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Research Article

Polyphenol Content, Physicochemical Properties, Enzymatic Activity, Anthocyanin Profiles, and Antioxidant Capacity of *Cerasus humilis* (Bge.) Sok. Genotypes

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Seven varieties of Chinese dwarf cherries were evaluated and compared with respect to their weight, diameter, titratable acidity, total soluble solids, color, polyphenol contents, ascorbic acid levels, anthocyanin profiles, enzymatic activity, and antioxidant capacity. The fruits are rich in phenolic content (339.07–770.30 mg/100 g fresh weight). Nine anthocyanins were obtained from fruits after chromatographic separation and their structures analyzed using HPLC-ESI-MS/MS. Cyanidin-3-glucoside was the major anthocyanin with 50.36–78.39% concentration. Three anthocyanins were reported for the first time in these cherries. They exhibit low polyphenol oxidase and peroxidase activities, but their superoxide dismutase activity is high (572.75–800.17 U/g FW). The highest amounts of soluble solid content (15.67 Brix %), total titratable acid (1.90%), ascorbic acid (18.47 mg/100 g FW), and total anthocyanin (152.66 mg/100 g FW) were observed. Three methods (DPPH-scavenging ability, oxygen radical absorbance capacity assay, and cellular antioxidant activity assay) were employed to evaluate the antioxidant capacity of the phenolic extracts of these cherries. Number 5 has the highest values of ORAC and CAA of 205.68 μmol TE/g DM and 99.67 μmol QE/100 g FW, respectively.

1. Introduction

Edible fruits express the number of species and numerous genotypes, accessions, types, and so forth occurring in most of the parts of the world. They accepted one of the most important plant genetic resources and elements of biodiversity [1]. The Chinese dwarf cherry (*Cerasus humilis* (Bge.) Sok.) is a small dwarf shrub belonging to the family *Rosaceae*. It is a typical perennial plant grown in the north of China which shows resistance to cold, draught, barren soil, and high salt. The plant is unique to China, with a cultivation history dated back to around 3,000 years [2]. The plant is used as a garden or landscape shrub because of its graceful flowers and tree crowns [3]. Its fruit is termed as the "calcium fruit," owing to its high calcium content [2], and exhibits a characteristic aroma as well as a sweet and sour taste. It is a rich source of nutrients, minerals (phosphorus, sulfur, potassium, calcium, manganese, iron, copper, zinc, rubidium, and strontium), soluble sugars (sucrose, glucose, fructose, and sorbitol), and organic acids (malic, succinic, tartaric, citric, and oxalic acids) [4]. Apart from its high ornamental value, the fruit bears significant importance in food, medicine, environmental protection, and energy production. It is consumed fresh or industrially processed and used as one of the best raw materials for brewing wine as well as for preparation of vinegar, beverages, jam, and other highly processed products [2].

Polyphenols represent a class of polymers having aromatic rings that are directly attached to the hydroxyl groups. Polyphenols play extensive bioactive roles, including those as antioxidants, anti-inflammatories, and antimicrobials, as well as antiradiation, cardioprotective, and gastroprotective agents. As natural antioxidants and free radical scavengers, they are useful in medicine, food, and cosmetic industries. The Chinese dwarf cherries are endowed with high phenolic content. Li et al. [5] investigated 17 genotypes of Chinese

dwarf cherry from Beijing, Inner Mongolia, Hebei, and Shanxi Province of China and reported that their total phenol content (851–1899 mg/100 g FW) was higher than that observed in red raspberry, blackberry, blueberry, and strawberry [6]. In addition, the study also measured the total anthocyanin (TA) content (1.6–39.3 mg/100 g FW) of the fruit. Prime factors influencing consumers to choose food are color, taste (flavor), and nutritional value. The color of a fruit is mainly determined by anthocyanins, formed through the condensation of anthocyanidins with sugar and are widely expressed in plant flowers, fruits, stems, leaves, and seeds. Anthocyanins are orange-red to deep purple in color produced by chemical combination of its C6-C3-C6 structure with glycosides, acyl groups, and other molecules [7]. However, there are many differences in polyphenol and ascorbic acid content, physical and chemical properties, and anthocyanin composition in the fruits among different genotypes and cultivars [5, 8]. Few studies have addressed the physical and chemical properties of Chinese dwarf cherry [4, 5]; however, very little is known about the new genotypes of Chinese dwarf cherry, especially their anthocyanin profiles.

In this study, we aimed to evaluate the differences in the active components, especially the concentration and composition of anthocyanins, in different genotypes of Chinese dwarf cherry. We determined the color, physicochemical indexes, antioxidant activity, and anthocyanin profiles in seven genotypes of Chinese dwarf cherry. The results will provide a theoretical reference for consumption of fresh fruits or industrial processing.

2. Materials and Methods

2.1. Plant Material. Seven genotypes of Chinese dwarf cherry (referred as number 1–7) were harvested from the following orchards of different districts of China: number 1, Hebei Normal University of Science and Technology University, Qinhuangdao City (39°34′13.99″N, 119°10′29.11″E), Hebei Province, China; numbers 2 and 3, Baishan City (41°56′17.79″N, 126°25′6.85″E), Jilin Province, China; numbers 4 and 5, Zhangjiakou City (41°42′16.29″N, 115°47′54.16″E), Hebei Province, China; and numbers 6 and 7, Dingxi City (35°53′34.55″N, 104°42′43.89″E), Gansu Province, China. All genotypes had access to the same level of fertilization, irrigation, pruning, and disease management. All samples of fully mature fruits were harvested by hand from several different trees during the period between September and October 2016. Following harvest, half of samples were immediately analyzed for their physical properties and the rest stored in plastic containers at −60°C for subsequent analysis.

2.2. Measurement of Physicochemical Properties. We measured various physicochemical properties for 30 fruit samples. The weight of each averaged size fruit was measured without the stalk, with an accuracy of 0.001 g using AL104, Mettler Toledo, Switzerland. The fruit diameter was determined with a Vernier caliper and the average diameter calculated with an accuracy of 0.001 cm. For color analysis, the sample (pulp with peel) was centrifuged at 10,000 ×g (TDZ5-WS, Xiangyi Centrifuge Instrument Co., Ltd., Changsha, China) for 10 min and the supernatant obtained analyzed using SMY-2000 color reader (Exact Science Inc., Beijing, China). Color parameters L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) were measured with the CIELAB color scale. A standard color plate with reflectance values $L^* = 52.16$, $a^* = 2.29$, $b^* = -1.45$ for distilled water was used as a reference and the total color change (ΔE) calculated [9]. Chemical analysis was performed as previously described [9, 10] with some modifications. Titratable acidity was estimated with potentiometric titration using 0.1 N sodium hydroxide (NaOH) solution. Total soluble solids (TSS) were analyzed from the supernatant obtained from centrifugation (10,000 ×g for 10 min) of 15 g sample using a saccharimeter (PAL-α, Atago Inc., Tokyo, Japan). The pH value was determined using a pH meter (FE20, Mettler Toledo, Switzerland).

2.3. Enzymatic Activity

2.3.1. Extraction of Enzymes. The sample (pulp with peel) (5 g) was weighed accurately and treated with 20 ml acetic acid-sodium acetate buffer (pH 5.6) at 4°C (refrigerator) for 0.5 h. The sample was centrifuged at 5,000 ×g for 20 min at 2–4°C using CF16RXII refrigerated centrifuge (Hitachi, Tokyo, Japan). The supernatant collected as an extracted enzyme solution was placed in an ice bath for subsequent analysis. The above steps were repeated thrice for each sample.

