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Differences in Chemical Composition and Antioxidant Capacity Among Different Genotypes of Autumn Olive (*Elaeagnus umbellate* Thunb.)

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Summary

Fruit from six genotypes of autumn olive (*Elaeagnus umbellate* Thunb.); Brilliant Rose, Delightful, Jewel, Natural 1, Natural 2 and Sweet N Tart; were evaluated for fruit quality, phenolic content, carotenoids, antioxidants, antioxidant capacity, and antioxidant enzyme activity. The fruit soluble solids content (SSC), titratable acids (TA), total carotenoids, and total phenolic content varied with genotypes. Soluble solids content (SSC) in six genotypes of autumn olive ranged from 10.6 to 18.4 %, while titratable acids ranged from 0.79 to 1.29 %. Jewel had the highest SSC and Sweet N Tart had the highest TA. Fructose and glucose were the two predominant sugars, and malic acid was the predominant organic acid found in autumn olive fruit. Jewel and Sweet N Tart cultivars had the highest sugar and organic acid content among the six genotypes. Autumn olive had potent free radical scavenging activities for 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl ($DPPH^{\cdot}$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+\cdot}$), peroxyl radical ($ROO^{\cdot}$), superoxide radicals ($O_2^{-\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen ($^1O_2$). Autumn olive also had high activities of antioxidant enzymes including glutathione peroxidase (GHS-POD), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (AsA-POD), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDAR). Among the six genotypes, Brilliant Rose and Jewel had the highest levels of antioxidants and antioxidant enzyme activity.

Key words: autumn olive, fruit quality, antioxidant capacity, antioxidant enzyme activity

Introduction

Autumn olive (*Elaeagnus umbellate* Thunb.) is native to Southern Europe and Central Asia and was introduced to the US in the 1830s from East Asia as an ornamental plant (*1*). Autumn olive shrubs grow from 18–20 feet tall and have been found from Maine to Virginia, and west to Wisconsin. The plant has been reported to develop a nitrogen fixing association with *Frankia* spp. (*2*) and is widely tolerant of varying environmental conditions (*3*). Autumn olives produce an abundance of small deep-red colored, sweet-tart fruit which are considered suitable for human consumption and which are highly attractive to birds. Autumn olive fruit can be used for preserves, condiments, fruit rolls, juice, flavoring, and other food products (*4*).

Fordham *et al.* (*4*) showed autumn olive fruit to contain carotenoids such as lycopene, α-cryptoxanthin, β-cryptoxanthin, β-carotene, lutein, phytoene, and phytofluene. The lycopene concentrations of red autumn olive fruit (15–54 mg/100 g) are considerably higher than of fresh tomato fruit (3 mg/100 g), and are similar to that of tomato paste (29 mg/100 g) (*4*). Epidemiological

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studies have shown that the increased consumption of foods rich in carotenoids is correlated with a diminished risk of several diseases (*5*–*7*). The antioxidant activity of carotenoids enables them to act as quenchers for singlet oxygen ($^1O_2$) and peroxyl radicals (*8*,*9*).

However, there has been little research conducted on autumn olive berry, and thus minimal information is available on the health benefits. The purpose of this study is to evaluate six genotypes of autumn olive berry (Brilliant Rose, Delightful, Jewel, Natural 1, Natural 2 and Sweet N Tart) with regards to fruit quality, antioxidant capacity and antioxidant enzyme activity that might be involved in oxygen detoxification.

Materials and Methods

Chemicals

Ascorbate, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+\cdot}$), histidine, hydrogen peroxide (30 %, by mass), hydroxylamine hydrochloride, *N*,*N*-dimethyl-*p*-nitrosoaniline, xanthine, xanthine oxide, ascorbate oxidase, dithiothreitol (DTT), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase, guaiacol, β-nicotinamide adenine dinucleotide (β-NADH, reduced form), β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), nitro blue tetrazolium (NBT), resveratrol (3,5,4′--trihydroxystilbene) and $FeSO_4$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). EDTA (ethylenediaminetetraacetic acid disodium salt dihydrate, $Na_2EDTA{\cdot}2H_2O$), 6-hydroxy-2,5,7,8-tetramethylchroman-2--carboxylic acid (Trolox), 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) and trichloroacetic acid were purchased from Aldrich (Milwaukee, WI, USA), and 2′,2′--azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA, USA).

