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ORIGINAL PAPER

Bioactive Compounds, Antioxidant and Binding Activities and Spear Yield of *Asparagus officinalis* L.

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Abstract The aim of this investigation was to find a proper harvesting period and establishing fern number, which effects the spear yield, bioactive compounds and antioxidant activities of *Asparagus officinalis* L. Spears were harvested at 2, 4, and 6 weeks after sprouting. Control for comparison was used without harvest. Spears and total yield increased with prolonged spear harvest period. In harvest of 6 weeks long optimum spear yield was the highest and fern numbers were 5~8. Bioactive compounds (polyphenols, flavonoids, flavanols, tannins and ascorbic acid) and the levels of antioxidant activities by ferric-reducing/antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays in asparagus ethanol extracts significantly differed in the investigated samples and were the highest at 6 weeks harvest period ($P<0.05$). The first and the second segments from the tip significantly increased with the increase of catalase (CAT). It was interesting to investigate *in vitro* how human serum albumin (HSA) interacts with polyphenols extracted from investigated vegetables. Therefore the functional properties of asparagus were studied by the interaction of polyphenol ethanol extracts with HSA, using 3D- FL. In conclusion, antioxidant status (bioactive compounds, binding and antioxidant activities) improved with the harvesting period and the first segment from spear tip. Appropriate harvesting is effective for higher asparagus yield and its bioactivity.

Keywords Asparagus production · Bioactive compounds · Antioxidant · Binding activities

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Introduction

The consumption of asparagus has increased as a rich source of bioactive compounds, decreasing the cholesterol in the human body and inhibiting the cancer [1–7]. It is known that caffeic and ferulic acids are relatively strong antioxidants. These acids were found in asparagus [2, 8, 9]. Antioxidants in asparagus protect human body against cancer, cardio- and cerebrovascular diseases [7]. Increasing spear yield has been used widely to prolong harvest period, especially in Korea [10]. In Asian and other countries prolonged harvest period from March to October increased asparagus yield production using modified mother-fern culture method [6, 11]. The proper harvesting duration and establishment of fern number are important strategies to produce optimum number of spear. In order to continue year-round asparagus production system it was important to elucidate asparagus yield associated with extending spear harvest period and establishing fern number. The length of harvested spear was about 20~25 cm from the spear tip. Asparagus spears are rich in bioactive compounds and minerals located in their edible parts [12, 13]. The degradation rate of peroxidase was gradually increased from bottom part of spear to spear tip of asparagus [14, 15]. Therefore, the

determination of antioxidant status (bioactive compounds and antioxidant activities) related with spear harvest period and different portion of the spear is important. The main objective of this work was to find a proper harvesting duration and to establish fern number on spear yield. Investigation of antioxidant status of spear was related with spear harvest treatment and different portions of the spears from the asparagus tip. Lotus samples were used for comparison of antioxidant status of asparagus.

Materials and Methods

Plant Material and Treatment

Two-year-old plants of asparagus (*Asparagus officinalis* L. cv Super Welcome) were cultivated at commercial asparagus planting at Hwasun province (Latitude 35.4, Longitude 126.5), Korea. Asparagus plants were planted to the bottom of 30 cm deep furrow. Two dripping hoses were set at the middle point, and the plants were irrigated as required. Soil water content was measured using Field Scout™ TDR 300 (Spectrum Technologies, USA). Average day and night temperatures were recorded during the experiment. The plants were grown in a glasshouse with a mean daily temperature of 22˚C. The establishing fern number treatments were 2, 5, 8, and 11 numbers per plant, respectively. Spears were harvested at 2, 4, 6 weeks after sprouting and then 2, 5, 8 and 11 ferns per plant were allowed to develop into mature plants. Measurements of spear growth characteristics, including spear number, diameter, head tightness and marketable quality were investigated by 2~3 days intervals. After trimming to 25 cm length spears were counted, weighted fresh weight and measured stem diameter 1 cm point from the base.

Lotus plant (*Nelumbo nucifera* Gaertn.) was purchased at the local market, Gwangju city, Republic of Korea, for comparison studies of bioactive compounds. The prepared vegetables were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight (DW) was determined. The samples were ground to pass through a 0.5 mm sieve and stored at −20 ° C until the bioactive substances were analyzed.