2.3.2. Polyphenol Oxidase (PPO) and Peroxidase (POD) Assay. The PPO and POD activity was measured using methods previously described [11, 12] with some modifications. A freshly prepared solution of 1 ml 0.2% (w/w) catechol (prepared in dark at 4°C) and 1 ml 0.1 M acetate buffer (pH 5.6) was placed in an electric-heated thermostatic water bath (HH-4, Jierui Electric Appliance Co., Ltd., Jintan, China) at 30°C for 3 min. For the PPO analysis, 0.5 ml enzyme extract was added to the above solution and its absorbance determined at 410 nm using a spectrophotometer (U-2910 Hitachi, Tokyo, Japan) to evaluate the absorbance change (ΔA) within the initial 1 min. For the POD assay, 1.67 ml of 30% hydrogen peroxide (H_2O_2) was measured at a constant volume of 100 ml. One milliliter of 1.5% (w/w) guaiacol solution (in 50% ethanol [v/v]) and 0.75 ml H_2O_2 solution were mixed and incubated at 30°C for 3 min. The enzyme extract (0.25 ml) was added to the above solution and the absorbance measured at 470 nm to determine the absorbance change (ΔA) within the initial 1 min. A solution without any enzyme served as a blank for both analyses. An absorbance increase of 0.01 was defined as one unit of enzymatic activity using the following formula:

$$\text{enzymatic activity (U/g FW)} = \frac{\Delta A}{0.01 \times w \times t \times n} \times D, \quad (1)$$

where ΔA is the absorbance change within 1 min, w is the weight of sample (g), t is reaction time (min), n is the dose of the enzyme solution (ml), and D is dilution factor.

2.3.3. Superoxide Dismutase (SOD) Assay. The total SOD (T-SOD) activity was determined as indicated in the manufacturer's kit (number A001-1, Nanjing Jiancheng Biological Engineering Research Institute, China). Briefly, 15 μl enzyme extract was added to 2 ml chromogenic agent and the absorbance measured at 550 nm wavelength using doubled distilled water (ddH_2O) as a control. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the initial reduction of nitroblue tetrazolium (NBT). Enzyme units were expressed as U/g FW of the sample.

2.4. Active Ingredient Analysis

2.4.1. Extraction Procedures for Anthocyanins and Phenolics. A mixture containing 1 g sample (pulp with peel) and 20 ml of 60% ethanol (containing 0.1% hydrochloric acid [HCl]) was incubated in dark at 30°C for 30 min in a water bath held at 160 rpm (SHA-B, Jinda Instrument Co., Ltd., Jintan, China). Following incubation, the extract was centrifuged at 3,000 ×g for 10 min. The above steps were repeated thrice, the filtrate combined, and its volume measured; each sample was stored at 4°C for subsequent analysis.

2.4.2. Total Anthocyanins. The TA content was measured using the pH differential method described by Liu et al. [9]. Briefly, 4 ml of KCl-HCl buffer (pH 1.0) and acetic acid-sodium acetate buffer (pH 4.5) solutions were added to 1 ml filtrate sample. Absorbance of the two reactions was measured at 520 and 700 nm with UV-Vis spectrophotometer using water as a blank. Anthocyanin contents were expressed in mg cyanidin-3-glucoside per 100 mg of fresh fruit using the following formula:

$$\begin{aligned}&\text{anthocyanin content (mg/100 g FW)}\\&= \left[\left(A_{520\,\text{nm}} - A_{700\,\text{nm}}\right)_{\text{pH 1.0}}\right.\\&\left. - \left(A_{520\,\text{nm}} - A_{700\,\text{nm}}\right)_{\text{pH 4.5}}\right] \times \text{MW} \times \text{DF} \times V\\&\times 100/m/\left(\varepsilon \times L\right),\end{aligned} \tag{2}$$

where MW is the molecular mass of cyanidin-3-glucoside, 449.2 g/mol; DF is dilution ratio; V is the total volume of extract; m is the weight of sample; ε is molar absorptivity of cyanidin-3-glucoside, 26,900; and L is the optical path length (usually 1 cm).

2.5. Total Polyphenols. The TP content was determined with some modification in the method described by Singleton et al. [13]. Briefly, 0.2 ml filtrate sample was diluted with 0.8 ml distilled water and incubated with a solution containing 5 ml distilled water, 1 ml of Folin-Ciocalteau reagent, and 3 ml 7.5% sodium carbonate ($NaCO_3$) in the absence of light for 2 h. Following incubation, its absorbance was measured at 765 nm wavelength. Using gallic acid as the standard, a standard curve was constructed over a concentration range of 0 to 1.0 mg/ml. The regression curve equation was $y = 98.75x + 0.1384$ ($R^2 = 0.9996$). Results were expressed as mg gallic acid equivalent per 100 g of sample (mg GAE/100 g).

2.6. Ascorbic Acid. Ascorbic acid content was determined by the method previously described [14, 15] with some modifications. Briefly, 1 g sample (pulp with peel) was treated with 15 ml of 1% acetic acid for 30 min. The extraction was repeated thrice to obtain a metered volume of 50 ml in a volumetric flask. The extract was incubated at 4°C for 15 min, followed by centrifugation at 3,000 ×g for 10 min. The supernatant obtained was diluted to a final volume of 25 ml and its absorbance measured at 245 nm using 1% acetic acid solution as a blank. Ascorbic acid was used as the standard over a concentration range of 1 to 15 μg/ml. The regression curve equation was $y = 52.80x + 0.0336$ ($R^2 = 0.9995$). Results were expressed in mg ascorbic acid per 100 g sample.

2.7. Monomeric Anthocyanins. Each sample (50 g) of Chinese dwarf cherry was mixed with 80% acidic ethanol extract (0.1% HCl) at a 1 : 10 solid-liquid ratio and stirred (HJ-5, Ronghua Instrument Co., Ltd., Jintan, China) at 30°C for 1 h. The extraction was repeated twice and the extract filtered. The filtrate was concentrated at 40°C using vacuum evaporation (Rotary evaporation instrument, N1100, Tokyo, Japan) until the alcohol was evaporated. The concentrated solution was filtered and separated using nonionic polystyrene-divinylbenzene resin (D101, Sigma) at 4°C. Water-soluble substances were removed using deionized water followed by elution of anthocyanins with 80% acidic ethanol. The eluent was concentrated through evaporation at 40°C and lyophilized. Samples were stored at −20°C for subsequent analysis [16].

Anthocyanin monomeric components were analyzed on an high performance liquid chromatography (HPLC) system equipped with a diode-array detector (DAD; Agilent 1200, USA). Optimized mobile phases used were 5% formic acid in water (v/v) (A) and acetonitrile (B). The elution program was as follows: 0–8 min, 5–10% B; 8–20 min, 10–20% B; 20–28 min, 20–25% B; 28–30 min, 25–5% B. Separation was performed on a 250 mm × 4.6 mm, 5 μm ZORBAX, Stable Bond C18 column (Agilent, USA), with the flow rate maintained at 1.0 ml/min. The injection amount was 10 μl and operating temperature 30°C. Data were recorded at 520 nm. A mass spectrometer equipped with an electrospray ionization (ESI) source was used to obtain MS/MS data (Ultimate 3000, Thermo Scientific LTQ-Orbitrap XL, USA). Anthocyanins were measured using the positive ionization mode. Sheath gas was set to 35 bar and the assist device to 10 bar. The flow velocity of the dry gas was 12 l/min and the dry gas temperature 320°C. The purging voltage was controlled at +4 kV. The range of MS/MS scanning and data collection was from 100 to 1500 m/z [9].