Sample material

Fruit from six genotypes of autumn olive berry (Brilliant Rose, Delightful, Jewel, Natural 1, Natural 2 and Sweet N Tart) were harvested from Hidden Spring Nursery, Cookeville, TN, USA and approximately 800 g to 1 kg of fruit were harvested per genotype. The fruit was picked around 9 am in the fall and shipped with dry ice overnight to the laboratory at Beltsville, MD, USA, frozen upon receipt, stored at –80 °C, and used for chemical analyses.

Sample preparation for assay

For analysis of sugars and organic acids, 4 g of fruit were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA) in 20 mL of imidazole buffer (20 mmol/L, pH=7.0). The extracts were centrifuged and the supernatants were used for sugar and organic acid determination.

For the assays of total phenolics, 2,2′-azinobis(3--ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+\cdot}$), 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl ($DPPH^{\cdot}$), and peroxyl radical ($ROO^{\cdot}$), triplicate 5 g of autumn olive fruit from each cultivar were extracted with 25 mL of 80 % acetone (containing 0.2 % formic acid) using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY, USA). The homogenized samples from the acetone extraction were then centrifuged at 14 000×*g* for 20 min at 4 °C. The supernatants were transferred to vials, stored at –80 °C, and later used for determination of total phenolics, $ROO^{\cdot}$ (ORAC), $DPPH^{\cdot}$ and $ABTS^{+\cdot}$ scavenging activity.

To prepare the samples for analyses of superoxide radicals ($O_2^{-\cdot}$), hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen ($^1O_2$), triplicate 35 g of autumn olive fruit from each cultivar were pulverized and then centrifuged at 14 000×*g* for 20 min at 4 °C. The supernatants were transferred to vials and stored at –80 °C until they were used for analysis.

Analysis of soluble solids content (SSC) and titratable acids (TA)

Autumn olive berries from each cultivar were pulverized with a cold mortar and pestle and pressed through four layers of cheese cloth to express the juice used for SSC and TA determination. The SSC of the fruit was determined on a Palette PR-100 (ATAGO-Spectrum Technologies, Plainfield, IL, USA) digital refractometer standardized with distilled water. TA was determined by diluting each 5-mL aliquot of autumn olive juice to 100 mL with distilled water and adjusting the pH to 8.2 using 0.1 M NaOH. Acidity was expressed as percent of malic acid equivalent.

Analysis of sugars and organic acids

Procedures described by Wang *et al.* (*10*) were used for the derivatization of sugars and organic acids. The sugars and organic acids were quantified by gas chromatographic techniques as previously described (*10*).

Total carotenoid and total phenolic content

For the total carotenoid content assay, five grams of autumn olive were extracted with 50 mL of tetrahydrofuran four times. Extracts were combined (200 mL) and concentrated to dryness on a Buchler Evapomix (Buchler Instruments, Fort Lee, NJ, USA), and then dissolved in 25 mL of methanol and partitioned three times with 25 mL of methylene chloride (with saturated salt water) in a separatory funnel. The organic phase (methylene chloride) was removed and the water phase washed with methylene chloride. The methylene chloride phase was removed, combined and dried over anhydrous sodium sulfate, filtered, and reduced in volume on a Buchler Evapomix. The concentrate was dissolved in methylene chloride and brought to 100 mL with methylene chloride and the absorbance of the sample was determined at 460 nm (Shimadzu UV-160A, Shimadzu Scientific Instruments, Columbia, MD, USA). Results were expressed as milligrams of β-carotene equivalent per 100 grams of fresh mass.

Total soluble phenolics in the fruit extracts were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (*11*) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 grams of fresh mass.

Free radical measurements

The ORAC assay was carried out using a high--throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader (*12*). The free radical DPPH scavenging capacity of autumn olive berry extract was evaluated as previously described (*13*) and using a calibration curve with different amounts of DPPH, the ED_{50} was calculated. The ED_{50} is the concentration of an antioxidant that is required to quench 50 % of the initial DPPH radicals under the experimental conditions given. The scavenging $ABTS^{+\cdot}$ radical capacity by fruit extract was performed by following the procedure described by Miller and Rice-Evans (*14*).

The assay for superoxide radicals ($O_2^{-\cdot}$) was performed using the methods of Gutteridge (*15*). The assay for hydroxyl radicals ($^{\cdot}OH$) was performed using the methods of Richmond *et al*. (*16*) and the production of singlet oxygen ($^1O_2$) by sodium hypochlorite and H_2O_2 was determined by using a spectrophotometric method according to Chakraborty and Tripathy (*17*).

