Aryal et al., 2019). After mixing 2.6 mL of distilled water and 0.2 mL of the sample extract, 0.2 mL of Folin-Ciocalteu's phenol reagent was added, vortexed, and allowed to react at room temperature for 6 min. Afterward, 2 mL of 7% Na_2CO_3 was added and vortexed, then reacted by incubation at room temperature for 90 min in the dark. Absorbance was measured at 750 nm using a spectrophotometer. Gallic acid standard was used to prepare the calibration curve. The total phenolics content was expressed as mg of gallic acid equivalents (GAE)/100 g FW.

Polyphenol analysis by HPLC-UV

The polyphenol content of extracts (mg/100 g FW) was analyzed using the method with some modifications (Yang et al., 2019). The extract was diluted five times with a diluted solution of 0.2 M phosphate buffer (pH 3.0):methanol:distilled water (2:3:15 v/v/v). The filtered samples were analyzed using an HPLC 1260 series system (Agilent, Santa Clara, CA, USA) equipped with an Agilent Eclipse XDBC-18 column (150×4.6 mm, 5 μm) at 40°C. The mobile phase gradients of 3% acetic acid in distilled water (mobile phase A) and methanol (mobile phase B) were used. The gradient was as follows: 0-4 mins, 100 to 90%; 4-15 mins, 90 to 45% and 15-18 mins 100% (for mobile phase A). The detector was set at 280 nm, and 10 μL of each sample was injected. Calibration curves were generated using each of the following standards: gallic acid, protocatechuic acid, catechol, catechin, chlorogenic acid, epigallocatechin gallate, caffeic acid, epicatechin, syringic acid, 4-methycatechol, epicatechin gallate, *p*-coumaric acid, ferulic acid, methyl gallate, and rutin.

DPPH radical scavenging activity analysis

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the sample was measured using the DPPH assay modified by Kim et al. (2015) and Mira-Sánchez et al. (2020). A 100-μM DPPH solution was prepared using DPPH and 80% methanol. After uniformly mixing 2.95 mL of the methanolic DPPH solution with 50 μL of the sample extract, it was left to react at ambient temperature for 30 min in the dark. Absorbance was measured at 517 nm wavelength using a spectrophotometer. The calibration curve was prepared using vitamin C as a standard. The DPPH radical scavenging ability was expressed as mg of vitamin C equivalents (VCE)/100 g FW.

ABTS radical scavenging activity analysis

The ABTS radical scavenging ability of the sample was measured according to the ABTS assay modified by Floegel et al. (2011) and Sridhar et al. (2019). Briefly, 1 mM of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2.5 mM ABTS were stirred with 100 mL of PBS and then reacted in a constant temperature water bath set at 70°C for 40 min to prepare an ABTS radical solution. After uniformly mixing 980 μL of ABTS solution with 20 μL of the sample extract, it was reacted at 37°C for 10 min. The absorbance was measured at a wavelength of 734 nm using a spectrophotometer. The calibration curve and ABTS radical scavenging ability units were the same as described above for the DPPH radical scavenging activity.

Statistical analysis

All data were statistically analyzed using the SPSS 20 program (SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed, and significant differences were analyzed using Duncan's multiple range test ($p<0.05$). Pearson's correlation coefficient (R) was calculated to assess the correlation between the mean values of each variable (antioxidant activity, total phenolics,