Assay of Antioxidant Enzyme

Spears of 20 cm long were divided into four segments: segment 1, segment 2, segment 3, and segment 4 proportionally indicated from spear tip to base by interval of 5 cm long. The superoxide dismutase (SOD) activity is based on the colorimetric assay for the measurement of total antioxidant capacity of crude aqueous fractions. The plate was incubated at 37 °C for 20 min, and the optical density was measured spectroscopically at 450 nm. For CAT activity the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 11 mM H_2O_2, and the crude enzyme extract [16]. The reaction was initiated by adding H_2O_2 to the mixture. The enzyme activity was determined by monitoring the decline in absorbance at 240 nm (ε=36 M^{-1} cm^{-1}), because of H_2O_2 consumption.

Ascorbate peroxidase (APX) activity was determined by monitoring the decline of absorbance at 290 nm as ascorbate (ε=2.8 mM^{-1} cm^{-1}). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 0.2 mM H_2O_2 [17].

Peroxidase (POX) activity was determined with guaiacol at 470 nm (ε=26.6 mM^{-1} cm^{-1}). The reaction mixture contained 40 mM potassium phosphate buffer (pH 6.9), 1.5 mM guaiacol, and 6.5 mM H_2O_2 in 1 mL with crude enzyme extract [18].

Assays of Bioactive Compounds

The contents of polyphenols, tannins, flavonoids, flavanols, and ascorbic acid in extracts of the studied vegetables were determined as previously described [19].

The lyophilized samples of asparagus (1 g) and lotus (1 g) were extracted with 100 mL of ethanol at room temperature in darkness for 24 h. The polyphenols were determined by Folin-Ciocalteu method with measurements at 750 nm with spectrophotometer (Hewlett-Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g dry weight (DW) [20]. The vegetable extracts of condensed tannins (procyanidins), extracted with 4 % methanol vanillin solution were measured spectroscopically at 500 nm. Flavonoids, extracted with 5 % $NaNO_2$, 10 % $AlCl_3 \cdot H_2O$, and 1 M NaOH, were measured at 510 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was measured [21, 22]. (+)-Catechin served as a standard for tannins, flavonoids and flavanols, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay in water extract (100 mg of lyophilized sample and 5 mL of water). The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm [23].

The AAs were Determined by Two Assays

1) In ferric-reducing/antioxidant power (FRAP) assay: 900 μL of reagent (2.5 mL of a 10 mmol ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol $FeCl_3 \cdot H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6) was mixed with 90 μL of distilled water

Table 1 Effect of harvest duration on spear quality characteristics, head tightness and marketable yield of asparagus

Harvest duration (Weeks)	Spear quality characteristics				Yield		
	Total spear (no.)	Mean weight (g)	Mean diameter (mm)	Head open (no.)	Total yield (g)	Marketable yield (g)	Reject yield (g)
0	9.6±3.2d	14.7±2.9b	6.83±1.31b	2.4±1.0c	222.4±72.8d	185.9±61.8d	36.4±14.5c
2	28.5±2.6c	23.6±0.8a	10.83±0.28a	5.1±0.9c	669.6±62.3c	592.9±54.8c	76.6±14.8c
4	55.1±3.5b	22.5±0.7a	10.65±0.19a	13.8±1.0b	1,238.2±81.8b	1,029.4±76.4b	208.7±16.5b
6	70.8±3.0a	22.9±0.7a	10.75±0.22a	18.1±1.4a	1,603.3±56.7a	1,335.7±54.3a	267.5±28.7a

Means sharing the same letter in a column are not significantly different by Duncan's multiple range test at $P \leq 0.05$

and 30 μL of investigated samples as the appropriate reagent blank. The absorbance was measured at 595 nm [24].

2) Cupric-reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper (II)–neocuproine [Cu (II)–Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank [25].

Fluorometric Measurements

Fluorometric measurements were used for the evaluation of *in vitro* binding properties of asparagus and lotus extracts to human serum albumin (HSA). Two dimensional (2D –FL) and three dimensional (3D-FL) fluorescence measurements for all extracts at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan, equipped with 1.0 cm quartz cells and a thermostat bath. The 2D- FL measurements were taken at emission wavelengths from 310 to 500 nm; and at excitation of 295 nm. The 3D-FL spectra were collected with subsequent scanning emission spectra from 250 to 500 nm at 1.0 nm increments by varying the excitation wavelengths from 200 to 350 nm at 10 nm increments. All solutions for protein interactions were prepared in 0.05 mol/L Tris–HCl buffer (pH 7.4), containing 0.1 mol/L NaCl. The final concentration of HSA was 2.0×10^{-6} mol/L. The HSA was mixed with catechin in the proportions of HSA: extract=1:1. Catechin was used as a standard [19].

Statistical Analysis

The experiments were conducted as randomized complete block design with two factors, harvesting period (no harvest, 2, 4, and 6 weeks) and established fern number (2, 5, 8, and 11 numbers per plant). Data were collected and analyzed using the procedure GLM of SAS package version 8.2 (SAS Institute, Cary, NC, USA). Duncan's multiple range test (DMRT) was used for mean separation at $P \leq 0.05$.

