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Comparison of Growth Patterns and Metabolite Composition of Different Ginseng Cultivars (Yunpoong and K-1) Grown in a Vertical Farm

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Abstract: This study analyzed growth patterns, biological compounds, antioxidant properties, ginsenoside contents, metabolites, and the annual net production of 'Yunpoong' and 'K-1' to find the optimal harvesting time of ginseng sprouts. One-year-old ginseng seedlings were cultivated in a container-type vertical farm under a temperature of 20 °C, a humidity of 60%, and average light intensity of 46.4 µmol m⁻² s⁻¹ (16 h photoperiod). Growth patterns at 2, 3, 4, and 5 weeks after transplanting (WAT) differed between cultivars. Regarding biological compounds and antioxidant properties, 'Yunpoong' took 5 WAT (43.59%; 2,2-diphenyl-1-picryl-hydrazine-hydrate radical scavenging activity, 1.47 OD_{593nm}; ferric reducing antioxidant power assay, 78.01%; 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity), and 'K-1' took 4 WAT (0.98 Re mg g⁻¹; total flavonoid contents, 35.93%; DPPH) to show a high content. Two cultivars showed the highest total ginsenoside contents at 5 WAT. Most of the analyzed metabolites had a higher content in 'Yunpoong' than in 'K-1'. In both cultivars, it was confirmed that the longer the growth period (3 – > 5 WAT), the lower the yield and the annual ginsenoside net production. Therefore, 'Yunpoong' and 'K-1' cultivars should be grown as ginseng sprouts in the vertical farms for approximately 3 WAT and 4 WAT, respectively.

Keywords: antioxidant properties; biological compounds; ginsenoside contents; *Panax ginseng*; plant factory

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer, National Institute of Horticultural and Herbal Science, Wanju-gun, Republic of Korea), a perennial plant belonging to the family Araliaceae and genus *Panax*, is an important medicinal crop cultivated in Asian countries, such as the Republic of Korea and China. The area of ginseng grown in the Republic of Korea was 14,729 hectares, with a production of 20,772 tons in 2021 [1]. The shape and growth features of ginseng differ depending on the cultivar [2]. The ginseng cultivars have various morphological and physiological properties [3], seed yield, ginsenoside content, and pest resistance [4,5]. 'Yunpoong' is a Jakyung species with a short plant length, multiple purple stems, one red fruit, and stipule-shaped leaves [6]. Their body of the root is cylindrical, with a medium length and large thickness, and has a high yield of fresh ginseng quality. Another Jakyung species, 'K-1', mostly has one dark-purple-colored stem with red fruits and long, triple-tipped oval leaves [7]. The structure of the K-1 root, a developed tap root with 2–3 lateral and numerous fine roots, is cylindrical, suitable for red ginseng production, and has stronger disease resistance.



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Ginsenosides are saponins and constitute the main components in ginseng with various physiological and pharmacological properties, such as anticancer [8], anti-stress [9], neuroprotective [10], and anti-diabetic [11]. The saponin content in ginseng differs based on the harvest time of ginseng leaves during general cultivation [12]. It can vary depending on the cultivar [3], age [13], planting location [14], and growth stage [15]. Ginseng leaves contain small amounts of ginsenoside-F1, ginsenoside-F2, and ginsenoside-F3 that are not found in the roots [15].

The ginseng leaves possess excellent physiological properties [16]. One-year-old ginseng leaves had higher ginsenoside content than roots [17,18]. A vertical farm is a multitier indoor plant production system that precisely controls all growth factors, including light, temperature, humidity, CO₂ concentration, water, and nutrients, to produce high volumes of plants [19]. By controlling the environment, such as light, temperature, and humidity, in vertical farms, it is possible to produce high-value-added crops year-round [20]. In particular, vertical farms are emerging as a new method for cultivating medicinal plants for medicines and functional foods, providing customized environmental conditions suitable for high yield and high-quality plants [21]. For example, ginseng grown in vertical farms has a shorter growth period [22] and higher saponin content than field-grown ginseng [23]. Vertical farms can increase the yield [24,25] of various crops and enable year-round production [19]. Additionally, ginseng sprouts have a short growing period and can be grown in a vertical farm where it is not affected by seasonal variations [26].

Many studies are being conducted on the comparison of ginseng varieties. Ahn et al. [3] examined the variation in ginsenoside content among different parts of ginseng roots based on the ginseng variety. The growth characteristics were compared to the 'Gopoong', 'Chunpoong', and 'Yunpoong' ginseng varieties [6]. However, there has yet to be a study on the changes in growth patterns and ginsenoside content for each ginseng variety. Therefore, this study was conducted to determine the optimal harvest time of the 'Yunpoong' and 'K-1' ginseng varieties grown in a vertical farm. Additionally, we compared the metabolite content and differences between 'Yunpoong' and 'K-1' through metabolite analysis.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

One-year-old ginseng seedlings ('Yunpoong' and 'K-1') were provided to the National Institute of Horticultural and Herbal Science, and approximately 7–10 cm ginseng seedlings were used as experimental material. Ginseng seedlings were immersed in 100 ppm of gibberellin aqueous solution (IAP, JahngRyu Industries Co., Ltd., Cheongju, Republic of Korea) for 30 min and then transplanted into a plastic tray ($51.5 \times 36 \times 8.5$ cm, L × W × H) filled with an artificial ginseng soil mix (Myeongpum-Insamsangto, Shinsung Mineral Co., Ltd., Goesan, Republic of Korea). For each cultivar, 42 ginseng sprouts for 3 repetitions were planted in a plastic container and cultivated in a smart farm cube (Dream PF Corp., Sacheon, Republic of Korea). Overhead sprays were performed with tap water 3–4 times weekly. The growth environment was maintained at 20 ± 3 °C, $60 \pm 10\%$ relative humidity, $46.4 \pm 0.3 \mu$ mol m⁻² s⁻¹ photosynthetic photon flux density (PPFD; Red and Blue LEDs), and 16 h photoperiod for 5 weeks after transplanting (WAT).

2.2. Growth Characteristics

The growth characteristics of ginseng sprouts were measured at 2, 3, 4, and 5 WAT by dividing the shoot and root, such as shoot and root fresh and dry weights—this is the method of Nguyen et al. [27]. The fresh weight of shoots and roots was measured with an electronic scale (PAG214C, Ohaus Corp., Parsippany, NJ, USA). Then, the samples were dried in an oven (WOF-155, Daihan Scientific, Seoul, Republic of Korea) at 70 °C for 3 days, and the dried weight was measured. The top/root ratio was divided as the fresh weight ratio of the top (shoot) to the root. The total yield was expressed by totaling the shoot and root fresh weights.

2.3. Analysis

2.3.1. Biological Compounds and Antioxidant Properties

Ginseng sprouts from 42 plants for 3 repetitions were harvested at 3, 4, and 5 WAT and prepared as follows. Dry powder samples (1 g) were extracted in 20 mL of 50% ethanol using a flask shaker (SH-800, SH Scientific Co., Ltd., Sejong, Republic of Korea) at about 25 °C for 12 h. The extract was centrifuged for 5 min, then the supernatant of 5 mL was filtered through a 0.45 µm membrane filter, and the supernatant was used for analysis [28].