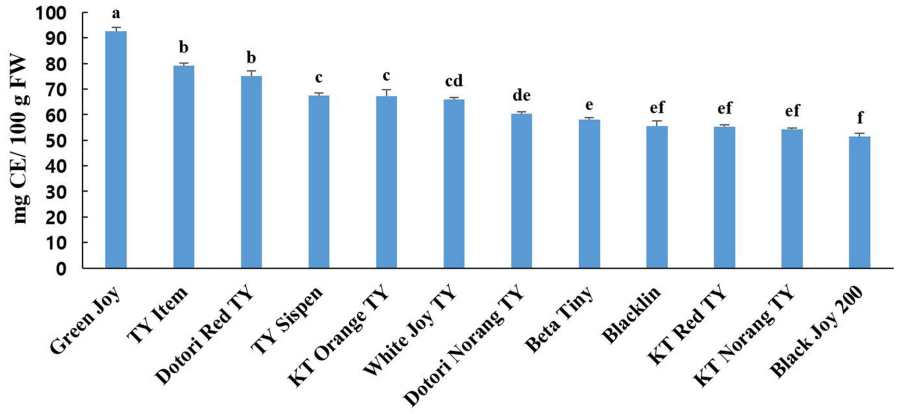

Fig. 1. Total flavonoids content of colored cherry tomatoes' pedicel and sepals. Error bars represent SD and different letter above the bars indicate significant differences based on Duncan's multiple range test (p<0.05).

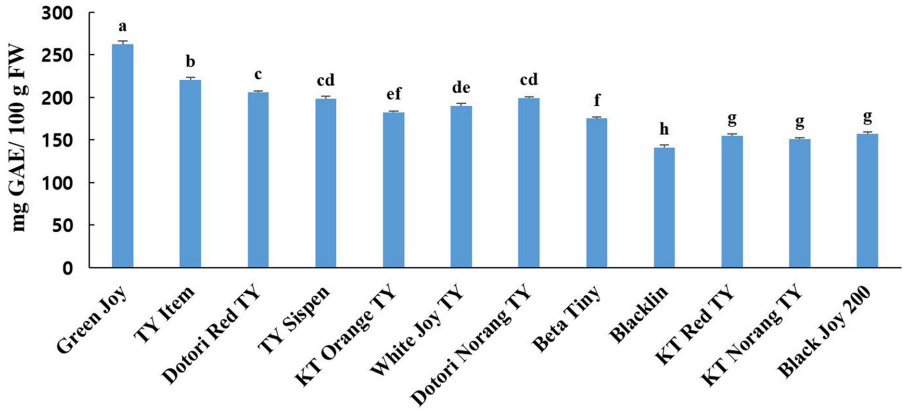

Fig. 2. Total phenolics content of colored cherry tomatoes' pedicel and sepals. Error bars represent SD and different letter above the bars indicate significant differences based on Duncan's multiple range test (p<0.05).

total flavonoid, individual polyphenolic compounds). The data are expressed as mean±standard deviation of triplicate determinations.

Results and Discussion

Total flavonoids content

The contents of total flavonoids found in the colored cherry tomato pedicel and sepals (CCTPS) are shown in Fig. 1. Among the 12 varieties, 'Green Joy' showed the highest value (92.55±3.2 mg CE/100 g FW), followed by 'TY Item' (79.06±2.12 mg CE/100 g FW), 'Dotori Red TY' (75.07±3.83 mg CE/100 g FW), 'TY Sispen' (67.48±2.18 mg CE/100 g FW), and 'KT Orange TY' (67.11±5.34 mg CE/100 g FW). 'Black Joy 200' had the significantly lowest at 51.45±2.7 mg CE/100 g FW. Noor Atiqah et al. (2014) detected a total flavonoids content of 1.88±0.26 mg CE/g dry weight (DW) in yellow cherry tomatoes and 1.71±0.09 mg CE/g DW in red cherry tomatoes, values lower those of the CCTPS examined in the current experiment. Lee et al. (2012) analyzed the peels of various fruits and measured a total flavonoids content of 4.5-40.3 mg quercetin equivalents (QE)/g DW. Gold kiwifruit skin had the highest content (40.3 mg), while citrus peel (4.5±0.1 mg) and banana peel (4.1±0.1 mg) had the lowest. Again, relatively higher contents of total flavonoids were detected in the CCTPS examined in the present experiment.