2.8. Analysis of Antioxidant Activity. Sample (5 g) was subjected to extraction using 100 ml of 60% ethanol (0.1% HCl) at 30°C using water bath held at 160 rpm for 30 min (in the absence of light). The extract was centrifuged at 3,000 ×g for 10 min. Extraction was repeated thrice and the extract filtered. The filtrate was concentrated at 40°C using vacuum evaporator. The concentrated solution was filtered and separated using nonionic polystyrene-divinylbenzene resin at 4°C. The eluent obtained was concentrated at 40°C and lyophilized.

All samples were stored at −20°C for subsequent analysis.

2.8.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Assay. The effect of Chinese dwarf cherry on DPPH radicals was estimated according to the method of Pan et al. [17]. Briefly, a mixture of 1 ml of sample (freeze-dried extract dissolved in ethanol) at different concentrations and 3 ml of 0.1 mmol/L DPPH solution (dissolved in ethanol) was vortexed and incubated for 30 min in dark. Following incubation, the absorbance of the sample was measured at 517 nm (A_{sample}) using distilled water as a blank (A_{blank}). Samples and ethanol mixed served as samples of zero and distilled water and ethanol mixed served as a blank of zero. The antioxidant capability of the sample was expressed as the percentage of DPPH reduced, calculated using the following formula:

$$\text{DPPH radical scavenging value (\%)} = \left(\frac{\left[1 - A_{\text{sample}}\right]}{A_{\text{blank}}}\right) \times 100\%. \quad (3)$$

IC_{50} value was calculated from the graph of scavenging effect percentage against extract concentration.

2.8.2. Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) Assay. The $ORAC_{FL}$ assay was performed using the method of Dávalos et al. [18], with some modification. Briefly, 20 μl of sample was diluted using 75 mM phosphate buffer (pH 7.4) and sodium fluorescein solution (120 μl, final concentration 70 nM) and incubated in a 96-well black microplate at 37°C for 30 min. The AAPH (2, 2′-azo 2 (2-amidine propane) 2 hydrochloride) solution (60 μl, final concentration 12 mM) was added to each well (reaction volume 200 μl) by multichannel pipettes and mixed well. Fluorescence was recorded at 485 nm (excitation wavelength) and 520 nm (emission wavelength) with a multifunctional fluorescence detector (FLUOstar Omega, BMG LABTECH Instrument Co., Ltd., Germany), at every 5-min interval over 60 mins at 37°C using phosphate buffer as a blank. The $ORAC_{FL}$ value was calculated as a Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent. A standard curve was plotted using Trolox at concentration ranging from 5 to 100 μM to calculate the antioxidant capacity of each ample. Results were expressed in μmol TE/g dried fruit.

2.8.3. Cellular Antioxidant Activity (CAA) Assay. The CAA assay was performed as previously described [19] with a few modifications. Briefly, HepG-2 cells were seeded in a 96-well microplate at 6×10^4 cells/well in a complete medium (89% Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum + 1% penicillin-streptomycin) and incubated at 37°C for 24 h in presence of 5% CO_2. Following incubation, cells were washed with 100 μl of phosphate buffered saline (PBS, pH 7.4) and treated with 100 μl of each extract sample at different concentrations in 25 μmol/l DCFH-DA (2′,7′-dichlorofluorescin diacetate) oxidation medium (prepared using 10 mM HEPES + DMEM). The blank and control groups were incubated at the same conditions in the oxidation medium without any sample. Following incubation, the wells were washed with 100 μl PBS. The blank well contained oxidation medium (100 μl, 10 mM HEPES + HBSS) and the other well contained HBSS (100 μl, 600 μmol/l ABAP). Fluorescence was recorded at 485 nm (excitation wavelength) and 520 nm (emission wavelength) at every 5-min interval over 60 min at 37°C. The blank and initial fluorescence values were adjusted to zero, and the curve of fluorescence value as a function of time was constructed. According to the area under the curve, sample CAA value was calculated using the following formula:

$$\text{CAA unit} = 1 - \left(\frac{\int \text{SA}}{\int \text{CA}}\right), \quad (4)$$

where $\int$ SA is the integrated area under the sample fluorescence-versus-time curve and $\int$ CA is the integrated area from the control curve. The median effective dose (EC_{50}) was determined from the median effect plot of log(fa/fu) versus log (dose), where fa is the fraction affected (CAA units) and fu is (1 − CAA units). EC_{50} values were converted to CAA values, which were expressed in μmol QE/100 g of fresh fruit.

2.9. Statistics. The statistical analysis was performed using one-way analysis of variance (ANOVA). Means were compared by Tukey's multiple-range test after the data were checked for normality of distribution and variance homogeneity. Data were expressed as mean ± standard deviation (SD). All experiments were performed in triplicate. Graphs were produced using OriginPro 8.6 software. The F values were significant at $p < 0.05$ and $p < 0.01$. Regression analysis and F tests were performed using SPSS software (IBM SPSS Statistics, IBM Corp., Version 17.0).

3. Results and Discussion

3.1. Physicochemical Characteristics. Physicochemical properties of different genotypes of Chinese dwarf cherry are shown in Table 1. Fruit weight is a key quality index and was reported to be significantly different ($p < 0.05$) among seven varieties of fruits. Of all the varieties, maximum weight was recorded for numbers 6 and 7 (10.11 and 9.11 g, resp.), which was approximately 2.75 times the weight recorded for the least weighed variety number 1 (3.67 g). Similar results were recorded with respect to diameter. The diameter of the largest fruit (number 7, 2.65 cm) was 1.38 times that of the smallest fruit (number 1, 1.85 cm). Fruit shape is also an important quality index owing to the differences in varieties, tree potential, and growth areas. In comparison to number 6, number 7 showed larger diameter ($p > 0.05$); however, the weight reported for number 6 was higher than that for number 7. These results indicate that under similar cultivation conditions in the same area, there exists a close relationship between the fruit shape and its germplasm. This is in line with the findings by Schmitz-Eiberger and Blanke [20] who reported diameter (2.64–2.93 cm) in five genotypes of sweet cherry. Titratable acidity determines the flavor quality of

Table 1: Physicochemical properties of different genotypes of Chinese dwarf cherry *Cerasus humilis* (Bge.) Sok.