For total phenolic contents, a slightly modified Folin–Denis method [29] was used. The ginseng extract (0.5 mL) was added to a 2 mL microcentrifuge tube, and 0.5 mL of 25% Na₂CO₃ solution was added. The mixture was allowed to stand for 3 min, whereafter 0.25 mL of 2N Folin–Ciocalteu reagent (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) was added. The mixture was left for 1 h at 30 °C for color development to occur and then centrifuged for 1 min. The absorbance of the sample was measured at 750 nm using a spectrophotometer (UV-1800 240 V, Shimadzu Corp., Kyoto, Japan). A standard calibration curve to estimate the total phenol content was drawn using gallic acid. Results are expressed in milligrams of gallic acid equivalents per gram on a dry weight basis (mg GAE g⁻¹ DW).

For total flavonoid contents, the method of Kim et al. [29] was used. Briefly, 0.5 mL of the ginseng extract was placed in a 2 mL microcentrifuge tube containing 1.0 mL of diethylene glycol (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA), followed by the addition of 0.01 mL of 1N NaOH. The mixture was placed in a constant temperature water bath at 37 °C for 1 h. Absorbance was measured at 420 nm using the spectrophotometer. A standard calibration curve was obtained to calculate the total flavonoid content. The contents were expressed as the average of three experiments.

For DPPH (2,2-diphenyl-1-picryl-hydrazine-hydrate; Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) radical scavenging activity, the method of Cho et al. [30] was used. DPPH solution (0.8 mL; 1.5×10^{-4} M) and the ginseng extract (0.2 mL) were dispensed into a 2 mL microcentrifuge tube, incubated in the dark for 30 min, and the absorbance value was measured at 525 nm using the spectrophotometer. The extraction solvent was used as the negative control. The radical scavenging activity was determined as follows:

Radical scavenging activity (%) = [1 - (absorbance value of experimental group/absorbance value of negative control group)] × 100 (1)

For FRAP (ferric reducing antioxidant power assay) determination, the method of Lee et al. [31] was used. The FRAP solution was prepared by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of TPTZ in 40 mM of HCl, and 20 mM of FeCl₃ in a ratio of 10:1:1. The prepared solution was preliminarily incubated at 37 °C for 15 min. Thereafter, 0.05 mL of diluted ginseng extract samples and 0.95 mL of the FRAP solution were mixed and incubated at 37 °C for 15 min. The reaction sample was measured at 593 nm using the spectrophotometer.

For ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); Sigma-Aldrich Co., Ltd., St. Louis, MO, USA] radical scavenging activity, the method of Cho et al. [30] was used. The ABTS (7 mM) solution and potassium persulphate (2.45 mM) were mixed in a 1:1 ratio and incubated in a dark room for 12–16 h to form ABTS cations. This ABTS+ solution was diluted with methanol to an absorbance value of 0.7 ± 0.02 at 732 nm to prepare the ABTS⁺ solution used in the experiment. After adding 0.9 mL of ATBS⁺ solution and 0.1 mL of ginseng extract to a 2 mL microcentrifuge tube and incubating for 3 min, the absorbance value was measured at 732 nm using the spectrophotometer. The extraction solvent was used as the negative control. The radical scavenging activity was determined by the same formula as for DPPH calculation.

2.3.2. Ginsenoside Analysis

For the analysis of pre-treated ginsenosides, ginseng sprouts from 42 plants for 3 repetitions were harvested at 3, 4, and 5 WAT and prepared as follows. The preprocessing involved drying the sprouts at 70 °C for 72 h, grinding them with a mixer, and storing them at 4 °C. The dried powder samples (5 g) were extracted with 70% HPLC (high-performance liquid chromatography) methanol (20 mL) in a constant temperature bath (70 °C) for 1 h. After centrifugation at 3000 rpm for 10 min using a centrifuge (1730R, GYROZEN Co., Ltd., Gimpo, Republic of Korea), the supernatant was filtered through a 0.45 μ m membrane filter. This process was repeated twice, and a total of 40 mL of ginseng sprout extract was obtained. The extraction samples were concentrated under reduced pressure at 60 °C, and the resulting extract was dissolved in 2 mL of HPLC water, filtered through a 0.45 μ m membrane filter, and used for HPLC analysis [32].

The quantification of ginsenosides in ginseng extract samples was carried out using HPLC analysis (HPLC Agilent 1260 series Co., Forest Hill, VIC, Australia) with a diode array detector, as described by Lee et al. [33] with a slight modification. Ginsenoside standards (Rg1, Re, Ro, Rf, F5, F3, Rg2, Rh1, Rb1, Rc, F1, Rb2, Rb3, Rd, Rd2, F2, Rg3, PPT, compound K, Rh2, and PPD) were purchased from KOC Biotech (Daejeon, Republic of Korea). HPLC-grade organic solvents used for analysis were purchased from J.T. Baker (Philipsburg, NJ, USA). Other reagents used were purchased at special grade or HPLC grades. The sample (20 μ L) and the 50% ethanol were injected onto an analytical reversed-phase C18 column (TSK-ODS100Z, 4.6 × 250 nm, 5 μ m; Tosoh Corp., Tokyo, Japan). The mobile phase was water (elution A) and acetonitrile (elution B), and the following gradient program was used: 0–10 min, 19% B; 15 min, 20% B; 30 min, 23% B; 42 min, 30% B; 75 min, 35% B; 80 min, 60% B; and 100 min, 90% B. The column was reconditioned with 100% B for 3 min. Other HPLC conditions were as follows: detection, 203 nm; flow rate, 1.0 mL/min; column temperature, 25 °C.

2.4. Analysis of Metabolites

2.4.1. Quantification Methods and Gas Chromatography–Mass Spectrometry (GC/MS) Conditions for Primary Metabolite

Extracts of sprouted ginseng ('Yunpoong' and 'K-1') were analyzed using GC/MS (Figure S1 and Table S1). The dry sample (25 mg) was homogenized with 80% methanol (800 uL) using a blender to extract metabolites. After centrifuging the extract for 10 min, 10 uL of the supernatant was dried using a speed vac (Labconco Co., Kansas, MO, USA). The dried residues were dissolved in 70 μ L of hydroxymethoxy amine and incubated for 90 min at 37 °C. After, samples were then derivatized by adding 70 μ L of N,O-bis (trimethylsilyl) trifluoroacetamide for 30 min at 70 °C. Then, the reaction product was centrifuged for 10 min, and the supernatant of 1 mL was used in GC/MS analysis. The derivatized metabolites were analyzed using a GC-2010 Plus (Shimadzu Corp., Kyoto, Japan) equipped with a DB-5MS column (30 m \times 0.25 mm, 0.25 μ m film thickness; Agilent Technologies, Santa Clara, CA, USA). The injector temperature was set at 200 °C, and helium was used as the carrier gas at a flow rate of 1 mL per min. The oven temperature program was set as holding at 70 °C for 2 min, increasing from 210 °C at 10 °C min, and holding at 320 °C for 7 min. The effluent was detected using a GCMS-TQ 8030 MS (Shimadzu Corp., Kyoto, Japan) system during 0.03 s with electron ionization at 15 eV. The ion source and interface temperatures were 200 and 280 °C, respectively. The primary metabolite analysis was performed for 5 repetitions.