Antioxidant enzyme measurements

For the measurement of glutathione peroxidase (GSH--POD, EC 1.11.1.9), and glutathione reductase (GR, EC 1.6.4.2), triplicate samples of fruit tissue (10 g of fresh mass) were homogenized in 10 mL of 0.1 M Tris-HCl buffer (pH=7.8) containing 2 mmol/L of EDTA-Na and 2 mmol/L of dithiothreitol (DTT). The homogenate was centrifuged at 20 000×*g* for 30 min at 4 °C, and the supernatant was used for the GSH-POD and GR assays. GSH-POD activity was determined using the method of Tappel (*18*). Enzyme activity was expressed as nmol of NADPH oxidized per mg of protein per min. GR activity was assayed according to Smith *et al*. (*19*). GR activity was expressed as nmol of NADPH oxidized per mg of protein per min.

For the measurement of superoxide dismutase (SOD, EC 1.15.1.1), triplicate samples of fruit tissue (10 g) were pulverized in a cold mortar and pestle with 10 mL of K-phosphate buffer (0.1 M, pH=7.3) containing 1 mmol/L of EDTA and 2 mmol/L of DTT. The homogenate was strained through 4 layers of miracloth and centrifuged at 12 000×*g* for 10 min at 4 °C. The supernatant was purified and total SOD activity was assayed according to Wang *et al*. (*20*).

For the measurement of ascorbate peroxidase (AsA--POD, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and monodehydroascorbate reductase (MDAR, EC 1.6.5.4), triplicate samples of fruit tissue (10 g) were pulverized in a cold mortar and pestle with 10 mL of K-phosphate buffer (0.1 M, pH=7.3) containing 1 mmol/L of EDTA and 2 mmol/L of DTT. The homogenate was centrifuged at 12 000×*g* for 10 min at 4 °C. The supernatant was used for the AsA-POD, DHAR, and MDAR assays. AsA-POD activity was assayed according to the method of Amako *et al*. (*21*). Enzyme activity was expressed as nmol of ascorbate oxidized per mg of protein per min. DHAR activity was assayed by measuring the rate of NADPH oxidation at 340 nm (*22*). Enzyme activity was expressed as nanomoles of NADPH oxidized per milligram of protein per min. MDAR activity was assayed by measuring the rate of NADH oxidation at 340 nm (*23*). Enzyme activity was expressed as nanomoles of NADH oxidized per milligram of protein per min.

For the determination of ascorbate (AsA) and glutathione (GSH), 4 g of fruit sample were homogenized with a cold mortar and pestle using 8 mL of ice-cooled 5 % trichloroacetic acid (TCA). The homogenate was filtered through four layers of miracloth and centrifuged at 16 000×*g* for 10 min at 4 °C. The supernatant was used for the AsA assays. AsA was determined using the methods of Arakawa *et al*. (*24*). A standard curve in the range 0–10 μmol of AsA was used. For measurement of GSH, triplicate fruit samples of 4 g were homogenized in 8.0 mL of ice-cold, degassed 7.57 mmol/L sodium ascorbate solution with chilled mortar and pestle under N_2 at 0 °C. The homogenate was filtered through four layers of miracloth and centrifuged at 30 000×*g* for 15 min at 0 °C. The supernatant was deproteined in glass test tubes by incubation in a water bath at 100 °C for 3 min and then centrifuged at 15 000×*g* for 15 min at 0 °C. The supernatants were used for the GSH assay. GSH was assayed using the method described by Castillo and Greppin (*25*).

Protein determination

Protein was determined according to Bradford (*26*), using bovine serum albumin (BSA) as a standard.

Statistical analysis

All experiments were conducted at least three times independently. Results were given as mean ± standard deviation of six independent determinations. All statistical analyses were performed with NCSS Statistical Analysis System (*27*). One-way analysis of variance (ANOVA) was used to compare the means, and differences were considered significant at $p \leq 0.05$. Correlation and regression analyses of free radical scavenging capacities *vs.* activities of antioxidant content, activities of antioxidant enzymes (SOD, GSH-POD, AsA-POD, MDAR, DHAR, and GR) and the nonenzyme components (AsA, GSH) were also performed using NCSS (*27*).

