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Evaluation of the differences in phenolic compounds and antioxidant activities of five green asparagus (*Asparagus officinalis* L.) cultivars

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RESEARCH ARTICLE

Abstract

Phenolic compounds and total antioxidant activities were analysed in five green asparagus cultivars (Grande, Altas, UC800, UC301 and UC157). In addition, the colours as well as the lignin, amino acid, and microelement contents of the asparagus cultivars were assessed. Cultivar 'Grande' had the best greenness (a^*=-12.47), the highest vitamin C (59.1 mg/kg) and microelement contents (282.3 mg/kg) and the lowest lignin content (0.92%). Cultivar 'UC301' had the highest amino acid content (3.95 g/kg), and cultivar 'UC157' had the highest total phenolic and total flavonoid contents. Correlations were determined to evaluate the relationship between phenolic compounds and total antioxidant activity. Phenolic compounds, particularly rutin, are major contributors to the antioxidant activity of green asparagus, with a correlation coefficient of r=0.977-0.982. A principal component analysis was then performed to investigate the interrelationships between the parameters and the investigated cultivars. The results showed that cultivars 'UC157' and 'Grande' had high total phenolic and rutin contents and consequently high antioxidant activity. The cultivar had a marked influence on bioactive constituents, particularly the phenolic compound content and composition, and on the antioxidant activity of green asparagus, which may provide a basis for improved health benefits and breeding programs.

Keywords: green asparagus, phenolics, antioxidant, correlation, PCA

1. Introduction

Fruits and vegetables are known to play a key role in protecting against cancer and cardiovascular disease. Epidemiological studies have identified a significant positive relationship between fruit and vegetable consumption and a reduced risk of certain chronic diseases (Borbalán *et al.*, 2003; Liu *et al.*, 2015). These protective effects have generally been attributed to different antioxidant constituents, such as vitamins C and E, carotenoids, flavonoids and phenolic acids (Mazzeo *et al.*, 2011). Therefore, researchers worldwide have shown increasing interest in the health benefits of compounds in fruits and vegetables. Most fruit and vegetable species have many cultivars, and many studies indicate that different cultivars vary in their bioactive compound content and antioxidant capacities (Flores *et al.*, 2015; Jorjong *et al.*, 2015; Kim *et al.*, 2015; Kou *et al.*, 2015; Park *et al.*, 2014; Wang *et al.*, 2015). In fact, even cultivars grown in the same geographic and climatic conditions differ significantly (Ercisli *et al.*, 2007).

Green asparagus is a popular vegetable that is consumed in many parts of the world whose edible shoots are used in salads, vegetable dishes, and soups (Wang *et al.*, 2003). Green asparagus has been shown to have a high nutrient/calorie ratio and can help prevent cancer, ageing or cardiovascular diseases due to its high content of antioxidants such as flavonoids (mainly rutin; RT), ascorbic acid, glutathione and other phenolic compounds (Makris and Rossiter, 2001; Sun *et al.*, 2007a; Wang *et al.*, 2003), having one of the highest antioxidant activities of 43 vegetables (Tsushida *et al.*, 1994). Although several studies have explored the bioactive compounds and antioxidant capacities of green asparagus (Drinkwater *et al.*, 2015; Mastropasqua *et al.*, 2016; Sun *et al.*, 2007a,b; Wang *et al.*, 2003), a comprehensive evaluation of green asparagus cultivars is still lacking. To our knowledge, one study related to the antioxidant activity of ethanolic extracts from several asparagus cultivars has been reported (Rodríguez *et al.*, 2005). The main objective of the present work was thus to characterise the main bioactive constituents and

antioxidant activities of five varieties of Chinese origin, which were collected in Jiangsu and Shandong. Colour parameters and the levels of lignin (Lg), amino acids (AA) and microelements (Me) were also determined. We analysed the data using Pearson's correlation and principal component analysis (PCA) to visualise the relationships between the investigated parameters and the five cultivars 'Grande', 'Altas', 'UC800', 'UC301', and 'UC157'. Thus, the results of this study will provide a good foundation for selecting parents for breeding programs and cultivars with health benefits.

2. Materials and methods

Chemicals

Coumaric acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, chlorogenic acid, gallic acid, RT, kaempferide, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the Folin-Ciocalteu reagent were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade or high-performance liquid chromatography (HPLC) grade.