2.4.2. Quantification Methods and Ultra-Performance Liquid Chromatography/Time-of-Flight Mass Spectrometry (UPLC/Q-TOF-MS) Conditions for Secondary Metabolites

Extracts of sprouted ginseng ('Yunpoong' and 'K-1') were analyzed using UPLC/Q-TOF-MS (Figure S2 and Table S2). The dry sample (50 mg) was homogenized with 80% methanol (800 uL) using a blender to extract the metabolites. Following centrifugation, the supernatants were analyzed using UPLC-Q-TOF MS (Waters Corp., Milford, MA, USA). The metabolites were separated using an Acquity BEH C18 column (2.1 mm \times 100 mm, 1.7 µm; Waters, Milford, MA, USA) equilibrated with water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The eluted metabolites were detected using Q-TOF MS with negative electrospray ionization mode. The desolation temperature

and flow rates were 400 °C and 900 L h⁻¹, respectively, and the source temperature was 100 °C. The sampling cone and capillary voltages were 20 V and 2.5 kV, respectively. Leucine-enkephalin (554.2615 Da) was used as the lock mass with an infusion flow rate of 0.35 mL per min. MS data were obtained with a scan range of 50 to 1500 m z^{-1} . The secondary metabolite analysis was performed for 5 repetitions.

2.4.3. Metabolite Data Processing

The spectra of substance peaks on GC/MS chromatograms were identified using the NIST 11 and Wiley 9 mass spectral libraries and retention indices, and calculated using n-alkanes. The skeptical peaks on UPLC/Q-TOF-MS chromatograms were evaluated using the Chemspider, Metlin (metlin.scripps.edu; accessed on 8 August 2022), human metabolome (www.hmdb.ca; accessed on 8 August 2022), and EZmass databases and authentic standards. The peaks were identified using the UNIFI software (version 1.9.2, Waters, Milford, MA, USA) connected to various online databases, version 3.9.

2.5. Statistical Analysis

The growth characteristics, biological compounds, antioxidant properties, and ginsenoside content were measured. Ginseng sprouts of 42 plants for 3 repetitions were harvested according to the WAT. Data were analyzed using the SAS 9.4 program (SAS Institute Inc., Cary, NC, USA) with variance analysis. Duncan's multiple range test was used to verify the significant differences (p < 0.05) in all treatments. All graphs were created using SigmaPlot 8.0 (Systat Software Inc., San Jose, CA, USA).

Multivariate statistical analysis was carried out using SIMCA-P+ v.16.0.2 (Umetrics, Umea, Sweden) and all variables were automatically transformed and scaled to unit variance. PCA, PLS-DA, VIP, permutation test, and *p*-value were used to visualize the graphs. Statistical differences between the experimental data were analyzed using one-way analysis of variance (ANOVA) with Duncan's test using SPSS 27.0 (SPSS Inc., Chicago, IL, USA). Boxplots and heatmaps of metabolite were calculated and created using the R software.

3. Results and Discussion

3.1. Growth Characteristics

Two ginseng cultivars ('Yunpoong' and 'K-1') showed different growth characteristic patterns at 2, 3, 4, and 5 weeks after transplanting (WAT) (Table 1). The shoot fresh weight of 'Yunpoong' showed the greatest value (1.0442 g) at 3 WAT, while 'K-1' showed the greatest value (0.8077 g) at 4 WAT. Overall, the shoot fresh weight was greater in 'Yunpoong' than in 'K-1'. The shoot dry weight showed a similar pattern to the shoot fresh weight. In the shoot dry weight, 'Yunpoong' showed the greatest value in the 3 WAT (0.1678 g) and 'K-1' in the 4 WAT (0.1271 g). The root fresh weights of 'Yunpoong' and 'K-1' showed the greatest values (0.7924 g, and 0.5502 g, respectively) at 3 WAT. Overall, the root fresh weight of 'Yunpoong' was greater than that of 'K-1'. Unlike the root fresh weight, the root dry weight showed a greater value in 'Yunpoong' at 3 WAT and 'K-1' at 5 WAT. The root dry weight of 'K-1' showed a similar trend to that of the root fresh weight of 'K-1'. Overall, the values of root dry weight were greater for 'Yunpoong' than for 'K-1'.

Table 1 presents the top/root ratio of 'Yunpoong' and 'K-1' at 2, 3, 4, and 5 WAT. A significantly higher (p < 0.05) top/root ratio was observed in 'K-1' than in 'Yunpoong' at 3 WAT. The top/root ratios of 'Yunpoong' and 'K-1' were the greatest at 4 WAT at 1.4058 and 1.5843, respectively. Similar to shoot fresh weight, there was a significantly difference regarding total yield between cultivars. The greatest total yield was 1.8367 g for 'Yunpoong' and 1.3315 g for 'K-1' at 4 WAT. Overall, the total yield value was greater in 'Yunpoong' than in 'K-1'.

		Shoo	ot (g)	Roc	ot (g)			
Cultivar	WAT	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight	lop/Koot Katio	Total Yield	
	2	$0.70\pm0.03\mathrm{b}$	$0.09\pm0.00\mathrm{b}$	$0.71\pm0.03~\mathrm{a}$	$0.10\pm0.00~\mathrm{c}$	$0.99\pm0.04~\mathrm{b}$	$1.42\pm0.05b$	
Vunnaana	3	$1.04\pm0.04~\mathrm{a}$	$0.17\pm0.01~\mathrm{a}$	$0.79\pm0.04~\mathrm{a}$	$0.13\pm0.01~\mathrm{ab}$	$1.34\pm0.08~\mathrm{a}$	$1.84\pm0.07~\mathrm{a}$	
runpoong	4	$1.01\pm0.07~\mathrm{a}$	$0.16\pm0.01~\mathrm{a}$	$0.72\pm0.05~\mathrm{a}$	$0.10\pm0.01~{ m bc}$	1.41 ± 0.06 a	1.73 ± 0.11 a	
	5	$0.95\pm0.05~\mathrm{a}$	$0.17\pm0.01~\mathrm{a}$	$0.74\pm0.04~\mathrm{a}$	$0.14\pm0.01~\mathrm{a}$	$1.29\pm0.04~\mathrm{a}$	$1.70\pm0.09~\mathrm{a}$	
	2	$0.40\pm0.02~{\rm c}$	$0.04\pm0.00~\mathrm{c}$	$0.42\pm0.02~\mathrm{b}$	$0.05\pm0.00\mathrm{b}$	$0.96\pm0.03\mathrm{b}$	$0.83\pm0.03~\mathrm{c}$	
K-1	3	$0.55\pm0.03~\mathrm{b}$	$0.08\pm0.01\mathrm{b}$	$0.55\pm0.05~\mathrm{a}$	$0.07\pm0.01~\mathrm{a}$	$1.03\pm0.06\mathrm{b}$	$1.10\pm0.07~\mathrm{b}$	
	4	$0.81\pm0.04~\mathrm{a}$	$0.13\pm0.01~\mathrm{a}$	$0.52\pm0.04~\mathrm{a}$	$0.08\pm0.01~\mathrm{a}$	1.58 ± 0.09 a	$1.33\pm0.07~\mathrm{a}$	
	5	$0.74\pm0.03~\mathrm{a}$	$0.12\pm0.01~\mathrm{a}$	$0.53\pm0.04~\mathrm{a}$	$0.08\pm0.01~\mathrm{a}$	$1.43\pm0.08~\mathrm{a}$	$1.26\pm0.06~\mathrm{a}$	