Results and Discussion

Total spear number, mean weight, mean diameter and total yield increased with prolonged spear harvest period (Table 1). The total yield of spears over the entire experiment period was higher for the 6 weeks harvest period treatment than in other harvest periods (Table 1). The experiment showed clearly that asparagus production was an increasing trend as duration of harvesting prolonged [26–28]. Total spear yield during several

Table 2 Effect of remaining fern number on spear quality characteristics, head tightness and marketable yield of asparagus

Fern (no.)	Spear quality characteristics				Yield		
	Total spear (no.)	Mean weight (g)	Mean diameter (mm)	Head open (no.)	Total yield (g)	Marketable yield (g)	Reject yield (g)
2	47.1±4.6a	24.1±0.9a	11.03±0.26a	10.7±1.4a	1,113.7±105.2a	960.5±92.5a	153.2±19.9a
5	40.1±6.3a	21.2±1.9ab	9.68±0.80ab	8.9±1.6a	924.6±142.0b	802.0±123.4b	122.5±23.1a
8	39.0±7.1a	21.1±1.8ab	9.90±0.79ab	9.5±1.9a	892.1±156.9b	744.0±130.0bc	148.1±32.1a
11	37.8±6.5a	17.3±2.0b	8.27±0.96b	10.3±2.1a	803.1±138.9b	637.5±109.4c	165.4±37.2a

Means sharing the same letter in a column are not significantly different by Duncan's multiple range test at $P \leq 0.05$

Table 3 Bioactive compounds and antioxidant activities in asparagus ethanol extracts with two spear harvest periods in comparison with lotus

Item	6 weeks	4 weeks	Lotus
Pol, mg GAE,	6.54±0.58a	5.56±0.54b	6.11±0.52a
FRAP, μM TE,	13.33±1.22a	11.33±1.16b	12.65±1.23ab
CUPRAC, μM TE	27.58±2.65a	23.44±2.32bc	18.93±1.88c
Flavan, μg CE	15.80±1.48a	13.43±1.23b	49.79±4.52c
Flavon, mg CE	1.73±0.18a	1.47±0.18b	0.790±0.12c
Tannin, mg CE	0.20±0.01a	0.17±0.01a	0.39±0.01b
VitC, mg Asc	11.09±1.14a	9.42±0.87b	5.23±0.56c

Pol, polyphenols; *GAE*, gallic acid equivalent; *FRAP*, ferric-reducing/antioxidant power; *TE*, trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; *CUPRAC*, cupric reducing antioxidant capacity; Flavan, flavanols; Flavon, flavonoids; *CE*, catechin equivalent; *VitC*, vitamin C; Mean±standard deviation. Averages in rows marked with different letters differ significantly ($P<0.05$)

seasons was the highest at 8 weeks, but if the harvest was either 1 week longer, or any shorter than 8 weeks, spear yield decreased [27]. Increasing duration of harvesting increased with significantly higher marketable yield (Table 1). The highest proportion of marketable yield was obtained when spear harvest period was 6 weeks. Remaining fern number treatments had no effect on spear quality characteristics, whereas significantly influenced on total and marketable yields (Table 2). Two remaining fern numbers improved mean weight, mean diameter and total yield compared to other treatments (Table 2). When fern was established, adjacent buds were emerged successive sequence and spears were harvested earlier than in other treatments. This implied that decreasing of fern number improved spear quality. The higher total yield from 6 weeks duration of harvesting resulted in a proportionally higher marketable yield in comparison with other treatments. Percentage of reject spears was relatively higher in spear harvest period at 4 and 6 weeks than other treatments (Table 1). These results confirm that higher yield of asparagus was at 6 weeks harvest period and 5~8 fern established in Korean climatic condition under plastic greenhouse (Tables 1–2).