Genotypes	Fruit weight (g)	Transverse diameter (cm)	Titratable acidity %	pH	TSS (Brix %)	L^*	a^*	b^*	ΔE	Color
No. 1	3.67 ± 0.23^{d}	1.85 ± 0.10^{c}	1.34 ± 0.04^{c}	2.48 ± 0.08^{b}	7.43 ± 0.06^{f}	23.60 ± 3.02^{c}	56.1 ± 5.08^{ab}	35.69 ± 4.71^{b}	52.36 ± 6.38^{b}	Red
No. 2	5.69 ± 0.34^{c}	2.09 ± 0.09^{b}	1.90 ± 0.04^{a}	2.42 ± 0.06^{bc}	13.40 ± 0.04^{b}	17.87 ± 3.34^{cd}	63.05 ± 8.03^{a}	58.4 ± 4.20^{a}	59.7 ± 4.69^{b}	Red
No. 3	5.45 ± 0.54^{c}	2.10 ± 0.08^{b}	0.84 ± 0.05^{d}	2.84 ± 0.03^{a}	13.07 ± 0.08^{c}	35.96 ± 2.38^{b}	19.13 ± 3.34^{d}	13.44 ± 1.05^{c}	21.84 ± 2.09^{c}	Red-yellow
No. 4	7.48 ± 0.66^{b}	2.30 ± 0.13^{b}	1.76 ± 0.11^{a}	2.57 ± 0.04^{b}	15.67 ± 0.06^{a}	14.09 ± 2.37^{d}	72.54 ± 3.68^{a}	51.88 ± 4.09^{a}	70.19 ± 4.29^{a}	Red
No. 5	7.71 ± 0.69^{b}	2.34 ± 0.17^{ab}	1.58 ± 0.04^{b}	2.57 ± 0.03^{b}	15.90 ± 0.09^{a}	25.03 ± 2.77^{c}	49.64 ± 2.19^{b}	36.21 ± 2.86^{b}	46.89 ± 2.31^{bc}	Red
No. 6	10.11 ± 0.58^{a}	2.55 ± 0.11^{a}	1.49 ± 0.05^{b}	2.64 ± 0.05^{ab}	12.73 ± 0.05^{d}	32.43 ± 0.19^{b}	28.93 ± 0.29^{c}	9.89 ± 0.78^{d}	24.13 ± 0.16^{c}	Red
No. 7	9.11 ± 0.67^{a}	2.65 ± 0.11^{a}	0.91 ± 0.06^{d}	2.68 ± 0.06^{a}	12.33 ± 0.06^{e}	46.39 ± 2.87^{a}	13.30 ± 2.20^{e}	5.21 ± 1.75^{e}	11.83 ± 2.42^{d}	Red-yellow

Numbers 1–7 indicate different genotypes. Data are expressed as mean ± SD ($n = 3$). Means in a row related to one test followed by different letters are significantly different ($p < 0.05$). TSS: total soluble solids.

fruits and can influence consumer choice. High acid content yields heavy taste, while low acidity results in lack of flavor. Titratable acidity was reported to be highest for numbers 4 and 2 (1.76% and 1.9%, resp.) and lowest for numbers 3 and 7 (0.84% and 0.91%, resp.). A significant difference in titratable acidity was observed between some varieties ($p < 0.05$), consistent with the findings of Li et al. [5]. The pH value reported for all the varieties of fruit ranged from 2.84 to 2.42, with number 3 exhibiting the lowest pH. Titratable acidity for Chinese dwarf cherry was similar to that observed for blackberry and raspberry in Croatia [21] and higher than that recorded for blue honeysuckle berries [22]. Thus, Chinese dwarf cherry belongs to sour fruits, with a distinct sour taste. Soluble solids reflect the fruit flavor as well as the internal quality. The TSS showed a significant difference ($p < 0.01$) between all fruit varieties, with values ranging from 7.43 to 15.90 Brix%. These results conclude that cultivars numbers 2–7 had more flavor than number 1 and coincide with those reported by Schmitz-Eiberger and Blanke [20] and Li et al. [5]. The TSS values observed for numbers 4 and 5 were higher than those reported in other studies for fruits such as blackberry [21], raspberry [21, 23], red currant, gooseberry, cornelian cherry [23], and sour and sweet cherry [24, 25]. Quality is similar between numbers 4 and 5. In general, the higher the sugar and acid content of the fresh fruit are, the better the flavor and quality are (e.g., numbers 4 and 5). The variety with high sugar and acid content is more suitable for food processing (e.g., numbers 2 to 5).

The color of food is one of the most important attributes [22]. Skin color is an important index of fruit quality as well as maturity and has a prime role in fruit marketing. Fruits with intense red coloration are likely to have greater consumer appeal. The color characteristics of the Chinese dwarf cherry genotypes are reported in Table 1. Results showed a significant difference ($p < 0.05$) in the L^* values of different genotypes, with highest values reported for numbers 7 and 3 (46.39 and 35.96, resp.) bearing red-yellow skin; the other varieties had red skin. The high values of a^* (ranging from 13.30 to 72.54) and b^* (ranging from 5.21 to 58.4) suggest that these cherries were mainly red and yellow. The results are in agreement with data for ΔE (the higher the values, the deeper the color) and color. The anthocyanin values reported in Figure 1 reveal a positive correlation between ΔE and anthocyanin ($r = 0.6393$, $p < 0.1$) as well as polyphenol ($r = 0.7211$, $p < 0.05$) contents. This variation could be attributed to the nature of pigments and anthocyanin contents in these cherries, which yielded a different color [22]. Cyanidin-3-glucoside was the main anthocyanin reported in Chinese dwarf cherry, which also occurs in other red fruits such as sweet cherry and red raspberry [12, 20].

3.2. Concentrations of Polyphenols, Anthocyanins, and Ascorbic Acid. Significant differences in the concentrations of ascorbic acid, TP, and TA were recorded not only among different genotypes but also among different locations ($p < 0.05$, Figure 1). The TP content was reported to be ranging from 339.07 to 770.30 mg/100 g FW, higher than that recorded for red raspberry, blackberry, and strawberry [6] and similar to that recorded for wild *Lonicera caerulea* [9]. The polyphenol content was highest for number 5 (up to 770.30 mg/100 g FW), which was 2.27 times the value reported for number 7 (lowest polyphenol content). A significant positive correlation was observed between the TP concentration and anthocyanins ($r = 0.9300$, $p < 0.01$). The ascorbic acid concentration observed for all the genotypes ranged from 14.33 to 18.47 mg/100 g FW (Figure 1), lower than that recorded for hawthorn, strawberries, blackberries, raspberries, oranges, and blueberries [6] but higher than that observed for sweet cherry fruits [20]. Ascorbic acid concentration reported was maximum for numbers 2 and 3 (18.47 and 17.55 mg/100 g, resp., $p > 0.05$) and least for numbers 6 and 7 (14.63 and 14.33 mg/100 g, resp.). In comparison to polyphenols and

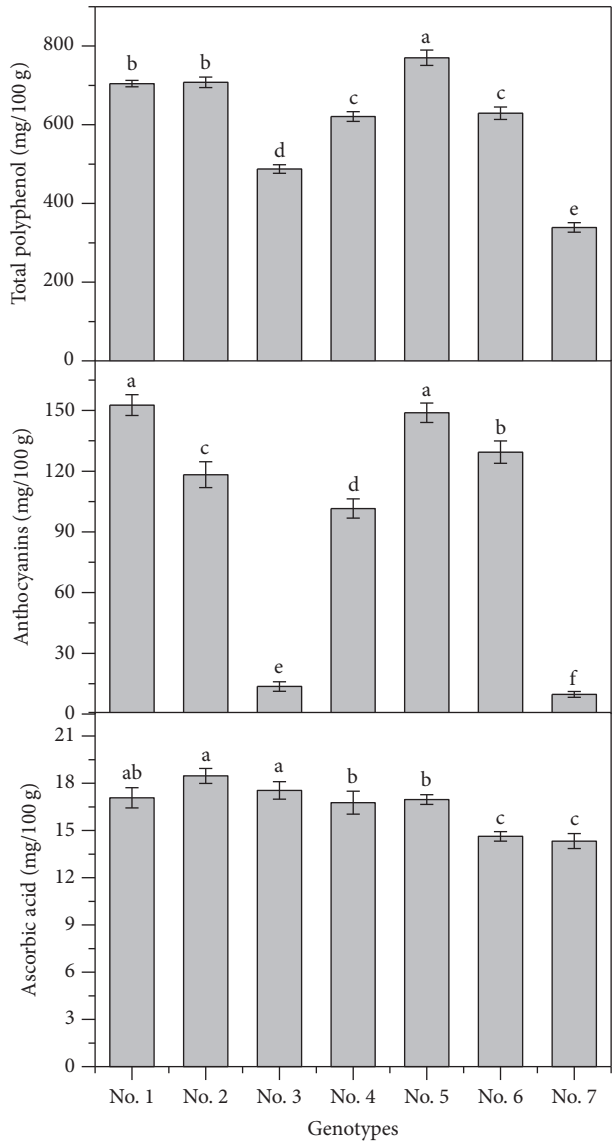

Figure 1: Contents of ascorbic acid, total polyphenols, and anthocyanins in Chinese dwarf cherry genotypes (*Cerasus humilis* (Bge.) Sok.). Numbers 1–7 indicate different genotypes. Different letters indicate significant differences ($p < 0.05$). Data are expressed as means ± SD ($n = 3$).

anthocyanins, ascorbic acid content between different varieties showed little variation (the maximum value was 1.29 times the minimum value).