Green asparagus cultivars

A representative sampling of green asparagus cultivation base was conducted. Two cultivars ('Grande' and 'Altas') of fresh green asparagus (*Asparagus officinalis* L.) were harvested from a local farm in Xuzhou, Jiangsu province, and 3 cultivars ('UC800', 'UC301', and 'UC157') were harvested from a farm in Caoxian, Shandong Province. Asparagus spears with a length of 20-30 cm were harvested on 15 May, 2014, and immediately transported to the lab. Random samplings (200 g each) of five green asparagus samples were used for analysis. Fresh spears (100 g) were subjected to analyse for colour, chlorophyll, carotenoids, and vitamin C (Vc). Spears (100 g) intended for Lg, AA, Me, phenolic compound and antioxidant activity analyses were stored at -80 °C until use.

Determination of colour parameters

Colour parameters were measured using a colour meter (WSC-S, Precision Scientific Instrument Co., Ltd., Shanghai, China). The chlorophyll and carotenoid contents were determined according to the method described by Mazzeo *et al.* (2011) with slight modifications. Green asparagus sample (2 g) were ground and extracted with 10 ml of 80% aqueous acetone solution in four times. The extracts were pooled and centrifuged at 12,000×*g* for 10 min. Total chlorophyll content was determined by measuring the absorbance of the solution at 663 and 645 nm. Carotenoid content was determined at 440 nm.

Determination of vitamin C, lignin, amino acids and microelements

The level of Vc was determined using the 2, 6-dichlorophenolindophenol assay (Nindo *et al.*, 2003) with slight modifications. One gram of green asparagus sample was homogenised with 5 ml of 5% trichloroacetic acid solution to extract Vc. The mixture was centrifuged and the supernatant was then taken and titrated with 0.1% aqueous sodium dichlorophenolindophenol solution to light pink colour persisted for 15 s. The Lg level was determined according to Van Soest (1963). AA were analysed using the ninhydrin colorimetric method (Huang *et al.*, 2010). Briefly, five grams of green asparagus sample was homogenised, diluted to 50 ml with distilled water, shaken, and centrifuged at 4,000×*g* for 10 min. Next, 4 ml of the collected supernatant was mixed with 1 ml of ninhydrin and 1 ml of acetic acid buffer (pH 6) and boiled for 15 min. The mixture was cooled, adjusted to 25 ml and incubated at room temperature for 15 min. The absorbance of the solution was determined at 670 nm.

Me content was determined as described by Šavikin *et al.* (2014) with atomic absorption spectrophotometer (TAS-990MFG, Beijing Purkinje General Instrument Co. Ltd., Beijing, China). Frozen asparagus samples were prepared by microwave digestion using a microwave system (XT-9900, Xintuo Microwave Testing Technology Co. Ltd., Shanghai, China). Two grams of asparagus sample, 1.0 ml of 30% hydrogen peroxide, and 5 ml of concentrated ultrapure nitric acid were mixed and poured into the microwave digestion vessel. After the effervescence had subsided, the sample was cooled, transferred into a clean volumetric flask, and diluted to 25 ml with ultrapure water. A blank was prepared in the same way.

Determination of total phenolic compounds and total flavonoids

Preparation of phenolic extracts

Phenolic compounds were extracted from green asparagus according to the method described by Wang *et al.* (2003), with slight modifications. Fifteen grams of green asparagus sample was ground and homogenised in 50 ml of 70% ethanol. The mixture was then treated with microwaves for 180 s at 200 W using an ultrasonic microwave-assisted extractor (CW-2000, Xintuo Microwave Decomposition and Testing Technology Co. Ltd). The insoluble components were separated by centrifugation at 4,000×*g* for 20 min, and the supernatant was collected for analysis.

Determination of total phenolic content

The total phenolic (TP) content was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Folin-Ciocalteu reagent was diluted 10 times with deionised water. Then, 750 µl of the diluted reagent, 100 µl of the ethanol extracts, and 750 µl of a 2% sodium carbonate solution were added to the tubes. The tubes were then incubated for 1 h at room temperature. The absorbance of the solution was determined at 750 nm. Gallic acid was used as a standard.

Determination of total flavonoid content

The total flavonoid (TF) concentration was determined using a colorimetric assay described by Singh *et al.* (2012), with slight modifications. RT was used as the standard. Briefly, 2 ml of the ethanol extracts was mixed with 0.75 ml of 5% sodium nitrite, vortexed, and incubated for 5 min. Then, 0.5 ml of 10% aluminium nitrate was added and incubated for 6 min. Finally, 4 ml of 5% sodium hydroxide was added. The resulting mixture was adjusted to 25 ml with distilled water and incubated for 15 min. The absorbance was read at 510 nm.