Table 1. Growth patterns of 'Yunpoong' and 'K-1' at 3, 4, and 5 weeks after transplanting.

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test.

The growth characteristic results at 3 WAT for 'Yunpoong' and 4 WAT for 'K-1' are similar to previous studies. In previous studies, Jang et al. [34] reported that the shoot dry weight increased up to 4 WAT and then decreased. Developing the seedling and mature ginseng canopies takes approximately 4 weeks [35]. The root dry weight decreased for the first 2 WAT and then gradually increased [34]. In our study, 'K-1' showed the same trend, but 'Yunpoong' decreased at 4 WAT. These inconsistent results may be due to the different environmental conditions and cultivars.

Well-grown plants have more shoot biomass than root biomass, which increases the top/root ratio [36]. The total yield was generally identical to the shoot fresh weight because the change in shoot fresh weight over time was much more significant than that of the root fresh weight. The total yield showed the same trend as the shoot fresh weight [37], consistent with our results. In two ginseng cultivars used in a previous study ('Chenpoong' and 'Geumpoong'), 'Chenpoong' had higher significant differences than 'Geumpoong' in the fresh and dry weight by cultivar and growth period [38]. Our results also showed that the growth characteristics of ginseng were affected by cultivar and growth period.

3.2. Biological Compounds and Antioxidant Properties

3.2.1. Biological Compounds (Total Phenolic and Flavonoid Contents)

The total phenolic and flavonoid contents differed between the two ginseng cultivars ('Yunpoong' and 'K-1') at 2, 3, 4, and 5 WAT (Table 2). The total phenolic contents of the shoot showed a higher range in 'Yunpoong' than in 'K-1'. The total phenolic contents in the roots of 'Yunpoong' gradually increased and showed the highest content at 5 WAT (2.10 GAE mg g⁻¹); however, 'K-1' showed the highest content at 3 WAT (1.60 GAE mg g⁻¹). During 3–5 WAT, the total phenolic contents of the 'K-1' roots did not change significantly. Overall, the total flavonoid contents were higher in 'Yunpoong' than 'K-1' in both the shoots and roots. For 'Yunpoong' at 3 WAT (1.17 RE mg g⁻¹), the total flavonoid contents of the shoots had a higher content than at other WATs, while no significant differences occurred at 4 (1.08 RE mg g⁻¹) and 5 WAT (1.06 RE mg g⁻¹). 'K-1' showed the highest total flavonoid contents in the roots of 'Yunpoong' and 'K-1' showed the highest values of 0.26 RE mg g⁻¹ at 4 and 5 WAT and 0.24 RE mg g⁻¹ at 5 WAT, respectively, showing a similar trend to the total flavonoid contents of the shoot.

Phytochemicals such as phenolic acids and flavonoids are widely present in various plants and are known as representative antioxidants derived from natural products [39,40]. Kim et al. [29] reported that the total phenolic and flavonoid contents of the shoot of ginseng sprouts were higher than those of the root; our results were similar to those of previous studies. The potential antioxidant activity was positively correlated with total phenolic and flavonoid contents [29,33,41,42]. However, our study showed different results depending on the growth period and cultivars. When comparing the total phenolic and flavonoid contents of the three cultivars in broccoli sprouts, the total phenolic contents differed significantly among the three cultivars. However, the total flavonoid contents did

not differ significantly among the three cultivars [43]. Our results suggested that ginseng's phenolic and flavonoid contents differed depending on the cultivars and periods.

	T 47.4 (T)	Total Phenolic Con	tents (GAE mg g^{-1})	Total Flavonoid Contents (RE mg g^{-1})			
Cultivar	WAT	Shoot	Root	Shoot	Root		
	3	$2.72\pm0.04b$	$1.43\pm0.02b$	$1.17\pm0.01~\mathrm{a}$	0.25 ± 0.00 a		
Yunpoong	4	$3.19\pm0.02~\mathrm{a}$	$1.86\pm0.04~\mathrm{a}$	$1.08\pm0.01~\mathrm{b}$	$0.26\pm0.01~\mathrm{b}$		
	5	$3.06\pm0.04~\mathrm{a}$	$2.10\pm0.09~\mathrm{a}$	$1.06\pm0.01~\text{b}$	$0.26\pm0.00~b$		
	3	$2.57\pm0.01~\mathrm{a}$	$1.60\pm0.02~\mathrm{a}$	$0.90\pm0.01~\text{b}$	$0.18\pm0.00~\text{b}$		
K-1	4	$2.46\pm0.01~\mathrm{b}$	$1.49\pm0.04~\mathrm{a}$	$0.98\pm0.02~\mathrm{a}$	$0.21\pm0.00~\mathrm{a}$		
	5	$2.32\pm0.02~\mathrm{c}$	$1.51\pm0.01~\mathrm{a}$	$0.79\pm0.01~\mathrm{c}$	$0.24\pm0.00~c$		

Table 2. Biological compounds of 'Yunpoong' and 'K-1' at 3, 4, and 5 weeks after transplanting.

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test.

3.2.2. Antioxidant Properties (DPPH, FRAP, and ABTS)

The DPPH of the shoot increased from 3 WAT (33.59%) to 5 WAT (43.59%) for 'Yunpoong' (Table 3), while that of 'K-1' was the highest at 4 WAT (35.93%). During the 3–5 WAT, DPPH of the shoots exhibited higher activity in 'Yunpoong' than 'K-1'. DPPH of the roots increased from 3 WAT to 5 WAT in both cultivars, and 'Yunpoong' showed 12.21% and 'K-1' 14.26% at 5 WAT.

Table 3. Antioxidant properties of 'Yunpoong' and 'K-1' at 3, 4, and 5 weeks after transplanting.