The attractive red color of the cherry and cherry-based products is one of the parameters used for determination of its quality and consequently consumers' preference. The Chinese dwarf cherry bears relatively large amount of anthocyanin compounds that differ significantly between the varieties at different locations (Figure 1). The concentration of TA varied from 9.69 to 152.66 mg/100 g FW, higher than that reported for sour cherries (2.7 to 28.0 mg/100 g FW) and red raspberry *(Prelude)* and similar to that observed for blackberry (125.6 to 152.2 mg/100 g FW) [8]. The lowest anthocyanin concentrations were reported for numbers 3 and 7 (13.61 and 9.69 mg/100 g FW, resp., $p < 0.05$). A strong correlation was observed between the anthocyanin content and color of the fruit peel and fruits; the skin color was red-yellow for fruits from numbers 7 and 3 and red for those from other genotypes. Li et al. [5] studied 17 genotypes of Chinese dwarf cherry from Beijing, Inner Mongolia, Hebei, and Shanxi Province in China and reported concentrations of total phenols (851–1899 mg/100 g FW), ascorbic acid (16.3 to 83.2 mg/100 g FW), and anthocyanins (1.6–39.3 mg/100 g FW). In comparison to our study, this study reported significantly higher values for polyphenols and ascorbic acid but lower values for anthocyanins. These discrepancies may be attributed to the differences in the varieties as well as the year of the harvest, resulting in alteration in the composition and content of active components. Picariello et al. [26] investigated five cherry biotypes (Rainier, Napoleon/Bigarreau, Bing, Montmorency, and Morello) and reported TP and TA values of 28.3 to 218.0 and 3.7 to 98.4 mg/100 g FW, respectively. Schmitz-Eiberger and Blanke [20] also reported TP and TA concentrations of 35.6 to 65.85 and 0.152 to 0.883 mg/100 g FW, respectively, in five genotypes of sweet cherry *(Prunus avium L.)*, namely, "Burlat," "Samba," "Earlise," "Souvenir de Charmes," and "Prime Giant." The above results suggest that polyphenol and anthocyanin contents of Chinese dwarf cherry are higher than those of average cherries. Thus, Chinese dwarf cherry is a rich source of polyphenols among fruit species.

3.3. Enzymatic Activity. The browning of fruits and vegetables during processing is attributed to the production of quinones from the action of PPO on polyphenols [27]. The PPO amount in the fruits and vegetables varies with varieties, kind of raw products, and maturity. Furthermore, oxidative enzymes such as PPO and POD are responsible for the deterioration of fruit color, flavor, and nutritional value [28]. Figure 2 shows details of PPO, POD, and SOD activities reported in the pulp samples of all the varieties. We observed a significant difference ($p < 0.05$) in the PPO values among all the varieties. The highest PPO values were reported for numbers 6 and 2 (62.01 and 56.01 U/g FW, resp.), which were approximately 2.38 times the value recorded for number 1 (lowest PPO). The PPO data indicated that browning occurred more often in numbers 6 and 2 during food processing, thereby decreasing the quality and nutritive value of the products. Higher POD activity was also observed

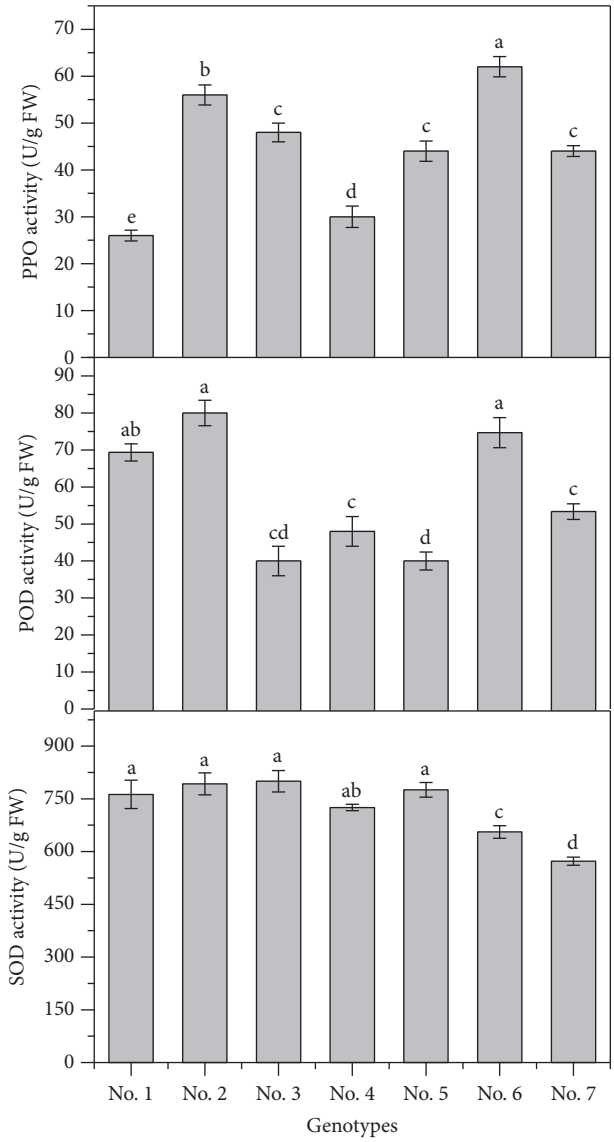

Figure 2: Enzymatic activity of PPO, POD, and SOD in Chinese dwarf cherry genotypes (*Cerasus humilis* (Bge.) Sok.). Numbers 1–7 indicate different genotypes. Different letters indicate significant differences ($p < 0.05$). Data are expressed as mean ± SD ($n = 3$).

in numbers 6 and 2 ($p > 0.05$). Cultivar number 1 showed values lower for PPO (25.99 U/g FW) but higher for POD (69.34 U/g FW). The higher POD activity recorded in our study may be attributed to the different varieties of Chinese dwarf cherry. Ortega-ortiz et al. [29] reported approximately 60 U/g FW POD activity in tomatoes ("Rio Grande" variety), while Liu et al. [9] reported higher PPO values for varieties with POD values higher than that for number 1 (PPO 164.23 and POD 172.19 U/g FW). Interestingly, PPO and POD activities influence the color stability of anthocyanins in food. Peng and Markakis [30] showed that the degradation rate of anthocyanins increases with an increase in the catechin concentration. The degradation pathway of anthocyanins by the action of polyphenol oxidase is as follows: under the influence of oxygen and polyphenol oxidase, catechols are

Table 2: The anthocyanins identified and the corresponding peak area percentages in anthocyanin extracts of different genotypes of Chinese dwarf cherry, *Cerasus humilis* (Bge.) Sok.