Total phenolics content

The contents of total phenolics detected in the CCTPS are shown in Fig. 2. Consistent with the trend of total flavonoids content, 'Green Joy' showed the significantly highest content (261.94±8.32 mg GAE/100 g FW) among the 12 varieties. The second, third and fourth highest was 'TY Item' (220.53±5.43 mg GAE/100 g FW), 'Dotori Red TY' (205.98±3.41 mg GAE/100 g FW), and 'Dotori Norang TY' (198.84±3.44 mg GAE/100 g FW), respectively. 'Blacklin' had the lowest content (140.70±5.98 mg GAE/100 g FW). For all the CCTPS, the results were higher than that recorded for pulp from cherry tomato variety 'Beta Tiny' of 11.02±1.98 mg/g DW (Kim et al., 2014) but similar to the total phenolics content of melon stalk of 143.4 mg/100 g DW (Kim et al., 2010). In other work, the calyx of 'Daebong' persimmon exhibited a total phenolics content of 73.00±0.01 mg GAE/g DW compared with 3.00±0.01 mg GAE/g DW in the flesh, a difference of more than 24-fold (Jo et al., 2010).

Individual phenolics content

Table 1 shows the individual polyphenols and their contents in the CCTPS. The major polyphenols identified were rutin, chlorogenic

Table 1. Polyphenol contents of colored cherry tomatoes' pedicel and sepals

Cultivar	Rutin (mg/100 g)	Chlorogenic acid (mg/100 g)	Methyl gallate (mg/100 g)
Green Joy	118.66±0.37[b 1)]	21.58±0.26[a]	0.91±0.02[c]
TY Item	146.74±4.65[a]	13.22±0.39[c]	0.74±0.02[e]
Dotori Red TY	106.40±2.91[d]	21.51±0.73[a]	1.31±0.05[a]
TY Sispen	145.10±3.87[a]	10.63±0.25[e]	0.89±0.01[c]
KT Orange TY	104.68±0.76[d]	11.89±0.14[d]	0.52±0.02[f]
White Joy TY	123.20±3.58[b]	5.22±0.14[h]	0.36±0.01[h]
Dotori Norang TY	95.32±2.87[e]	7.75±0.2[f]	0.84±0.01[d]
Beta Tiny	99.06±3.06[e]	15.79±0.32[b]	0.36±0.01[h]
Blacklin	78.97±3.11[g]	3.06±0.16[i]	0.44±0.01[g]
KT Red TY	77.42±1.46[g]	10.19±0.24[e]	0.96±0.04[b]
KT Norang TY	89.45±2.74[f]	6.39±0.17[g]	0.51±0.02[f]
Black Joy 200	112.56±1.47[c]	6.54±0.21[g]	0.31±0.01[i]

[1)]Means with different letters in the same column are significantly different (p<0.05).

acid, and methyl gallate. Rutin content was significantly the highest in 'TY Item', followed by 'TY Sispen', 'White Joy TY', and 'Green Joy', with values of 146.74±4.65, 145.10±3.87, 123.20±3.58, and 118.66±0.37 mg/100 g FW, respectively. The three varieties with the highest chlorogenic acid content were in the order of 'Green Joy' (21.58±0.26 mg/100 g FW), 'Dotori Red TY' (21.51±0.73 mg/100 g FW), and 'Beta Tiny' (15.79±0.32 mg/100 g FW). The methyl gallate content was significantly highest in 'Dotori Red TY' (1.31±0.05 mg/100 g FW). Kim et al. (2020) reported a rutin content of 0.25~11.55 mg/100 g in the fruit from 12 varieties of commercial cherry tomatoes in Korea. Furthermore, Di Lecce et al. (2013) confirmed that the chlorogenic acid content of the red cherry tomato fruit was 2.38±0.20 μg/g. Compared with both of these previous studies, higher values were recorded for the CCTPS analyzed in the current experiment.