Analysis of phenolic compounds

Agilent HPLC (Agilent 1100, Santa Clara, CA, USA) analysis was performed to evaluate polyphenol concentrations in green asparagus samples. The HPLC included a quaternary pump, thermostatic column compartment, vacuum degasser, and a UV detector. The ethanol extracts were filtered through a 0.45-µm membrane filter prior to injection into the HPLC. The samples were separated on an Agilent ODS XDB C_{18} column (150 mm×4.6 mm i.d., 5-µm particle size) at 30 °C. The mobile phase consisted 5% formic acid (solvent A) and 80% acetonitrile (solvent B), at a flow rate of 0.9 ml/min. The total running time was 30 min. The gradients were 0 min 80% solvent A and 20% solvent B, 20 min, 60% A, 40% B, 30 min, 40% A, 60% B (Wang *et al.*, 2003). The injection volume was 10 µl, and the detector was set at 280 or 320 nm for analysis. Phenolic contents were quantified using external standards (0.01-1 mg/ml in methanol, the linear correlation coefficients of the standard curves are from 0.9911 to 0.9999). All standards were filtered through a 0.45-µm membrane filter before injection. Each sample was measured in triplicate and the experiment was conducted twice.

Determination of antioxidant activity

One gram of green asparagus sample was ground and homogenised in 5 ml of 70% ethanol. The resulting mixture was centrifuged at 4,000×*g* for 10 min. The supernatant was collected to analyse the free radical-scavenging capacity.

The DPPH radical-scavenging capacity was analysed according to the method of Brand-Williams *et al.* (1995). One millilitre of asparagus sample (0.20 g/ml) was added to 2 ml of DPPH-free radical solution (0.2 mmol/l) in ethanol and incubated in the dark for 30 min at room temperature. The absorbance of the solution was determined at 517 nm.

The hydroxyl (•OH) radical-scavenging capacity was determined using the salicylic acid method (Smirnoff and Cumbes, 1989). Briefly, 3 ml of asparagus sample (0.20 g/ml), 2 ml of 6 mmol/l salicylic acid, and 2 ml of 6 mmol/l ferrous sulphate were mixed, and 0.2 ml of 6 mmol/l hydrogen peroxide was then added to start the reaction. The reaction solution was incubated in a room-temperature water bath for 30 min. The absorbance of the solution was determined at 510 nm.

For the two free radical-scavenging capacity assays, the scavenging capacity was calculated using the following equation:

$$\text{Free radical-scavenging capacity (\%)} = (A_0 - A_t) / A_0 \times 100$$

A_0 was the absorbance of the DPPH/•OH radical solution at time zero and A_t was the absorbance of the DPPH/•OH radical solution after 30 min.

Statistical analysis

The experiments were performed using a completely randomised design. All data were expressed as the mean±standard error and subjected to statistical analysis in SPSS/PC version 18.0 (SPSS Inc., Chicago, IL, USA). The data were analysed by one-way analysis of variance. Mean separations were analysed by Duncan's multiple-range test, and differences at $P<0.05$ were considered significant. PCA was applied to separate the cultivars using SPSS version 18.0.

3. Results and discussion

Colour, lignin and amino acids

The colour and the Lg and AA contents of five cultivars of green asparagus are listed in Table 1. Colour is an important quality attribute of green asparagus. The L^* value represents the lightness of fruit and vegetable products. Cultivars 'Grande' and 'Altas' showed the highest L^* values of the analysed green asparagus cultivars. The negative a^* value represents the intensity of greenness, which is the natural colour of green asparagus. Cultivar 'Grande' had the lowest a^* value, showing an intense green colour. No significant ($P>0.05$) differences in the a^* value were observed among the other four cultivars. Chlorophyll and carotenoids are important pigments that affect the colour quality of green asparagus. Cultivar 'Grande' had the highest chlorophyll

content, which was consistent with its displaying the lowest *a** value ($P<0.05$). A trend of decreasing chlorophyll content was observed for 'UC800', 'UC157', 'UC301', and 'Altas'. The highest carotenoid content was recorded in cultivar 'Altas', followed by 'Grande', 'UC301', 'UC157', and 'UC800'. These results indicate that the cultivar significantly influences the colour of green asparagus. Similar results for colour were obtained in summer squash (Martínez-Valdivieso *et al.*, 2015).