No.	T_R (min)	Tentative identification	MS[M]$^+$ (m/z)	MS/MS (m/z)	Peak area (%)						
					No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
1	13.286	5-Methylpyranocyanidin-3-glucoside	487	325	3.77	3.26	nd	nd	2.71	nd	nd
2	13.622	Cyanidin-3-glucoside	449	287	69.05	78.39	76.98	66.82	65.62	50.36	71.03
3	14.403	Cyanidin-3-rutinoside	595	449, 287	2.58	4.34	1.07	5.64	3.47	11.02	3.603
4	14.926	Pelargonidin-3-rutinoside	579	433, 271	0.427	1.21	nd	0.78	0.93	0.38	nd
5	15.604	Pelargonidin-3-glucoside	433	271	14.39	7.98	13.19	15.61	16.97	21.83	8.48
6	19.881	Cyanidin-3-acetyl glucoside	491	287	3.020	nd	0.77	1.08	nd	0.84	1.73
7	20.938	Delphinidin-3-glucoside	465	303	4.525	3.72	5.10	6.37	6.30	8.66	11.40
8	22.879	Pelargonidin-3- acetylglucoside	475	271	1.56	1.10	2.88	3.43	4.00	6.47	3.76
9	23.174	Delphinidin-3-acetylglucoside	507	303	0.691	nd	nd	0.25	nd	0.44	nd

Nd: not detected. Peak areas of anthocyanins were determined at 520 nm by HPLC; numbers 1–7 indicate different genotypes.

first oxidized to generate benzoquinone structure. Further oxidation results in conversion of anthocyanins into colorless compounds by benzoquinone. Therefore, the processing parameters for different varieties of Chinese dwarf cherry vary owing to the differences in their PPO and POD activities.

Superoxide dismutase (SOD) catalyzes the conversion of superoxide ions to O_2 and H_2O_2 through a disproportionation reaction, thereby exhibiting a protective effect on the product quality. The SOD activity recorded in all the varieties of Chinese dwarf cherry ranged from 572.75 to 800.17 U/g FW (Figure 2) and was higher than that for *Lonicera caerulea* (470.74 U/g FW) reported by Liu et al. [9]. Highest SOD activities were recorded for numbers 2 and 3 (792.59 and 800.17 U/g FW, resp.), which were approximately 1.4 times the activity for number 7 (572.75 U/g FW). Thus, the Chinese dwarf cherry has relatively low PPO and POD activities but higher SOD activity, which can better protect the color and nutritional value of the product during beverage processing.

3.4. Anthocyanin Profiles. Anthocyanin profiles obtained for the Chinese dwarf fruit showed nine compounds. The analytical retention time, identities, and peak areas of these compounds are presented in Table 2, while the chromatogram is shown in Figure 3. Using HPLC -ESI-MS/MS, we separated anthocyanins and analyzed their chemical structures. The m/z of the molecular ions $[M]^+$ and fragment ions for peak 1 were m/z 487 and 325, respectively, as 487 lost a hexose ($487 - 325 = 162$ U). The m/z was 287 for the parent nucleus of anthocyanins and, thus, the peak 1 was identified as 5-methylpyranocyanidin-3-glucoside [31]. The m/z of $[M]^+$ and fragment ions for peak 2 were 499 and 287, respectively; the peak corresponded to the compound cyanidin-3-glucoside. Peaks 3, 4, 5, and 7 were cyanidin-3-rutinoside, pelargonidin-3-rutinoside, pelargonidin-3-glucoside, and delphinidin-3-glucoside, respectively. For peaks 6, 8, and 9, the m/z of $[M]^+$ were 491, 475, and 507, respectively, and the m/z of fragment ions were 287, 271, and 303, respectively. All of them were molecular ions that lost acetyl-hexoside (204). According to spectral data on compounds in the database and reference NCBI PubChem Compound [31, 32], peaks 6, 8, and 9 were cyanidin-3-acetylglucoside, pelargonidin-3-acetylglucoside, and delphinidin-3-acetylglucoside, respectively. Cyanidin-3-glucoside was the main anthocyanin in Chinese dwarf cherry and accounted for 50.36–78.39% of the TA content, as shown in Table 2. Pelargonidin-3-glucoside and cyanidin-3-rutinoside accounted for 8.48–21.83% and 1.07–11.02% of TA, respectively. The anthocyanin species and amounts were different among the seven varieties. For instance, 5-methylpyranocyanidin-3-glucoside was undetected for numbers 3, 4, 6, and 7; pelargonidin-3-rutinoside for numbers 3 and 7; cyanidin-3-acetylglucoside for numbers 2 and 5; and delphinidin-3-acetylglucoside for numbers 2, 3, 5, and 7. However, a total of nine monomeric components were detected for number 1, while numbers 3 and 6 showed only six monomeric components. These results suggest that the lower the anthocyanin content in a cultivar is, the lesser the diversity in monomers is. In addition, the variation in the anthocyanin composition and individual type among different genotypes, as recorded in blueberry genotypes [33], may be attributed to the difference in the genotype—one of the major determinants of the anthocyanin composition in plants [34].

Little has been reported about the anthocyanin composition of Chinese dwarf cherry. Based on the HPLC-ESI-MS data, Chen et al. [8] speculated that Chinese dwarf cherry contains four kinds of anthocyanins, including cyanidin-3-glucoside, cyanidin-3-xylosyl-rutinoside, cyanidin-3-rutinoside, and pelargonidin-3-glucoside. In agreement with our results, cyanidin-3-glucoside was reported to be the most abundant of anthocyanins (90.3%). However, we were unable to detect cyanidin-3-xylosyl-rutinoside in our study. Chang et al. [32] employed LC-DAD-IT-TOF-MS technology and identified six anthocyanin monomers in the Chinese dwarf cherry, with cyanidin-3-glucoside being the most abundant (62.6%). As shown in our study, 5-methylpyranocyanidin-3-glucoside, delphinidin-3-glucoside, and delphinidin-3-acetylglucoside were detected for the first time in Chinese dwarf cherry.

3.5. Antioxidant Capacity. The antioxidant capacity of foods such as vegetables and fruits is determined by their chemical