Results and Discussion

Sugar and acid content

Sugars and organic acids have an important impact on the sensory quality of fruit. The general flavor selection criteria for fruits have been combined in sensory--perceived high sweetness and high acidity. Fruit from the 6 genotypes of autumn olives tested had good and pleasing flavors, suggesting that there are many combinations of SSC and TA that confer good flavor. Autumn olives have high sugar and acid content. SSC among the six genotypes of autumn olive ranged from 10.6 to 18.4 % and TA ranged from 0.79 to 1.29 %. Jewel had the highest SSC and Sweet N Tart had the highest TA (Table 1). Autumn olive fruit also contains reducing sugars such as fructose, glucose and sucrose, and organic acids such as malic, quinic and citric acid. Fructose and glucose were the predominant sugars and malic acid was the predominant organic acid found in autumn olive fruit. Jewel and Sweet N Tart cultivars had the highest

Table 1. The content of sugar, organic acid, soluble solids content (SSC), and titrable acidity (TA) in various genotypes of autumn olive berry fruit[ab]

Genotype	*w*(sugar) (mg/100 g of fresh mass)			*w*(organic acid) (mg/100 g of fresh mass)			*w*(SSC) %	*w*(TA) %
	Fructose	Glucose	Sucrose	Malic	Quinic	Citric		
Brilliant Rose	62.8c	55.1c	0.62a	4.26d	0.09a	0.04a	12.5c	0.98b
Delightful	60.1c	50.1b	1.12d	3.89c	0.27b	0.03a	13.9d	0.93b
Jewel	78.0d	65.3d	1.02c	5.12e	0.07a	0.04a	18.4f	1.12c
Natural 1	48.7a	38.6a	0.69a	2.54b	0.29b	0.03a	10.6a	0.84a
Natural 2	56.9b	49.6b	1.19d	2.02a	0.07a	0.02a	11.5b	0.79a
Sweet N Tart	63.6c	56.8c	0.83b	6.88f	0.28b	0.04a	14.7e	1.29d
Significant genotype	*	*	*	*	*	ns	*	*

[a]Data is expressed as the mean of three replicates
[b]Means within the same column followed by different letters are significantly different at 5 % level
* or ns, significant or non-significant, at $p \leq 0.05$, respectively

sugar and organic acid content among the six genotypes. The proportions of fructose, glucose and sucrose are important in the perception of fruit quality since fructose is 1.8 times sweeter than sucrose (*28*), while the sweetness of glucose is only 60 % of that of sucrose (*29*). Carbohydrate and SSC contents in autumn olive fruit were positively correlated (R^2=0.8756).

Malic, quinic and citric acid were found in autumn olive and malic acid was the primary organic acid (Table 1). Sweet N Tart contained the highest malic acid and Natural 2 had the lowest. The total organic acid level was positively correlated with titratable acidity (R^2= =0.9483). The ratios of SSC to TA ranged from 11.40 to 16.43 with Jewel having the highest SSC:TA ratio and Sweet N Tart having the lowest (Table 1).

Total carotenoid and total phenolic content

Significant differences were found in total carotenoids and total phenolic content among different cultivars of autumn olive (Table 2). The Brilliant Rose genotype had the highest content of carotenoids followed by Jewel, Natural 2, Sweet N Tart, Natural 1 and Delightful. Lycopene concentrations of red autumn olive fruit are considerably higher than those of fresh tomato fruit (*4*). Epidemiological studies have shown that high tomato intake was associated with a 50 % reduction of mortality from cancers at all sites in elderly Americans (*30*). Lycopene has been shown to have an exceptionally high singlet oxygen quenching ability, twice that of β-carotene and 10 times that of α-tocopherol (*31–33*). Total phenolics were variable among different genotypes of autumn olive with Brilliant Rose having the highest total phenolics (Table 2). Phenolics are secondary plant metabolites. They protect the plant against damaging photodynamic reactions by quenching the excited state of active oxygen species (*34*). Thus, phenolic compounds in autumn olive may help protect cells against the oxidative damage caused by free radicals.