Green asparagus is gaining in popularity due to its unique texture and flavour (Lau *et al.*, 2000). Postharvest green asparagus has a short shelf life due to deterioration, particularly lignification and toughening texture. The toughness of asparagus is a major factor that determines its quality. Changes in the texture of green asparagus are closely related to changes in Lg content. Significant differences were observed in the Lg contents of different green asparagus cultivars (Table 1). Cultivar 'Grande' had the lowest Lg content, indicating its tender and succulent texture. Cultivars 'UC157', 'UC301' and 'UC800' had the highest Lg contents, with no significant differences among them. Cultivar 'Altas' had a medium Lg content. Being a vegetable, green asparagus is also rich in AA. The highest AA contents were found in cultivars 'Grande' and 'UC301', whereas no significant ($P>0.05$) differences were found among the other three cultivars (Table 1).

Vitamin C, total phenols and total flavonoids

Vc is an important component of our nutrition and is used as additive in many foods because of its antioxidant properties (Zheng *et al.*, 2011), and vegetables are a major source of Vc. The Vc content in green asparagus samples ranged from 24.2 to 59.1 mg/kg (Table 1). These values are slightly lower than those from green asparagus reported by Mastropasqua *et al.* (2016) and Nindo *et al.* (2003). Cultivar 'Grande' had the highest Vc content, whereas cultivar 'UC800' had the lowest. The other three analysed cultivars showed no significant ($P>0.05$) differences. Clear differences in Vc content were also observed among strawberry cultivars (Kim *et al.*, 2015). Asparagus contains flavonoids and other phenolic compounds (Makris and Rossiter, 2001). Different TP and TF contents were observed in each of the analysed cultivars, with significant ($P<0.05$) differences among them (Table 1). The TP content ranged from 124.09 to 425.82 mg/kg with the lowest value in 'UC800' and highest value in 'UC157'. These concentrations are comparable to those found in the literature (Rodríguez *et al.*, 2005; Sun *et al.*, 2007c). Similar changes were also observed in the TF content, which ranged from 70.53 to 279.66 mg/kg, consistent with those reported by Sun *et al.* (2007c). Cultivar 'UC157' showed the highest value, followed by 'Grande', 'Altas', 'UC301', and 'UC800'. Similarly, a large variation in TP and TF contents has been observed among peach cultivars (Liu *et al.*, 2015), Mao-Luang cultivars (Jorjong *et al.*, 2015), strawberry cultivars (Šamec *et al.*, 2016), and sweet potato cultivars (Shekhar *et al.*, 2015).

Microelements

Minerals have both direct and indirect effects on human health. The direct effects of minerals stem from the consequences of their consumption on human nutrition, whereas the indirect effects involve their contribution to fruit quality and subsequent consumer acceptance (Vicente *et al.*, 2009). Asparagus is an adequate food source for mineral nutrients (López *et al.*, 1999). The Me compositions of the different green asparagus cultivars are shown in Table 2. The main Me in the tested samples were Zn, Cr, Mn, Fe, Cu, and Se. Green asparagus from cultivar 'Grande' exhibited the highest Zn, Mn, Cu and Se contents. The highest Fe content was observed in cultivar 'UC301', and the lowest was in cultivar 'Altas'. The element Se is especially abundant in green asparagus. The highest Se content was found in cultivars 'Grande' and 'UC800', the lowest in cultivar 'Altas'. Our results indicate that Me concentrations varied widely between cultivars, in agreement with previous reports (Ekholm *et al.*, 2007; Šavikin *et al.*, 2014; Vicente *et al.*, 2009). Because the studied green asparagus samples were collected from two different environments, the different Me patterns clearly

Table 1. Colour and quality parameters of five green asparagus cultivars.[1]