Cultivar	WAT	DPPH Radica Activi	al Scavenging ity (%)	Ferric Reducir Power (C	ng/Antioxidant OD _{593nm})	ABTS Radical Scavenging Activity (%)		
		Shoot	Root	Shoot	Root	Shoot	Root	
	3	$33.59\pm0.22c$	$10.01\pm0.38\mathrm{b}$	$1.30\pm0.01~\mathrm{c}$	$0.34\pm0.00b$	$75.22\pm0.32b$	$25.02\pm0.72~\mathrm{c}$	
Yunpoong	4	$37.70\pm0.53\mathrm{b}$	$11.43\pm0.11~\mathrm{ab}$	$1.40\pm0.01~\mathrm{b}$	$0.41\pm0.01~\mathrm{a}$	$73.95\pm0.56~\mathrm{c}$	$35.02\pm0.47\mathrm{b}$	
	5	$43.59\pm0.05~\mathrm{a}$	$12.21\pm0.23~\mathrm{a}$	$1.47\pm0.02~\mathrm{a}$	$0.40\pm0.01~\mathrm{a}$	$78.01\pm0.42~\mathrm{a}$	$39.17\pm1.00~\mathrm{a}$	
	3	$32.73\pm0.31~\mathrm{b}$	$11.16\pm0.23~\mathrm{c}$	$1.12\pm0.01~\mathrm{b}$	$0.42\pm0.00~\mathrm{c}$	71.03 ± 0.30 a	$36.13\pm0.28\mathrm{b}$	
K-1	4	$35.93\pm0.76~\mathrm{a}$	$12.99\pm0.16b$	$1.25\pm0.01~\mathrm{a}$	$0.48\pm0.00b$	$61.35\pm0.40~b$	$35.44\pm0.35~\mathrm{b}$	
	5	$33.35\pm0.29~\mathrm{ab}$	$14.26\pm0.42~\mathrm{a}$	$1.26\pm0.01~\mathrm{a}$	$0.53\pm0.00~\mathrm{a}$	$62.09\pm0.10b$	$39.74\pm0.61~\mathrm{a}$	

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test.

The FRAP of the shoot content was higher in 'Yunpoong' than 'K-1', but the FRAP of the root content was higher in 'K-1' than in 'Yunpoong'. FRAP of the shoots increased from 3 to 5 WAT for 'Yunpoong' (1.30 and 1.47 OD_{593nm} , respectively) and 'K-1' (1.12 and 1.26 OD_{593nm} , respectively). FRAP of the roots showed a higher content (0.51 OD_{593nm}) at 4 WAT in 'Yunpoong' than in 'K-1'. FRAP of the roots increased from 3 to 5 WAT in 'K-1' (0.42 and 0.53 OD_{593nm} , respectively).

The ABTS of the shoots and roots in 'K-1' was higher than in 'Yunpoong' during the WAT. The ABTS of the shoots was the highest at 5 WAT and 3 WAT for 'Yunpoong' (78.01%) and 'K-1' (62.09%), respectively. The highest root activity for 'Yunpoong' (39.17%) and 'K-1' (39.74%) was observed at 5 WAT.

Antioxidant properties are used to determine the antioxidant capacity of fruits [44] and vegetables [45]. DPPH is a method to confirm the activity of scavenging anion radicals [46]. It is a relatively stable free radical with a deep purple color and is reduced by sulfurcontaining amino acids and L-ascorbic acid to confirm antioxidant power. FRAP is a method that uses the fact that Fe^{3+} can donate a hydrogen ion, and the radical is stabilized and reduced to Fe^{2+} [47]. ABTS is a method for confirming the activity of scavenging cation radicals. It reacts with hydroxyl, peroxyl, alkoxyl, and inorganic radicals to form stable cation radicals, enabling the measurement of the anti-oxidation of hydrophilic and hydrophobic substances [48]. Kang et al. [49] concluded that DPPH radical scavenging activity is an indicator of antioxidant activity for the total phenolic contents; the higher the reducing power, the higher the activity. Our results showed no correlation between biological compounds and antioxidant properties. In grains and fruits, the correlation between total phenol content and antioxidant activity is high. However, it has been reported that there is little correlation between total phenol content and antioxidant activity in medicinal plants and fruits containing a large number of anthocyanins [50]. Judging from the report that phenolic compounds contained in ginseng have structural characteristics that make it difficult to access the DPPH radical center, the types and contents of phenolic compounds differed [51]. Presumably, this is because trace components other than phenolic compounds have a combined effect. There was no significant difference in the DPPH, FRAP, and ABTS contents between the shoots and roots [47]. Our results showed that the shoot (leaves) had higher antioxidant properties than the roots in all WAT.

3.3. Ginsenoside

The ginsenoside contents showed significant differences between the ginseng cultivars ('Yunpoong' and 'K-1') at 3, 4, and 5 WAT (Tables 4 and 5). Regarding the protopanaxatrioltype (PPT-type) compounds of the shoot, the contents of 'Yunpoong' and 'K-1' showed an increase in Rg1 and Re. The ginsenoside Rh1 was not detected in 'Yunpoong' at 4 WAT. The total PPT-type content of the shoot was 25.26 and 34.28 mg g^{-1} for 'Yunpoong' and 'K-1', respectively, showing approximately 1.36 times higher content in 'K-1' at 5 WAT. Among the protopanaxadiol (PPD-type) compounds in the shoot, Rb2, Rb3, and F2 tended to increase over time. The compound K (C.K) was not detected in 'Yunpoong' at 3 WAT, and C.K and ginsenoside Rd2 did not differ significantly between 'Yunpoong' and 'K-1' at 3-5 WAT. The total PPD-type of the shoot was approximately 1.45 times higher for 'K-1' (69.44 mg g^{-1}) than for 'Yunpoong' (47.75 mg g⁻¹). Oleanane types (OA-type) in the 'Yunpoong' shoots decreased from 3 WAT (0.38 mg g⁻¹) to 5 WAT (0.24 mg g⁻¹). OA-type in the 'K-1' shoots increased from 3 WAT (0.32 mg g^{-1}) to 5 WAT (0.49 mg g^{-1}). The total ginsenoside contents of the shoots were 47.75 mg g^{-1} and 69.44 mg g^{-1} for 'Yunpoong' and 'K-1', respectively, at 5 WAT. The major ginsenoside derivatives of the shoots (at concentrations > 9 mg g⁻¹) were Re and Rd in 'Yunpoong' and 'K-1'at 3-5 WAT. The individual ginsenoside content distributions of the 'Yunpoong' shoots at 5 WAT were PPT-type (25.26 mg g^{-1}) >PPD-type $(22.25 \text{ mg g}^{-1})$ >OA-type (0.24 mg g^{-1}) , while those of 'K-1' were PPD-type $(34.68 \text{ mg g}^{-1})$ > PPT-type $(34.28 \text{ mg g}^{-1})$ > OA-type (0.49 mg g^{-1}) .