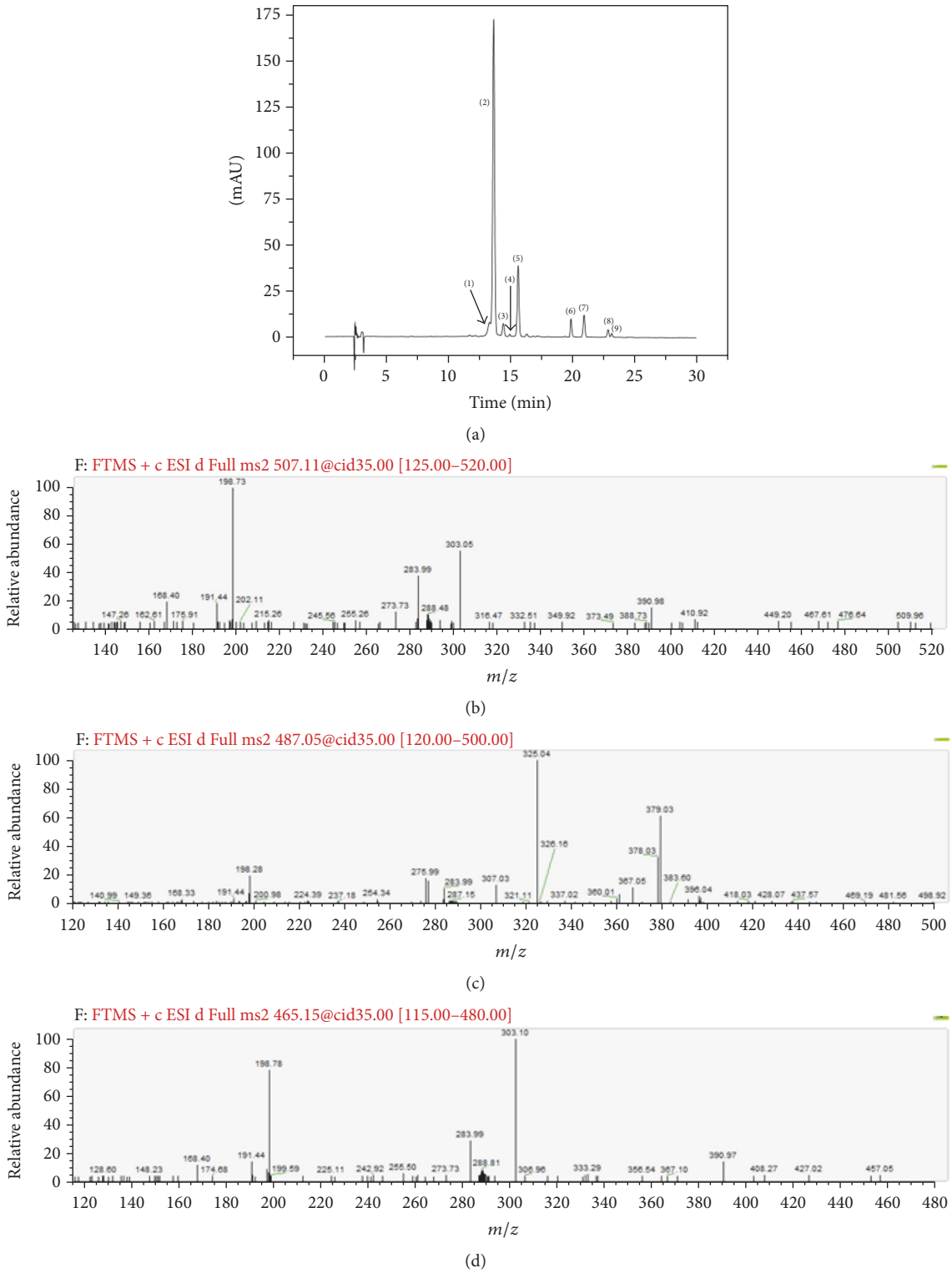

Figure 3: A chromatogram (acquired at 520 nm) of HPLC-DAD in anthocyanin extracts of fresh Chinese dwarf cherry (*Cerasus humilis* (Bge.) Sok.) of genotypes number 1. (a) (1) 5-Methylpyranocyanidin-3-glucoside; (2) cyanidin-3-glucoside; (3) cyanidin-3-rutinoside; (4) pelargonidin-3-rutinoside; (5) pelargonidin-3-glucoside; (6) cyanidin-3-acetyl glucoside; (7) delphinidin-3-glucoside; (8) pelargonidin-3-acetylglucoside; (9) delphinidin-3-acetylglucoside. (b)–(d) ESI-MS spectra of delphinidin-3-acetylglucoside (MS = 507; MS^2 = 303), 5-methylpyranocyanidin-3-glucoside (MS = 487; MS^2 = 325), and delphinidin-3-glucoside (MS = 465; MS^2 = 303), respectively.

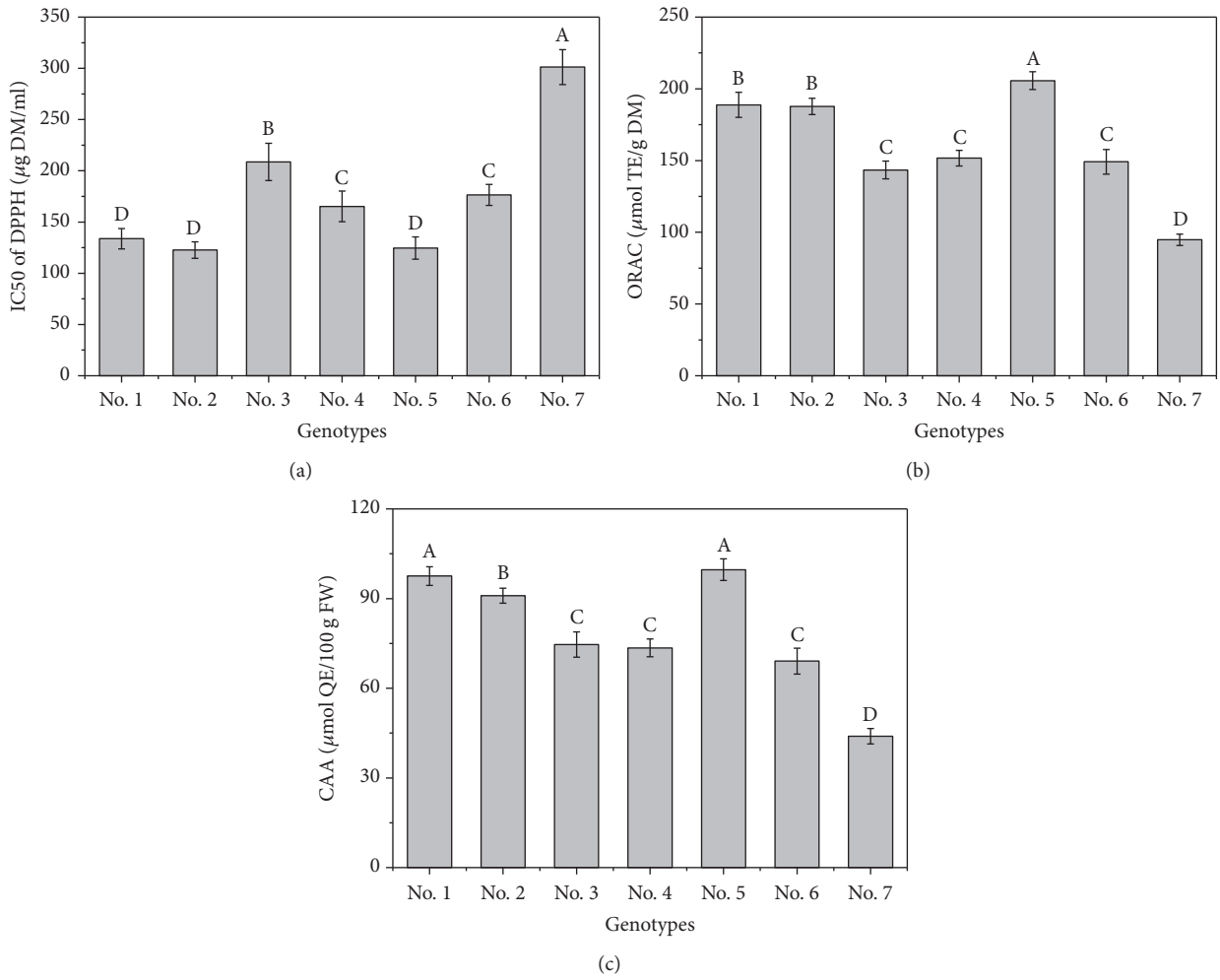

Figure 4: Antioxidant capacity of Chinese dwarf cherry genotypes (*Cerasus humilis* (Bge.) Sok.). Numbers 1–7 indicate different genotypes. Different letters indicate significant differences ($p < 0.05$). Data are expressed as mean ± SD ($n = 3$). DM means the dry weight of Chinese dwarf cherry. FW means the fresh fruits weight of Chinese dwarf cherry.

and enzymatic composition as well as the type of assay employed, owing to the lack of a single validated assay [35]. Therefore, we employed three different methods (DPPH-scavenging abilities, $ORAC_{FL}$ assay, and CAA assay) to evaluate the antioxidant capacity for the extracts of Chinese dwarf cherry. The results are shown in Figure 4.