Table 2. Antioxidant activities, total phenolic and total carotenoid content in different genotypes of autumn olive berry fruit[a]

Genotype	ORAC[b] μmol TE/g of fresh mass			ABTS[b]	DDPH[c]	*w*(total phenolics)[d]	*w*(total carotenoids)[e]
	Soluble	Insoluble	Total		ED_{50}	mg/100 g of fresh mass	
Brilliant Rose	62.5e[f]	4.13e	66.6d	29.9d	2.42a	258.1e	59.3e
Delightful	42.9a	3.15b	46.0a	23.6b	5.37f	168.9a	43.4a
Jewel	54.4d	4.02d	58.5c	25.5c	2.65b	241.8d	56.6d
Natural 1	44.1a	3.07a	47.2a	20.3a	4.76e	188.2b	49.3a
Natural 2	47.7b	3.05a	50.8b	23.9bc	3.69d	180.2b	52.6c
Sweet N Tart	50.4c	3.58c	54.0b	24.2c	3.02c	208.6c	49.5b
Significant genotype	*	*	*	*	*	*	*

[a]Data expressed as the mean of three replicates
[b]Data expressed as μmol of Trolox equivalents per g of fresh mass
[c]The ED_{50} is the concentration of an antioxidant (mg of fresh mass of autumn olive berry) which is required to quench 50 % of the initial DPPD radicals under the experimental conditions given
[d]Data expressed as mg of gallic acid equivalents per 100 g of fresh mass
[e]Data expressed as mg of β-carotene equivalents per 100 g of fresh mass
[f]Means within the same column followed by different letters are significantly different at 5 % level
*Significant at $p \leq 0.05$

Free radical scavenging capacity

Autumn olive berries had high antioxidant capacity against radicals of $DPPH^{\cdot}$, $ABTS^{+\cdot}$, $ROO^{\cdot}$, $O_2^{-\cdot}$, $^{\cdot}OH$, and $^1O_2$ (Tables 2 and 3). Significant DPPH-radical scavenging activity was detected in autumn olive extracts, although the different varieties differed in their activity to react and quench DPPH radicals (Table 2). The ED_{50} value is used to express the concentration of an antioxidant required to quench 50 % of the initial DPPH radicals under the experimental conditions given. A smaller ED_{50} value corresponds to greater DPPH-radical scavenging activity. The ED_{50} values ranged from 2.42 to 5.37 mg of fresh mass for six autumn olive genotypes. The fruit of Brilliant Rose extract had the greatest free radical scavenging activities against the DPPH radical with an ED_{50} of 2.42 mg of fresh mass.

The $ABTS^{+\cdot}$ scavenging activity was another method used in our study to measure antioxidant activity in autumn olive. In our study, we used the decolorization assay based on inhibition by antioxidants of absorbance of radical cation ($ABTS^{+\cdot}$), which was generated through chemical reduction by manganese dioxide (*14*). The autumn olive genotype Brillant Rose had the highest radical cation $ABTS^{+\cdot}$ scavenging capacity, followed by Jewel, Sweet N Tart, Natural 2, Delightful and Natural 1 (Table 2). The DPPH-radical and $ABTS^{+\cdot}$ scavenging activity were correlated to ORAC values with R^2 equal to 0.8053 and 0.8274, respectively. This indicated that antioxidant capacity of autumn olive could be measured either by the ORAC or DPPH-radical or $ABTS^{+\cdot}$ scavenging assays.

Common oxidants produced in organisms include reactive oxygen species (ROS), such as superoxide radicals ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$). Imbalances in the production and metabolism of ROS can cause oxidative stress and lead to cell death (*35*). For this reason, recent studies have focused on antioxidants because they may help protect the body against ROS damage (*36*). The scavenging capacities against peroxyl radicals ($ROO^{\cdot}$), hydroxyl radicals ($^{\cdot}OH$), superoxide radicals ($O_2^{-\cdot}$) and singlet oxygen ($^1O_2$) in six autumn olive genotypes are shown in Tables 2 and 3. The scavenging activity for $ROO^{\cdot}$ radical was expressed as ORAC activity. The ORAC values from the hydrophilic fraction for autumn olive extracts ranged from 42.9 to 62.5 μmol of Trolox equivalents (TE)/g of fresh berries, and the lipophilic fraction from 3.05 to 4.13 μmol TE/g of fresh berries, with the cultivar Brilliant Rose yielding the highest ORAC value (Table 2). There was a correlation between total phenolic contents and free radical scavenging activity (ORAC) (R^2=0.8846).