The ginsenoside contents of the roots of the two ginseng cultivars ('Yunpoong' and 'K-1') were not significantly affected by WAT. PPT-type compounds of the 'Yunpoong' roots contained ginsenoside Re (3, 4, and 5 WAT; 1.09, 1.08, and 1.52 mg g⁻¹, respectively), F5 (5 WAT; 4.45 mg g⁻¹) and F3 (3 WAT; 2.75 mg g⁻¹) and the remaining components were detected at low levels (<2.0 mg g⁻¹). Ginsenoside Re was detected in the 'K-1' roots at 3, 4, and 5 WAT (3.78, 4.32, and 3.33 mg g⁻¹, respectively), and the remaining PPT-type compounds were detected at low levels. The PPD-type of the 'Yunpoong' roots was higher at 5 WAT (10.61 mg g⁻¹) than at 3 WAT (6.47 mg g⁻¹), while 'K-1' showed a high value at 3 WAT (9.89 mg g⁻¹). The OA-type of the roots was higher at 3 WAT (0.92 and 1.48 mg g⁻¹, respectively) than at 5 WAT (0.39 and 0.58 mg g⁻¹, respectively) for 'Yunpoong' and 'K-1'. Total ginsenoside contents of the 'Yunpoong' roots increased from 3 WAT (18.51 mg g⁻¹) to 5 WAT (25.52 mg g⁻¹), while that of the 'K-1' roots declined from 3 WAT (16.89 mg g⁻¹) to 5 WAT (15.29 mg g⁻¹). The individual ginsenoside content distributions of the 'Yunpoong' roots at 5 WAT were in the following order: PPT-type (13.44 mg g⁻¹) > PPD-type (9.41 mg g⁻¹) > PPT-type (5.30 mg g⁻¹), > OA-type (0.58 mg g⁻¹).

			Protopanaxatriol Types (mg g ⁻¹)									
	Cultivar	WAT	Rg1	Re	Rf	F5	F3	Rg2	Rh1	F1	РРТ	Total PPT-Type
		3	$2.71\pm0.03~\mathrm{c}$	$12.62\pm0.02\mathrm{b}$	$0.19\pm0.00~\mathrm{a}$	$1.10\pm0.01~\mathrm{b}$	$1.61\pm0.01~\mathrm{b}$	$0.59\pm0.01~\mathrm{b}$	$0.05\pm0.03~\mathrm{a}$	$1.38\pm0.00~\mathrm{c}$	$0.13\pm0.00\mathrm{b}$	$20.38\pm0.01~\mathrm{c}$
	Yunpoong	4	$2.85\pm0.01~\mathrm{b}$	$15.25\pm0.08~\mathrm{a}$	$0.54\pm0.08~\mathrm{a}$	1.26 ± 0.04 ab	$1.88\pm0.02~\mathrm{a}$	$0.69\pm0.01~\mathrm{a}$	ND	1.52 ± 0.01 a	$0.16\pm0.00~\mathrm{a}$	$24.16\pm0.23b$
Ch t	1 0	5	$3.67\pm0.02~\mathrm{a}$	$15.31\pm0.09~\mathrm{a}$	$0.58\pm0.20~\text{a}$	$1.45\pm0.09~\mathrm{a}$	$1.91\pm0.00~\mathrm{a}$	$0.70\pm0.01~\mathrm{a}$	$0.05\pm0.02~\mathrm{a}$	$1.47\pm0.00~\mathrm{b}$	$0.12\pm0.00b$	$25.26\pm0.27~\mathrm{a}$
Shoot		3	$4.50\pm0.01~{\rm c}$	$14.87\pm0.02~\mathrm{c}$	$1.08\pm0.02~\mathrm{a}$	$1.77\pm0.03~\mathrm{a}$	$1.83\pm0.01~{\rm c}$	$0.75\pm0.02\mathrm{b}$	$0.08\pm0.00~\mathrm{b}$	$1.69\pm0.01~\mathrm{b}$	$0.15\pm0.01~\mathrm{b}$	$26.73\pm0.02~\mathrm{c}$
	K-1	4	$5.28\pm0.01\mathrm{b}$	$17.79\pm0.03~\mathrm{b}$	$0.31\pm0.21\mathrm{b}$	$1.72\pm0.07~\mathrm{a}$	$2.12\pm0.02b$	$0.90\pm0.01~\mathrm{a}$	$0.13\pm0.01~\mathrm{a}$	$1.72\pm0.02\mathrm{b}$	$0.14\pm0.01~{ m b}$	$30.10\pm0.20\mathrm{b}$
		5	$6.67\pm0.31~\mathrm{a}$	$19.67\pm0.29~\mathrm{a}$	$0.40\pm0.15b$	$1.95\pm0.10~\text{a}$	$2.41\pm0.02~\text{a}$	$0.93\pm0.04~\mathrm{a}$	$0.13\pm0.00~\text{a}$	$1.94\pm0.04~\mathrm{a}$	$0.19\pm0.00~\mathrm{a}$	$34.28\pm0.19~\text{a}$
		3	$1.09\pm0.01~\mathrm{b}$	$4.95\pm0.01~\mathrm{b}$	$0.92\pm0.10~\mathrm{a}$	$0.47\pm0.03~\mathrm{b}$	$2.75\pm0.04~\mathrm{a}$	$0.82\pm0.08~\mathrm{a}$	ND	$0.05\pm0.02~\mathrm{a}$	$0.06\pm0.00~\mathrm{a}$	$11.12\pm0.21\mathrm{b}$
	Yunpoong	4	$1.08\pm0.06~\mathrm{b}$	$4.68\pm0.06~{\rm c}$	$0.89\pm0.03~\mathrm{a}$	$0.51\pm0.02\mathrm{b}$	$0.19\pm0.10~{ m c}$	$0.56\pm0.03\mathrm{b}$	ND	$0.05\pm0.00~\mathrm{a}$	$0.03\pm0.00~\mathrm{b}$	$7.99\pm0.13~{\rm c}$
Root		5	$1.52\pm0.05~\mathrm{a}$	$5.71\pm0.03~\mathrm{a}$	$0.94\pm0.03~\mathrm{a}$	$4.45\pm0.06~\text{a}$	$0.66\pm0.07b$	ND	ND	$0.09\pm0.01~\mathrm{a}$	$0.06\pm0.00~\mathrm{a}$	$13.44\pm0.13~\mathrm{a}$
		3	$0.99\pm0.01~\mathrm{a}$	$3.78\pm0.01~\text{b}$	$0.61\pm0.03~\mathrm{a}$	$0.43\pm0.00~\mathrm{b}$	$0.27\pm0.01~\mathrm{a}$	$0.44\pm0.00~\mathrm{b}$	ND	$0.05\pm0.00~\mathrm{a}$	$0.03\pm0.00~\text{b}$	$6.61\pm0.04~\mathrm{b}$
	K-1	4	$0.86\pm0.01\mathrm{b}$	$4.32\pm0.05~\mathrm{a}$	$0.59\pm0.01~\mathrm{a}$	$0.53\pm0.01~\mathrm{a}$	$0.19\pm0.02~{ m b}$	$0.55\pm0.01~\mathrm{a}$	ND	$0.04\pm0.00~\mathrm{b}$	$0.07\pm0.00~\mathrm{a}$	$7.14\pm0.09~\mathrm{a}$
		5	$0.77\pm0.02~\mathrm{c}$	$3.33\pm0.01~\mathrm{c}$	$0.47\pm0.02\mathrm{b}$	$0.35\pm0.00~\mathrm{c}$	ND	$0.31\pm0.00~\mathrm{c}$	ND	$0.04\pm0.00b$	$0.03\pm0.00b$	$5.30\pm0.05~\mathrm{c}$

Table 4. Protopanaxatriol types in the 'Yunpoong' and 'K-1'shoots at 3, 4, and 5 weeks after transplanting.