The IC_{50} values of the DPPH radical-scavenging activity recorded for the seven genotypes ranged from 122.63 to 301.20 μg DM/ml, as shown in Figure 4(a). The activity recorded was strongest for numbers 2 and 5 ($p > 0.05$) and weakest for number 7, which may be attributed to the polyphenol content of these fruits. Studies have reported strong correlations between the antioxidant capacity and phenolic compounds in sweet cherry [5], blackberry, and hybrid berry cultivars [36]. Our study showed a significant negative correlation between the IC_{50} value of DPPH radical-scavenging activity of the fruit and concentrations of anthocyanins ($r = -0.8633$, $p < 0.01$), polyphenols ($r = -0.979$, $p < 0.001$), and ascorbic acid ($r = -0.7668$, $p < 0.05$). Thus, anthocyanins, polyphenols, and ascorbic acid are the active substances responsible for DPPH radical-scavenging effect. The IC_{50} value reported in our study is higher than that reported for mulberry, sea buckthorn, black currant, yellow raspberry, red raspberry *(Nova)*, red raspberry *(Prelude)*, and black raspberry *(Hull)* [8] as well as sweet cherry [5]. There exists a close association between the antioxidant capacity and structure of anthocyanins and polyphenols. The scavenging of free radicals and the antioxidant mechanism are related to the phenolic hydroxyl structure of polyphenols, which can offer electrons or protons to free radicals, leading to the downregulation of free radicals through three phases as follows: (1) a reaction between polyphenol and superoxide anion free radical ($^{\bullet}O_2^-$) to prevent free radicals from appearing; (2) sequestration of transition metal ions mediated by polyphenols to stop generation of hydroxyl free radical ($^{\bullet}OH$); and (3) a reaction between polyphenol and lipid-free oxygen radicals ($ROO^{\bullet}$) to prevent lipid peroxidation [37].

Oxygen radical absorbance capacity ($ORAC_{FL}$) is one of the important methods for evaluation of total antioxidant activity. As shown in Figure 4(b), the $ORAC_{FL}$ value recorded for all the varieties ranged from 94.79 to 205.68 μmol TE/g DM ($p < 0.01$), higher than that observed for blackberry,

raspberry, black raspberry, and strawberry fruits [38]. The highest $ORAC_{FL}$ value was recorded for number 5, which was 2.17 times that recorded for number 7 (lowest $ORAC_{FL}$). The data reveal a significant difference in the antioxidant capacity among different varieties of Chinese dwarf cherry. In addition, a significant positive correlation was observed between the $ORAC_{FL}$ activity and anthocyanin ($r = 0.8296$, $p < 0.05$) as well as polyphenol ($r = 0.9617$, $p < 0.001$) contents, highlighting the active role of these compounds in $ORAC_{FL}$ activity. Ou et al. [39] analyzed $ORAC_{FL}$ activity in a total of 927 samples from 13 different vegetable types (e.g., white cabbage, peppers, carrots, and peas) and reported the highest $ORAC_{FL}$ value in green pepper (160 μmol/g DM) and the lowest value in peas (18 μmol/g DM). The $ORAC_{FL}$ value was reported to range from 18 to 262.4 μmol TE/g of fresh haskap berries [40]. Thus, Chinese dwarf cherry exhibits strong antioxidant capacity.

We further explored the antioxidant capacity of Chinese dwarf cherry with the CAA assay, which reflects the antioxidants involved in processes such as absorption, metabolism, and cell distribution. The CCA assay shows greater biological relevance than the DPPH and $ORAC_{FL}$ assays and is widely used for evaluation of antioxidant activity in fruit and vegetable food products. As shown in Figure 4(c), the CAA value for all the varieties ranged from 43.91 to 99.67 μmol QE/100 g FW. The highest values were recorded for numbers 5 and 1 (97.54 and 99.67 μmol QE/100 g FW, resp., $p > 0.05$), which were approximately 2.27 times the value recorded for number 7 (lowest CCA). These results are similar to those of the $ORAC_{FL}$ assay. We observed a significant positive correlation between CAA activity and anthocyanin ($r = 0.7680$, $p < 0.05$) as well as polyphenol ($r = 0.9175$, $p < 0.01$) contents, indicating their important role in CAA activity. A large number of experimental studies have shown that CAA values are closely related to the total phenolic content of tested samples. Wolfe and Liu [19, 41] determined the antioxidant activity of extracts from dozens of common fruits and vegetables. The CCA values for apples were reported as 21.9 ± 4.0 and 17.2 ± 2.0 μmol QE/100 g and those for wild blueberry were reported as 292 ± 11 and 74.1 ± 12.5 μmol QE/100 g, as determined by two different processing methods (with and without PBS washing). These results coincide with our findings using Chinese dwarf cherry (PBS washing). The CAA method is used for the evaluation of antioxidant activity of pure chemicals in plants; flavonoids of 3,4,-*O*-dihydroxy-4-ketone with the 2,3-double bond in the combination group and 3-hydroxy structure have higher CAA values [41]. Therefore, berries such as wild blueberries, strawberries, blackberries, and raspberries bear higher CCA values.

4. Conclusions

Variation in the physicochemical properties was observed among all the seven genotypes of Chinese dwarf cherry. Concentrations of anthocyanins and polyphenols in genotypes numbers 1–7 were higher than those in normal cherries. Genotypes numbers 2–7 had more flavor, while numbers 1, 4, and 5 exhibited higher sugar and acid contents as well as better flavor and quality. Furthermore, numbers 2–5 are deemed suitable for food processing, owing to their high sugar and acid contents. The Chinese dwarf cherry shows relatively lower PPO and POD activity but higher SOD activity, which can protect the color and nutritional value of the product during beverage processing. However, all varieties display varied suitability for food processing because of the differences in their PPO and POD activities. In this study, nine anthocyanins were identified; cyanidin-3-glucoside was the major anthocyanin in all the varieties studied. Three anthocyanins, 5-methylpyranocyanidin-3-glucoside, delphinidin-3-glucoside, and delphinidin-3-acetylglucoside were found for the first time in Chinese dwarf cherry. In addition, our study highlights the strong antioxidant capacity of Chinese dwarf cheery through its DPPH-scavenging abilities as well as high ORACFL and CAA values. It appears that the investigated Chinese dwarf cherry species have a great potential in terms of biodiversity, nutritive value, and biomedical research. Further studies are required for the identification of the polyphenol composition and evaluation of their functions among the seven genotypes.

Additional Points

Practical Applications. The Chinese dwarf cherry (*Cerasus humilis* (Bge.) Sok.) is a rich source of polyphenol, minerals, soluble sugars; the fruit bears significant importance in food processing. There are many differences in polyphenol and ascorbic acid content, physical and chemical properties, and anthocyanin composition in the fruits among different genotypes; genotypes, used as raw materials in different production processes of different types of dwarf cherry, may contribute to the different chemical profiles and color attribute. In this study, we aimed to evaluate the differences in the active components, the color, physicochemical indexes, enzymatic activity, antioxidant activity, and anthocyanin profiles in seven genotypes of Chinese dwarf cherry. The results will provide a theoretical reference for consumption of fresh fruits or food industrial processing. In addition, the results of this study can be used to provide additional data for the purpose of standardization and quality improvement of beverage processing production.

Conflicts of Interest

The authors have declared no conflicts of interest.

Acknowledgments

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