Cultivar	*L**	*a**	Chlorophyll (mg/kg)	Carotenoids (mg/kg)	Lignin (%)	Amino acids (g/kg)	Vitamin C (mg/kg)	TP (mg/kg)	TF (mg/kg)
Grande	51.21±1.51^{ab}	-12.47±0.27^{b}	39.6±0.71^{a}	20.2±0.04^{b}	0.92±0.09^{c}	3.65±0.31^{a}	59.1±1.48^{a}	380.98±4.47^{b}	270.09±3.08^{b}
Altas	54.77±2.90^{a}	-9.94±2.61^{a}	16.6±0.42^{d}	33.0±0.54^{a}	1.22±0.19^{b}	3.16±0.01^{b}	35.5±.3.21^{b}	320.55±0.29^{c}	251.92±1.01^{c}
UC157	48.28±1.36^{b}	-9.60±0.52^{a}	25.0±1.20^{b}	14.7±.1.20^{c}	1.44±0.08^{a}	3.21±0.23^{b}	36.2±.0.91^{bc}	425.82±5.07^{a}	279.66±4.16^{a}
UC301	50.49±1.91^{b}	-9.04±0.17^{a}	23.3±0.74^{c}	14.9±0.33^{c}	1.53±0.11^{a}	3.95±0.02^{a}	34.2±.0.84^{bc}	213.40±3.10^{d}	96.37±2.79^{d}
UC800	49.68±2.74^{b}	-9.27±1.40^{a}	26.1±0.44^{b}	13.1±0.32^{d}	1.48±0.10^{a}	3.05±0.00^{b}	24.2±0.13^{c}	124.09±2.01^{e}	70.53±0.95^{e}

[1] The values are the means±SD, n=3. Different letters in the same column indicate a significant difference ($P<0.05$). *L** = lightness; *a** = greenness; TP = total phenolics; TF = total flavonoids.

Table 2. Microelement contents (mg/kg) of five green asparagus cultivars.[1]

Cultivar	Zn	Cr	Mn	Fe	Cu	Se
Grande	62.6±1.52^{a}	22.8±1.10^{b}	49.9±0.78^{a}	78.3±0.54^{d}	47.2±0.49^{a}	21.5±0.51^{a}
Altas	41.6±0.34^{b}	35.0±1.04^{a}	27.0±0.96^{b}	53.8±0.32^{e}	37.8±0.13^{b}	13.7±0.33^{d}
UC157	6.3±0.12^{d}	ND[2]	ND	91.0±0.61^{b}	17.5±0.27^{d}	16.8±0.42^{c}
UC301	9.0±0.12^{c}	ND	ND	113.0±1.33^{a}	19.8±0.24^{c}	19.0±0.38^{b}
UC800	6.5±0.09^{d}	ND	ND	82.0±0.50^{c}	17.3±0.13^{d}	20.8±0.52^{a}

[1] The values are the means±SD; n=3. Different letters in the same column indicate a significant difference (P<0.05).
[2] ND = not detectable.

demonstrated the differential capacity of each cultivar to absorb ions from the soil, as well as differences in the ion composition of different soils (Šavikin *et al.*, 2014). As shown in Table 2, the soil and climatic conditions were the dominant factors that influenced the concentrations of Zn, Fe, Cu, and particularly Cr and Mn, in the investigated green asparagus cultivars. The Cr concentration ranged from 22.8 to 35.0 mg/kg and the Mn concentration ranged from 27.0 to 49.9 mg/kg in green asparagus from farmland in Xuzhou, Jiangsu province. However, Cr and Mn were not detected in green asparagus from farmland in Caoxian, Shandong province. The measured concentrations of Zn (41.6 to 62.6 mg/kg) and Cu (37.8 to 47.2 mg/kg) in green asparagus from Xuzhou were higher than those in green asparagus from Caoxian. In contrast, the Fe content (82.0 to 113.0 mg/kg) in the green asparagus from Caoxian was higher than in those from Xuzhou (53.8 to 78.3 mg/kg). Konczak and Roulle (2011) demonstrated that the mineral content of fruits and vegetables varies according to soil conditions, weather and agricultural practices.