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test. ND: Not detected.

Table 5. Protopanaxadiol types, oleanane types	, and total ginsenoside in the 'Yunpoons	g' and 'K-1' shoots at 3, 4, and 5 weeks after trans	planting.
		, ,	

			Protopanaxadiol Type (mg g ⁻¹)										Oleanane Type	Total		
	Cultivar	WAT	Rb1	Rc	Rb2	Rb3	Rd	Rd2	F2	Rg3	С.К	Rh2	PPD	Total PPD-Type	Ro	Ginsenoside
Shoot	Yunpoong	3 4 5	$\begin{array}{c} 0.55 \pm 0.01 \ c \\ 0.63 \pm 0.02 \ b \\ 0.68 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 2.48 \pm 0.08 \ \text{b} \\ 2.84 \pm 0.01 \ \text{a} \\ 2.71 \pm 0.05 \ \text{a} \end{array}$	$\begin{array}{c} 3.49 \pm 0.03 \text{ b} \\ 3.95 \pm 0.03 \text{ a} \\ 3.99 \pm 0.04 \text{ a} \end{array}$	$\begin{array}{c} 0.40 \pm 0.04 \text{ a} \\ 0.45 \pm 0.02 \text{ a} \\ 0.49 \pm 0.05 \text{ a} \end{array}$	$\begin{array}{c} 11.17 \pm 0.22 \text{ b} \\ 12.50 \pm 0.09 \text{ a} \\ 12.20 \pm 0.28 \text{ a} \end{array}$	$\begin{array}{c} 0.22 \pm 0.02 \text{ a} \\ 0.28 \pm 0.01 \text{ a} \\ 0.27 \pm 0.08 \text{ a} \end{array}$	$\begin{array}{c} 0.51 \pm 0.09 \text{ a} \\ 0.58 \pm 0.01 \text{ a} \\ 0.70 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.18 \pm 0.03 \text{ a} \\ 0.17 \pm 0.01 \text{ a} \\ 0.15 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} \text{ND} \\ 0.01 \pm 0.00 \text{ a} \\ 0.01 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.10 \pm 0.00 \text{ a} \\ 0.07 \pm 0.00 \text{ b} \\ 0.06 \pm 0.00 \text{ b} \end{array}$	$\begin{array}{c} 0.92 \pm 0.01 \ b \\ 1.10 \pm 0.01 \ a \\ 0.99 \pm 0.04 \ b \end{array}$	$\begin{array}{c} 20.03 \pm 0.44 \text{ b} \\ 22.59 \pm 0.15 \text{ a} \\ 22.25 \pm 0.39 \text{ a} \end{array}$	$\begin{array}{c} 0.38 \pm 0.01 \text{ a} \\ 0.37 \pm 0.00 \text{ a} \\ 0.24 \pm 0.01 \text{ b} \end{array}$	$\begin{array}{c} 40.80 \pm 0.45 \ b \\ 47.12 \pm 0.38 \ a \\ 47.75 \pm 0.50 \ a \end{array}$
Shoot	K-1	3 4 5	$\begin{array}{c} 0.95 \pm 0.02 \text{ a} \\ 0.94 \pm 0.03 \text{ a} \\ 1.04 \pm 0.04 \text{ a} \end{array}$	$\begin{array}{c} 3.23 \pm 0.04 \ c \\ 4.20 \pm 0.03 \ b \\ 4.77 \pm 0.10 \ a \end{array}$	$\begin{array}{c} 4.98 \pm 0.03 \text{ c} \\ 5.89 \pm 0.01 \text{ b} \\ 6.54 \pm 0.03 \text{ a} \end{array}$	$\begin{array}{c} 0.52 \pm 0.01 \ b \\ 0.68 \pm 0.06 \ a \\ 0.75 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 13.46 \pm 0.08 \text{ c} \\ 14.63 \pm 0.40 \text{ b} \\ 18.62 \pm 0.22 \text{ a} \end{array}$	$\begin{array}{c} 0.19 \pm 0.02 \text{ a} \\ 0.26 \pm 0.01 \text{ a} \\ 0.40 \pm 0.08 \text{ a} \end{array}$	$\begin{array}{c} 0.54 \pm 0.01 \ \text{b} \\ 0.69 \pm 0.06 \ \text{b} \\ 0.87 \pm 0.03 \ \text{a} \end{array}$	$\begin{array}{c} 0.28 \pm 0.11 \text{ a} \\ 0.19 \pm 0.02 \text{ a} \\ 0.26 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.01 \pm 0.00 \text{ a} \\ 0.01 \pm 0.01 \text{ a} \\ 0.01 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 0.13 \pm 0.00 \text{ a} \\ 0.12 \pm 0.00 \text{ b} \\ 0.11 \pm 0.00 \text{ b} \end{array}$	$\begin{array}{c} 0.84 \pm 0.01 \text{ b} \\ 1.38 \pm 0.05 \text{ a} \\ 1.30 \pm 0.03 \text{ a} \end{array}$	$\begin{array}{c} 25.14 \pm 0.17 \text{ c} \\ 28.99 \pm 0.49 \text{ b} \\ 34.68 \pm 0.07 \text{ a} \end{array}$	$\begin{array}{c} 0.32 \pm 0.01 \ b \\ 0.46 \pm 0.02 \ a \\ 0.49 \pm 0.06 \ a \end{array}$	$\begin{array}{c} 52.19\pm 0.18\ c\\ 59.55\pm 0.47\ b\\ 69.44\pm 0.20\ a\end{array}$
Root	Yunpoong	3 4 5	$\begin{array}{c} 0.01 \pm 0.00 \ b \\ 3.92 \pm 0.17 \ a \\ 0.37 \pm 0.01 \ b \end{array}$	$\begin{array}{c} 2.82 \pm 0.02 \ b\\ 2.61 \pm 0.03 \ c\\ 4.37 \pm 0.03 \ a \end{array}$	$\begin{array}{c} 1.73 \pm 0.02 \ \text{b} \\ 1.36 \pm 0.01 \ \text{c} \\ 2.72 \pm 0.03 \ \text{a} \end{array}$	$\begin{array}{c} 0.27 \pm 0.00 \; ab \\ 0.17 \pm 0.08 \; b \\ 0.40 \pm 0.00 \; a \end{array}$	$\begin{array}{c} 0.80 \pm 0.01 \ b \\ 0.77 \pm 0.01 \ b \\ 1.60 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 0.04 \pm 0.00 \text{ a} \\ 0.05 \pm 0.02 \text{ a} \\ 0.07 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.01 \pm 0.00 \ \mathrm{b} \\ \mathrm{ND} \\ 0.04 \pm 0.01 \ \mathrm{a} \end{array}$	$\begin{array}{c} 0.06 \pm 0.01 \text{ a} \\ 0.04 \pm 0.01 \text{ b} \\ 0.05 \pm 0.01 \text{ ab} \end{array}$	$\begin{array}{c} 0.03 \pm 0.00 \ c \\ 0.05 \pm 0.00 \ b \\ 0.11 \pm 0.00 \ a \end{array}$	$\begin{array}{c} 0.25 \pm 0.00 \text{ b} \\ 0.23 \pm 0.00 \text{ c} \\ 0.67 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 0.45 \pm 0.01 \text{ a} \\ 0.48 \pm 0.16 \text{ a} \\ 0.22 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 6.47 \pm 0.03 \text{ b} \\ 9.66 \pm 0.38 \text{ a} \\ 10.61 \pm 0.08 \text{ a} \end{array}$	$\begin{array}{c} 0.92 \pm 0.01 \text{ a} \\ 1.24 \pm 0.15 \text{ a} \\ 1.48 \pm 0.39 \text{ a} \end{array}$	$\begin{array}{c} 18.51 \pm 0.25 \text{ b} \\ 18.90 \pm 0.34 \text{ b} \\ 25.52 \pm 0.18 \text{ a} \end{array}$
	K-1	3 4 5	$\begin{array}{c} 3.59 \pm 0.01 \text{ a} \\ 3.42 \pm 0.05 \text{ b} \\ 3.59 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 2.85 \pm 0.01 \text{ a} \\ 2.51 \pm 0.04 \text{ b} \\ 2.51 \pm 0.01 \text{ b} \end{array}$	$\begin{array}{c} 1.63 \pm 0.02 \text{ a} \\ 1.16 \pm 0.02 \text{ c} \\ 1.51 \pm 0.01 \text{ b} \end{array}$	$\begin{array}{c} 0.27 \pm 0.01 \text{ a} \\ 0.23 \pm 0.01 \text{ b} \\ 0.27 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.74 \pm 0.02 \text{ a} \\ 0.57 \pm 0.01 \text{ b} \\ 0.70 \pm 0.01 \text{ a} \end{array}$	$\begin{array}{c} 0.04 \pm 0.00 \ b \\ 0.04 \pm 0.00 \ ab \\ 0.06 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 0.01 \pm 0.00 \text{ a} \\ 0.02 \pm 0.01 \text{ a} \\ 0.03 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 0.03 \pm 0.00 \text{ a} \\ 0.03 \pm 0.00 \text{ a} \\ 0.04 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 0.06 \pm 0.00 \text{ b} \\ 0.03 \pm 0.00 \text{ c} \\ 0.09 \pm 0.00 \text{ a} \end{array}$	$\begin{array}{c} 0.36 \pm 0.00 \text{ a} \\ 0.31 \pm 0.01 \text{ b} \\ 0.31 \pm 0.01 \text{ b} \end{array}$	$\begin{array}{c} 0.31 \pm 0.01 \text{ b} \\ 0.42 \pm 0.01 \text{ a} \\ 0.30 \pm 0.00 \text{ b} \end{array}$	$\begin{array}{c} 9.89 \pm 0.02 \text{ a} \\ 8.74 \pm 0.15 \text{ c} \\ 9.41 \pm 0.03 \text{ b} \end{array}$	$\begin{array}{c} 0.39 \pm 0.05 \ b \\ 0.43 \pm 0.03 \ ab \\ 0.58 \pm 0.01 \ a \end{array}$	$\begin{array}{c} 16.89 \pm 0.05 \text{ a} \\ 16.31 \pm 0.27 \text{ a} \\ 15.29 \pm 0.07 \text{ b} \end{array}$