DPPH radical scavenging activity

The DPPH radical scavenging activities of the CCTPS are shown in Fig. 3. Consistent with the highest total phenolics content, the DPPH radical scavenging activity of 'Green Joy' (118.83±4.39 mg VCE/100 g FW) was significantly highest among the 12 varieties. It was about 2.5-fold more than that of 'KT Norang TY', which was the significantly lowest (48.08±2.83 mg VCE/100 g FW). 'Green Joy', which recorded the highest DPPH activity among the 12 cherry tomato varieties, showed a 2.7-fold higher activity relative to regular tomato, which had 44.2±1.1 mg VCE/100 g DW (Floegel et al., 2011). It was also higher than that showed in apple peel powder (95±4 mg/100 g FW) prepared by three different processing methods (Youn et al., 2017). According to Kim et al. (2009)'s research on the radical scavenging activity and antioxidant compounds in extracts from various parts of oriental melon, the DPPH antioxidant activity is dependent on the content of total phenolic compounds (R=1.00) and less so on the total flavonoid content (R=0.84). In the context of this previous study (Kim et al., 2009), the results of the current study showed a similar trend.

ABTS radical scavenging activity

The ABTS radical scavenging activities showed by the CCTPS are shown in Fig. 4. Consistent with the DPPH radical scavenging activity results, 'Green Joy' showed the significantly highest ABTS activity (368.96±11.68 mg VCE/100 g FW) among the 12 varieties. It was about twice as high as 'Blacklin', which had the significantly

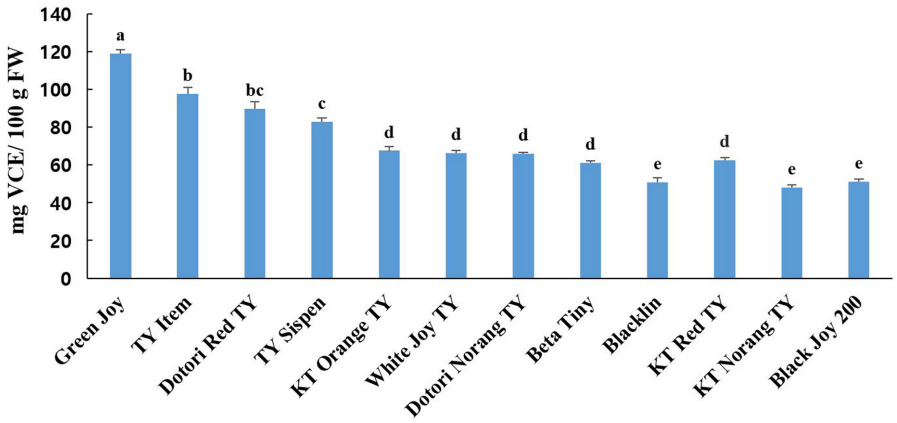

Fig. 3. DPPH free radical scavenging activities of colored cherry tomatoes' pedicel and sepals. Error bars represent SD and different letter above the bars indicate significant differences based on Duncan's multiple range test (p<0.05).

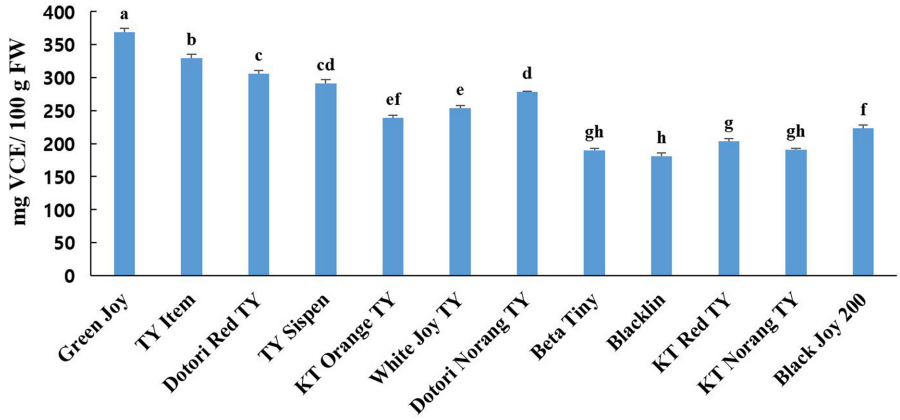

Fig. 4. ABTS free radical scavenging activities of colored cherry tomatoes' pedicel and sepals. Error bars represent SD and different letter above the bars indicate significant differences based on Duncan's multiple range test (p<0.05).