Phenolic compounds

A high intake of antioxidant and bioactive compounds, particularly polyphenols, may play a crucial role in preventing several diseases, such as cancer, cardiovascular and neurodegenerative diseases, and other chronic pathologies (Kou *et al.*, 2015). The phenolic compound contents of the different green asparagus cultivars are presented in Table 3. Five phenolic acids (chlorogenic acid, ferulic acid, caffeic acid, *p*-hydroxybenzoic acid, and coumaric acid) and three flavonoid compounds (RT, quercetin and kaempferide) were detected in green asparagus, which was similar to the results of Liu and Jiang (2005) in the green asparagus cultivar 'Mary Washington 500'. Chlorogenic acid was detected only in cultivars 'Grande' (93.18 mg/kg) and 'UC157' (71.44 mg/kg). Caffeic acid was found only in cultivar 'Altas' (15.29 mg/kg). For ferulic acid, the highest content was found in cultivar 'UC157' (58.17 mg/kg), followed by 'UC301' (37.49 mg/kg), 'Altas' (31.38 mg/kg), and 'UC800' (31.28 mg/kg). It was not detected in cultivar 'Grande'. The highest *p*-hydroxybenzoic acid content was observed in cultivar 'UC301', with a value of 2.56 mg/kg. There were no significant differences among the other four cultivars. The highest coumaric acid content was observed in cultivar 'UC301' (23.29 mg/kg), followed by 'UC800' (19.27 mg/kg), 'Grande' (11.12 mg/kg), and 'Altas' (6.43 mg/kg). Coumaric acid was not detected in cultivar 'UC157'. RT was the main flavonoid compound of green asparagus, as shown in the data in Table 3, which was consistent with previous reports (Makris and Rossiter, 2001; Sun *et al.*, 2007b). The total RT content ranged from 29.26 to 237.86 mg/kg, which was in good agreement with the values reported by Drinkwater

Table 3. Phenolic compound contents (mg/kg) of five green asparagus cultivars.[1]

Cultivar	Chlorogenic acid	Ferulic acid	Caffeic acid	p-Hydroxybenzoic acid	Coumaric acid	Rutin	Quercetin	Kaempferide
Grande	93.18±0.18^{a}	ND	ND	2.09±0.05^{b}	11.12±0.03^{c}	232.09±3.05^{b}	17.18±0.25^{b}	15.24±0.21^{d}
Altas	ND[2]	31.38±0.23^{c}	15.29±0.16^{a}	2.12±0.03^{b}	6.43±0.08^{d}	201.18±4.32^{c}	23.26±0.09^{a}	17.36±0.11^{b}
UC157	71.44±0.33^{b}	58.17±0.09^{a}	ND	2.08±0.02^{b}	ND	237.86±1.07^{a}	15.29±0.36^{c}	17.30±0.22^{b}
UC301	ND	37.49±0.16^{b}	ND	2.56±0.04^{a}	23.29±0.12^{a}	50.09±0.78^{d}	13.47±0.10^{d}	21.48±0.35^{a}
UC800	ND	31.28±0.17^{c}	ND	2.03±0.03^{b}	19.27±0.21^{b}	29.26±1.24^{e}	15.32±0.30^{c}	16.39±0.27^{c}

[1] The values are the means±SD; n=3. Different letters in the same column indicate a significant difference (P<0.05).
[2] ND = not detectable.

et al. (2015) and Wang *et al.* (2003) Cultivar 'UC157' had the highest RT content; cultivar 'UC800', the lowest. A trend of increasing RT content was observed for cultivars 'UC301', 'Altas', and 'Grande'. The highest quercetin content was observed in cultivar 'Altas' (23.26 mg/kg), followed by 'Grande' (17.18 mg/kg), 'UC800' (15.32 mg/kg), 'UC157' (15.29 mg/kg), and 'UC301' (13.47 mg/kg). The highest kaempferide content was observed in cultivar 'UC301' (21.48 mg/kg), the lowest in cultivar 'Grande' (15.24 mg/kg). These results indicated that the cultivar influences the composition (Espin *et al.*, 2016; Jorjong *et al.*, 2015) and content (Flores *et al.*, 2015; Kim *et al.*, 2015; Liu *et al.*, 2015; Wang *et al.*, 2015) of phenolic compounds.