The scavenging capacity for $^{\cdot}OH$ ranged from 35.7 to 79.5 μmol TE/g of fresh mass, reflecting a 2.23-fold difference among genotypes (Table 3). Brilliant Rose and Jewel had the highest scavenging antioxidant capacity on $^{\cdot}OH$ values and the highest percent inhibition of $^{\cdot}OH$ activity at 82.5 to 82.8 %, respectively, among all of the autumn olive genotypes used in this study (the percent inhibition of $^{\cdot}OH$ activity in the presence of berry fruit extract was expressed against blanks which had been prepared similarly but without berry fruit extract). Meanwhile, Delightful had the lowest $^{\cdot}OH$ scavenging efficiency with only 38.0 % inhibition of $^{\cdot}OH$ production. The scavenging capacity values against $^1O_2$ in autumn olive ranged from 1.06 to 2.14 μmol TE/g of fresh mass, and ranged from 13.7 to 52.1 μmol TE/g of fresh mass against $O_2^{-\cdot}$ (Table 3). The relative scavenging efficiency (% inhibition of radical production) on $^1O_2$ and $O_2^{-\cdot}$ ranged from 24.6 to 50.0 % and 31.5 to 79.9 %, respectively. Brilliant Rose consistently had the best scavenging capacity for reactive oxygen species not only for $ROO^{\cdot}$ and $^{\cdot}OH$, but also for $^1O_2$, and $O_2^{-\cdot}$ with 2.14 and 52.1 μmol TE/g of fresh mass, respectively (Table 3), and the inhibition of radical production was 50.0 % for $^1O_2$ and 79.9 % for $O_2^{-\cdot}$.

Antioxidant enzymes

The antioxidant enzyme defense system consists of hundreds of different substances and mechanisms. Antioxidant enzymes have the capacity to lower the free radical burden and neutralize excess free radicals created by stress conditions. Autumn olive berry had high antioxidant enzyme activities as well as the nonenzyme components (AsA and GSH) (Table 4). Genotype Bril-

Table 3. Scavenging capacity in different genotypes of autumn olive berry fruit on active oxygen species ($O_2^{-\cdot}$, $^{\cdot}OH$ and $^1O_2$)[a]

Genotype	Active oxygen species μmol Trolox/g fresh mass[b]			Inhibition[c] %		
	$O_2^{-\cdot}$	$^{\cdot}OH$	$^1O_2$	$O_2^{-\cdot}$	$^{\cdot}OH$	$^1O_2$
Brilliant Rose	52.1e[d]	79.5d	2.14e	79.9e	82.8d	50.0e
Delightful	19.0b	35.7a	1.06a	31.5b	38.0a	24.6a
Jewel	42.0d	79.2d	1.76d	69.5d	82.5d	42.6d
Natural 1	13.7a	47.8b	1.16a	22.7a	51.1b	26.6a
Natural 2	27.9c	43.5b	1.21b	46.1c	46.4b	30.1b
Sweet N Tart	34.4c	58.3c	1.45c	54.9c	62.2c	35.2c
Significant genotype	*	*	*	*	*	*

[a]Data expressed as the mean value of three replicates
[b]Data of $O_2^{-\cdot}$, $^{\cdot}OH$ and $^1O_2$ expressed as μmol of Trolox equivalents per g of fresh mass
[c]Data expressed as percent inhibition of radical ($O_2^{-\cdot}$, $^{\cdot}OH$ or $^1O_2$) production in the present per g of fruit extract
[d]Means within the same column followed by different letters are significantly different at the 5 % level
*Significant at $p \leq 0.05$

Table 4. Activities of antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GSH-POD), ascorbate peroxidase (AsA-POD), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR)) and non-enzyme antioxidants (ascorbic acid (AsA) and reduced glutathione (GSH)) in different genotypes of autumn olive berry fruit[ab]

Genotype	SOD (U/mg protein)	GSH--POD	AsA--POD	MDAR	DHAR	GR	GSH (μmol/g fresh mass)	AsA (nmol/g fresh mass
		(nmol/mg protein per min)						
Brilliant Rose	32.3e	98.3d	57.7e	8.12e	42.1e	33.1e	36.3e	12.4d
Delightful	15.8b	45.2a	26.9a	3.37a	28.6b	12.2a	18.8a	7.4a
Jewel	22.9c	79.2c	48.6d	6.89cd	37.7d	26.9c	28.9d	11.9cd
Natural 1	13.6a	59.6a	34.1b	4.38b	25.2a	15.7b	15.9a	7.2a
Natural 2	17.5b	68.2b	41.5c	6.01c	32.8c	12.1a	25.8c	9.4b
Sweet N Tart	27.2d	61.4b	47.2d	7.15d	38.7d	29.4d	22.6b	10.7bc
Significant genotype	*	*	*	*	*	*	*	*