Results of Bioactive Compounds

The results of the determination of the contents of the bioactive compounds in all studied samples are summarized in Table 3. The significant highest contents ($P<0.05$) of polyphenols, flavonoids, flavanols, tannins, and ascorbic acid, were at 6 weeks after spear harvest period in asparagus ethanol extracts. The AAs values by FRAP and CUPRAC assays (μMTE/g) at 6 weeks spear harvest for asparagus ethanol extracts were the highest in comparison with other samples (Table 2). As it was calculated, a very good correlation was found between the antioxidant activities and the contents of total polyphenols (R^2 from 0.96 to 0.83). The determined indices of asparagus after 4 weeks of harvest were lower than after 6 weeks. Our obtained results were not in a full agreement with others [29], where the antioxidant activity was higher (65.77 μmol TE/g DW). Such difference can be explained by the experimental conditions such as the material used was fresh green asparagus and the antioxidant activity was determined in asparagus buffer extract. The investigated polyphenol extracts of asparagus were slightly lower than in our samples, but the flavonoids were higher. The antioxidant activity of asparagus by CUPRAC assay was similar to the data of Sun et al. [9], where the samples were determined by ABTS method. Our results were in full agreement with others, reported the nutritional and *in vitro* antioxidant properties of asparagus [30]. Artichoke, spinach, broccoli, and asparagus also showed considerable antioxidant activity [31]. The comparison of asparagus with white lotus showed similar results (Table 3). The contents of phenolic compounds ranged from 1.2 mg GAE/g (carrot) to 16.9 mg GAE/g (lettuce). The results obtained for asparagus are within this range. We observed the antioxidant activity in asparagus and our results pointed out its importance in the diet. Ascorbic acid was more than fivefold higher (vs. 23 mg/100 g). Phenolic compounds were more abundant in *A. acutifolius* spears (41.97 vs. 27.62 mg/100 g) as well as the radical scavenging activity against DPPH radical and nitric oxide. *A. acutifolius* appears nutritionally interesting for its high content in phenols and for its antioxidant status in comparison with other vegetables.

Table 4 Antioxidant activities of asparagus samples with different spear segment from spear tip to spear base

Segments	SOD % Inhibition, mg protein^{-1}	CAT H_2O_2decomposed, μmol min^{-1} mgprotein^{-1}	APX Ascorbate oxidized, μmol min^{-1} mgprotein^{-1}	POX Tetraguaiacol formed, μmol min^{-1} mgprotein^{-1}
Segment 1	83.2±0.45a	11.0±1.15ab	535.2±81.01a	1.87±0.09c
Segment 2	71.0±3.08c	12.3±0.98a	491.3±34.42a	2.38±0.01ab
Segment 3	82.1±3.69ab	8.1±0.72b	585.7±65.50a	2.21±0.16b
Segment 4	75.4±1.18bc	2.39±0.53c	552.4±24.57a	2.46±0.05a

SOD, superoxide dismutase; *CAT*, catalase; *APX*, ascorbate peroxidase; *POX*, peroxidase. Means sharing the same letter in a column are not significantly different by Duncan's multiple range test at $P\leq0.05$