Antioxidant activity and correlation with phenolic content

The role of antioxidants in maintaining health and preventing disorders and disease has gained much attention. Free radical scavenging is one of the important functions of antioxidants because free radicals induce the oxidation of lipids, proteins, and DNA, which results in disturbances to and the loss of function of biological membranes and enzymes, as well as the production of toxic compounds (Kou *et al.*, 2015). Thus, the role of free radical-scavenging antioxidants has attracted much attention from scientists and the general public. The total antioxidant activity in green asparagus was evaluated using DPPH and •OH free radical-scavenging capacity assays, and the results are reported in Figure 1. The findings of the DPPH and •OH assays were similar: cultivars 'Grande' and 'UC157' showed the highest DPPH and •OH free radical-scavenging capacity, indicating that these cultivars had the highest antioxidant activities. As shown above, these two cultivars also have the highest TP contents among the studied cultivars (Table 3). In addition, a decreasing trend in the DPPH and •OH free radical-scavenging capacity was observed among the other three cultivars ('Altas'>'UC301'>'UC800'), which could be related to differences in their phenolic composition and content (Espin *et al.*, 2016). Park *et al.* (2014) reported that kiwi cultivars with higher phenolic contents exhibited higher antioxidant activity. Thus, the correlations between antioxidant activity (DPPH and •OH) and Vc, TP, TF, total phenolic acid (TPA), RT, Zn, and Se contents were analysed using the Pearson's correlation coefficient (r) (Table 4). Strong correlations were observed between TP and TF (r=0.963), TP and RT (r=0.965), and TF and RT (r=0.999). The antioxidant activity measured by the DPPH assay was positively and strongly correlated with TP, TF, TPA, and RT, with correlation coefficients of r=0.969, 0.981, 0.884, and 0.977, respectively. The antioxidant activity measured by the •OH assay was positively and strongly correlated with TP, TF, and RT, with correlation coefficients of r=0.985, 0.983, and 0.982, respectively. No relationship was found between the antioxidant elements (Zn, Se) and antioxidant activity. These results suggest that phenolic compounds, particularly RT, are major contributors to the antioxidant activity of green asparagus, which is consistent with the result of Tsushida *et al.* (1994). Sun *et al.* (2007c) detected a strong correlation between antioxidant activity and flavonoid content in asparagus, with no relationship found between antioxidant activity and TP content. Drinkwater *et al.* (2015) demonstrated that RT, TP and antioxidant activity were all highly correlated. Rodríguez *et al.* (2005) observed a significant correlation between antioxidant activity and TP content, with r=0.9178. One possible explanation for these results is that the sample origin and cultivar (Rodríguez *et al.*, 2005), processing (Drinkwater *et al.,* 2015), and the

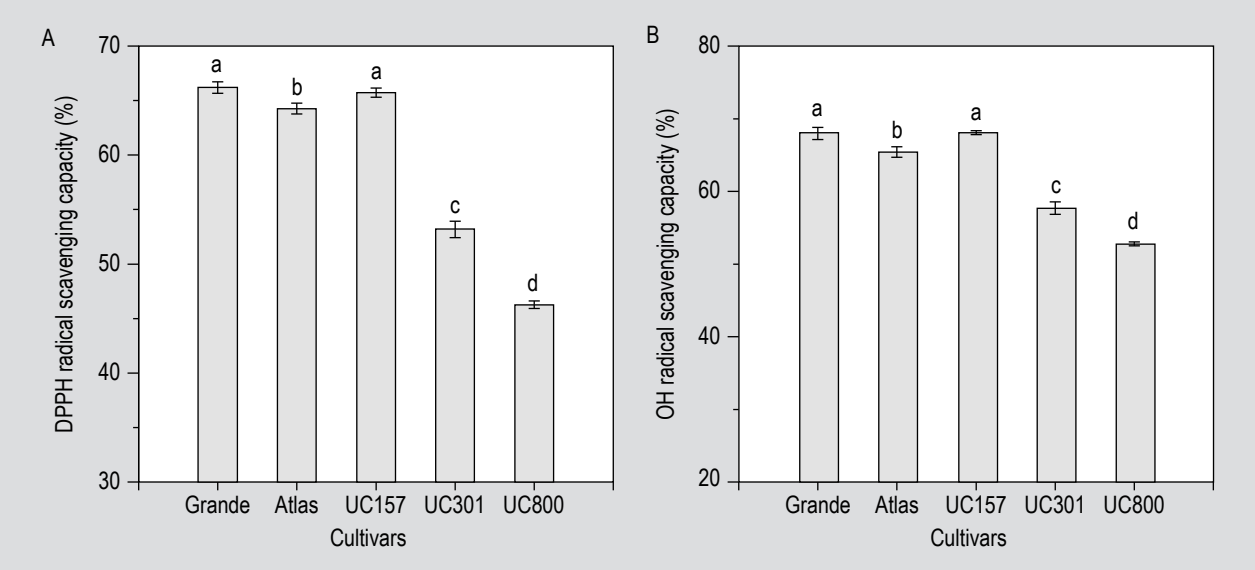

Figure 1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (A) and hydroxyl (•OH) (B) radical-scavenging capability of five green asparagus cultivars.