[a]Data is expressed as the mean of three replicates
[b]Means within the same column followed by different letters are significantly different at the 5 % level
*Significant at $p \leq 0.05$

liant Rose had the highest enzyme activities, as well as AsA and GSH. Brilliant Rose had SOD of 32.3 U/mg protein, GSH-POD of 98.3 nmol/mg protein per min, AsA-POD of 57.7 nmol/mg protein per min, MDAR of 8.12 nmol/mg protein per min, DHAR of 42.1 nmol/mg protein per min, and GR of 33.1 nmol/mg protein per min (Table 4). Brilliant Rose also contained ASA of 12.4 nmol/g of fresh mass and GSH of 36.3 μmol/g of fresh mass (Table 4). Among the genotypes tested, the Delightful, Natural 1 and Natural 2 genotypes had lower antioxidant enzyme activities and ASA and GSH content compared to the other genotypes (Table 4).

SOD catalyzes the breakdown of $O_2^{\cdot-}$ to O_2 and H_2O_2, removes singlet oxygen as well as $O_2^{\cdot-}$, prevents formation of $^{\cdot}OH$ and has been implicated as an essential defense against the potential toxicity of oxygen (*37*). The SOD activities in autumn olive extracts ranged from 13.6 to 32.3 U/mg protein. High SOD activity also correlated with high antioxidant activity (ORAC) with R^2=0.9577.

Glutathione peroxidase (GSH-POD) is another of the body's major protectors against free radicals. GSH-POD may be responsible for scavenging H_2O_2, catalyzing the peroxidation of reduced glutathione (GSH), and forming the oxidized disulfide form of glutathione (GSSG) as a product. This antioxidant enzyme consists of the amino acid glutathione and the trace mineral selenium. These two nutrients team up to combat a specific class of free radicals called peroxides. GSH-POD prevents the destruction of cell membranes by removing several classes of these lipid peroxides. The effect of excess peroxidation in our cells is diverse and dangerous and must be limited to maintain cellular health (*38*). The GSH-POD activities in autumn olive extracts ranged from 45.2 to 98.3 nmol/mg protein per min. High GSH-POD activity correlated with high antioxidant activity (ORAC) with R^2=0.9549.

AsA-POD is a heme-containing protein and is highly specific for ascorbate as the electron donor (*39*). AsA-POD activity positively correlated with ascorbic acid content in autumn olive extracts (R^2=0.9052). The close correlations between ORAC values and AsA content (R^2=0.9286) and AsA-POD (R^2=0.9686) were also evident.

Ascorbic acid (AsA) serves as an excellent antioxidant and plays a fundamental role in the removal of hydrogen peroxide *via* the ascorbate-glutathione cycle and produces dehydroxyascorbic acid (DHAsA). DHAsA is reduced to ascorbic acid by MDAR or DHAR at the expense of NADH and glutathione (GSH) (*40*). Autumn olive had high activities of MDAR and DHAR with Brilliant Rose having the highest and Delightful and Natural 2 the lowest. The ORAC value correlated with activities of MDAR (R^2=0.8125) and DHAR (R^2=0.9283).

Glutathione reductase (GR) is a ubiquitous NADPH--dependent enzyme and is present in the cells of both plants and animals (*41*). It has been suggested that in higher plants, GR may be a rate-limiting enzyme for the defense against active O_2 toxicity (*42*). Cultivars with high activity of GR were also found to have high ORAC values (R^2=0.9512) and high GSH contents (R^2=0.9712). These suggest that antioxidants and antioxidant enzymes in autumn olive fruit may serve as the first line of defense against free radicals to avoid unnecessary cellular and tissue damage. Therefore, it is possible that these antioxidant enzymes in autumn olive can prevent cellular and tissue damage in the human body.

Conclusions

Collectively, our results indicate that autumn olive berry has good fruit quality which is variable among different genotypes. Autumn olive had high content of carotenoid, total phenolics, and high antioxidant capacities against radicals of DPPH, $ABTS^{+\cdot}$, $ROO^{\cdot}$, $O_2^{\cdot-}$, $^{\cdot}OH$, and $^1O_2$. Autumn olive berry also had high antioxidant enzyme activities as well as nonenzyme components (ascorbic acid and glutathione). These activities were different among the autumn olive genotypes. Dietary supplementation with various fruits and vegetables including autumn olive berry could have benefits to human health.

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