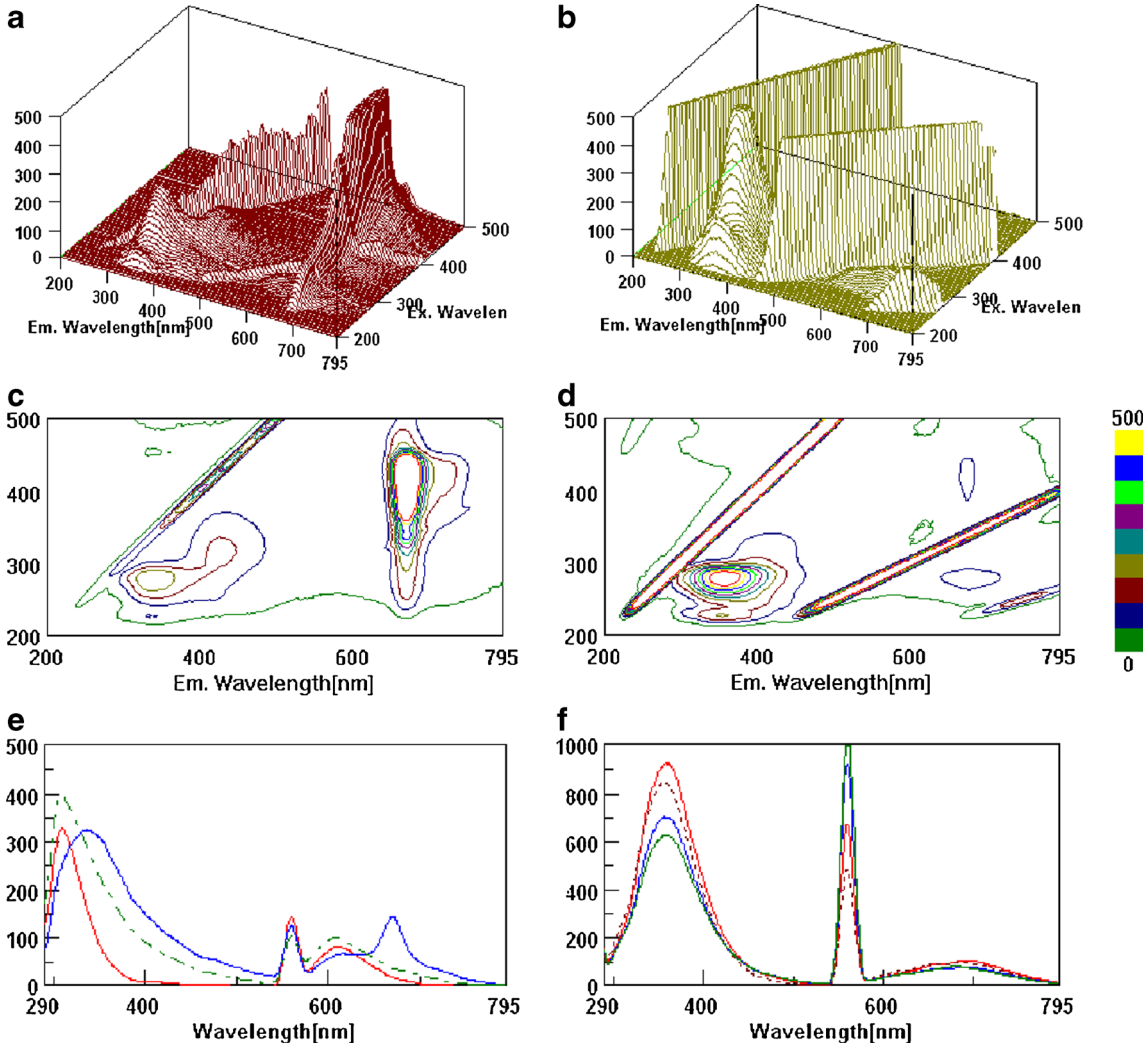

Fig. 1 Three dimensional fluorescence spectra (3D-FL) and cross section view of **a** and **c**, ethanol extracts of asparagus after 6 weeks harvest period; **b** and **d**, asparagus ethanol extract and interaction with human serum albumin (HSA); **e**, fluorescence intensity (FI) of lotus ethanol extracts (first line from the top with FI of 396.65); catechin (second line from the top with FI=329.31), asparagus (third line, FI=325.51). **f**, FI after interaction with HSA (first line from the top with FI of 925.73), HSA+catechin (second line from the top with FI of 846.55), HSA+lotus ethanol extract (third line from the top with FI of 702.86), HSA+asparagus ethanol extract (fourth line from the top with FI of 627.57)

Harvesting increase in the quality and yields and our results were similar [32]. The first segment from the spear tip increased SOD and CAT enzyme activities (Table 4). In contrast, the first segment from the spear tip was lower in POX enzyme activity than other segments. Overall, the first segment from the spear tip contained the highest amount of antioxidant enzyme compounds.

In fluorescence studies asparagus samples after 6 weeks harvest with the highest polyphenol contents and antioxidant activities were used. The 2D-FL of lotus and asparagus ethanol extracts slightly differs by the wavelengths of the main peaks and their fluorescence intensities (FIs). All main fluorescence peaks were located between λ em from 309 and 670 nm. According to the values of FIs in the main peaks the ethanolic extracts showed an increase from lotus, catechin and asparagus (Fig. 1a, c, and e). The binding properties of the lotus and asparagus samples in comparison with the pure flavonoid catechin are shown in 3D-FL (Fig.1b and d). One of the main peaks for HSA was at λex/em of 220/358 nm (Fig. 1f). The interaction of HSA and the ethanol extracts of lotus and asparagus (Fig. 1b, d, f) showed slight change in the position of the main peak at the wavelength of 358 nm and the decrease in the FI. The following changes appeared when asparagus ethanol extracts were added to HSA: initially the main peak at λ em of 358 nm and FI of 925.73 (Fig. 1f, the upper line is HSA) and after interaction (Fig. 1f, the lowest line with FI of 627.57). The following decrease in FIs (%) occurred during the interaction of ethanol extracts with HSA: HSA+catechin=8.55 %; HSA+lotus=24.08 %; HSA+asparagus=32.21 %. These results are in direct relationship with the antioxidant properties of the extracts. The synergism of bioactive compounds is shown when to the mixture of HSA and

extracts of kiwi fruit catechin was added. The fluorescence is significantly quenched, because of the conformation of proteins, phenolic acids and flavonoids, as it was described in our very recent results [22]. In conclusion, bioactive compounds, antioxidant and binding activities were improved during the harvesting period. Appropriate harvesting time is effective for higher yield and bioactivity of asparagus.

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Conflict of Interest We don't have any conflict of interest.

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