Table 2. Pearson correlation among antioxidant compounds and activities of colored cherry tomatoes' pedicel and sepals

	Phenolic	DPPH	ABTS	Rutin	Chlorogenic acid	Methyl gallate
Flavonoid	0.933**	0.930**	0.902**	0.561**	0.722**	0.498**
Phenolic		0.912**	0.953**	0.618**	0.712**	0.493**
DPPH			0.898**	0.622**	0.648**	0.492**
ABTS				0.678**	0.627**	0.589**
Rutin					0.265^{ns}	0.104^{ns}
Chlorogenic acid						0.626**

Pearson correlation (R): **, significance at p<0.01, ns, not significant

lowest content (181.09±8.76 mg VCE/100 g FW). In a study by Chun et al. (2005) on daily phenolic intake and total antioxidant activity from fruits and vegetables in the US diet, the ABTS radical scavenging activity of tomato was 29.44±1.60 mg VCE/100 g DW. In addition, Youn et al. (2017) verified that the ABTS radical scavenging activity of apple peel powder was 87±5 mg/100 g FW. The current results were higher compared with these two studies.

Correlation between the antioxidant substances and antioxidant activities

Table 2 shows the correlations between the antioxidant substances and antioxidant activity of the CCTPS. The correlation between the total flavonoids and total phenolics was very high (R=0.933). High correlations also existed between the total phenolics and chlorogenic acid (R=0.712), total flavonoids and radical scavenging activities (DPPH, R=0.930; ABTS, R=0.902), and the total phenolics and radical scavenging activities (DPPH, R=0.912; ABTS, R=0.953). A high positive correlation was also found between the DPPH and ABTS antioxidant capacities (R=0.898). Based on these data, the flavonoids and phenolic constituents in CCTPS contributed to the antioxidant ability.

The current results are consistent with several previous studies. For instance, Fidrianny et al. (2014) described a high correlation between the total flavonoids content and antioxidant activity (DPPH, R=0.894; ABTS, R=0.905) and, similarly, the total phenolic content and antioxidant activity (DPPH, R=0.855; ABTS, R=0.866) of white dragon fruit peel extracts. It was surmised that the antioxidant activities (DPPH and ABTS) could be estimated indirectly by measuring the total flavonoid and total phenolic content in the samples. Kim et al. (2010) noted a high correlation (R≥0.900) between antioxidant activity (DPPH, ABTS, reducing power) and total phenolic content, but particularly between ABTS and total phenolic content (R=0.990). Furthermore, other studies showed a correlation between the DPPH and ABTS antioxidant activities (R=0.90) in peach peels (Liu et al., 2015) and between antioxidant activities in tropical fruit peel powders (Can-Cauich et al., 2017).

Conclusion

This study analyzed the antioxidant constituents and antioxidant capacities of the non-edible parts (pedicle and sepals) of 12 cherry tomato varieties grown in Korea. 'Green Joy' samples had the highest total flavonoids content (92.55±3.2 mg CE/100 g FW), total phenolics content (261.94±8.32 mg GAE/100 g FW), and antioxidant activities (DPPH, 118.83±4.39 mg VCE/100 g FW; ABTS 368.96±11.68 mg VCE/100 g FW). Among the 12 varieties, 'TY Item' and 'TY Sispen' had the highest rutin content, whereas the chlorogenic acid content was highest in 'Green Joy' and 'Dotori Red TY', and methyl gallate was highest in 'Dotori Red TY'. The correlation between the total flavonoids and total phenolics was R=0.933, and the correlation between the antioxidant capabilities (DPPH and ABTS) was R=0.898. In conclusion, the non-edible parts of cherry tomato have high potential as functional food materials because they contain similar or more antioxidants and antioxidant properties than the pulp of tomato and cherry tomato or other fruits.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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