Table 4. Correlation coefficients between phytochemicals and antioxidant activity.[1,2]

	Vc	TP	TF	TPA	RT	Zn	Se	DPPH	•OH
Vc	1	0.659	0.648	0.890*	0.666	0.835	0.273	0.706	0.713
TP		1	0.963**	0.804	0.965**	0.448	-0.333	0.969**	0.985**
TF			1	0.798	0.999**	0.582	-0.409	0.981**	0.983**
TPA				1	0.796	0.788	-0.172	0.884*	0.869
RT					1	0.585	-0.367	0.977**	0.982**
Zn						1	0.044	0.610	0.579
Se							1	-0.415	-0.367
DPPH								1	0.997**
•OH									1

[1] * values are significantly different (P<0.05); **Values are significantly different (P<0.01).
[2] DPPH = 2,2-diphenyl-1-picrylhydrazyl; •OH = hydroxyl; RT = rutin; Se = selenium TF = total flavonoids; TP = total phenolics; TPA = total phenolic acids; Vc = vitamin C; Zn = zinc.

properties of the extracting solvents (Sun *et al.,* 2007c) significantly affected the antioxidant activity.

Principal component analysis

A PCA was performed to explain the differences between the analysed green asparagus cultivars by combining the Me, AA, Lg, Vc, TP, TPA, TF, and RT data with the antioxidant parameters (DPPH and •OH free radical-scavenging capacity). The PCA produced four components that accounted for 88.1, 9.3, 2.2, and 0.4% of the variance. The first two principal components explained 97.4% of the total variability. The most important variables integrated in the first component were RT, TF, and TP and the second were Me, Vc, TPA, and Lg. The points in the plot are the data observations, which are similar when they are near each other and are dissimilar when further apart (Flores *et al.*, 2015). The first component was positively correlated with the Lg, AA, and Me contents and negatively and strongly correlated with the RT, TF, and TP contents. The second component was positively and strongly correlated with the Me, Vc, and TPA contents and negatively and strongly correlated with the Lg content. As shown in Figure 2, Vc, phenolic compounds and antioxidant activity are grouped together on the left side of the PCA plot. It is evident that cultivar 'UC157' was on the fourth axis as a result of its high TP, TF, and RT contents and DPPH and •OH free radical-scavenging capacity, which are important parameters for selecting cultivars with more significant health-promoting characteristics (Šamec *et al.*, 2016). Cultivar 'Grande' was on the third axis, with higher TPA, Vc, and Me contents and DPPH and •OH free radical-scavenging capacity. Cultivar 'Altas' was also on the third axis. The remaining two cultivars, 'UC301' and 'UC800', were clearly separated.

4. Conclusions

The present study demonstrated that the cultivar had a marked influence on bioactive constituents, particularly the phenolic compound content and composition, and on the antioxidant activity. The correlation and principal component analyses revealed differences between the green asparagus cultivars. Cultivars 'UC157' and 'Grande' were characterised as having high TP and RT contents and, consequently, high antioxidant activity. The results of this study provide a good foundation for selecting parents for breeding programs and cultivars with health benefits.

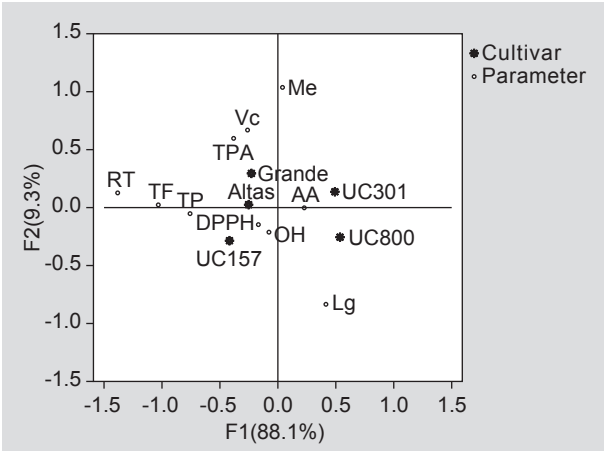

Figure 2. Principal component analysis plot based on data obtained from analyses of the lignin (Lg), amino acid (AA), microelement (Me), vitamin C (Vc), total phenolic (TP), total phenolic acid (TPA), total flavonoid (TF) and rutin (RT) contents and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (•OH) free radical-scavenging capacity of five green asparagus cultivars.

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