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test. ND: Not detected.

Ginsenosides are generally classified into two groups based on their chemical structure: the four-cyclic structure dammarane type and the five-cyclic structure OA-type. In addition, dammarane-type ginsenosides are divided into two groups, PPD-type and PPT-type [52]. Ginseng leaves had a 6–8 times higher total ginsenoside content than the roots [18]. Kim et al. [29] and Hwang et al. [53] reported on the growth in a vertical farm; total ginsenoside contents of the shoots (61.31–110 and 101.73 mg g⁻¹) were higher than that of the roots (25.81–34.19 and 37.39 mg g⁻¹). Our results showed that the total ginsenoside contents in 'Yunpoong' and 'K-1' were higher in the shoots (40.80–47.75 mg g⁻¹ and 52.19–69.44 mg g⁻¹, respectively) than in the roots (18.51–25.52 mg g⁻¹ and 15.29–16.89 mg g⁻¹, respectively). The major ginsenoside compounds in ginseng sprout leaves were ginsenoside Re and Rd [37,54,55]. In our study, the Rd and Re contents were higher in 'K-1' than in 'Yunpoong' (Rd, 11.17–12.50 mg g⁻¹ and 13.46–18.62 mg g⁻¹, respectively; Re, 12.62–15.31 mg g⁻¹ and 14.87–19.67 mg g⁻¹, respectively). Choi et al. [12] reported that the total ginsenoside contents were 97.29, 66.42, 67.61, and 36.24 mg g⁻¹ at 5, 7, 9, and 15 weeks, showing a gradually decreasing content.

3.4. Metabolites

The primary metabolites measured at 7 WAT differed for the two ginseng cultivars ('Yunpoong' and 'K-1') (Figure 1). The identification of 'Yunpoong' and 'K-1' metabolites at 7 WAT using GC/MS was visualized using a partial least-squares discriminant analysis (PLS-DA) score plot (Figure S3). The goodness of fit ($R_2X = 0.721$; $R_2Y = 0.993$), predictability ($Q_2 = 0.974$), *p*-value (0.001), and cross-validation determined using the permutation test (*n* = 5) indicated that the PLS-DA model was statistically acceptable. In the PLS-DA score plot, 'Yunpoong' and 'K-1' were separated by t1 and t2. The VIP and *p*-values of individual metabolites were analyzed to find primary metabolites that contributed to the difference between ginseng cultivars (Figure S1 and Table S1). In total, 18 metabolites were detected: 7 amino acids, 1 inorganic acid, 4 organic acids, and 6 sugars were detected; their VIP was VIP ≥ 0.86 and their *p*-values were p < 0.05. The primary metabolomic differences between 'Yunpoong' and 'K-1' are shown using boxplots and a heatmap (Figure 1). It showed that the content of primary metabolites differed between ginseng cultivars. 'Yunpoong' had a higher metabolite content than 'K-1', except for malic acid (an organic acid) and maltose (a sugar).



Figure 1. Box plots for primary metabolites in 'Yunpoong' and 'K-1' cultivars.

At week 7, the secondary metabolite patterns differed between the two ginseng cultivars ('Yunpoong' and 'K-1'), as detected using UPLC/Q-TOF-MS (Figure 2). The UPLC/Q-TOF-MS result was visualized using a PLS-DA to discriminate between the two ginseng cultivars ('Yunpoong' and 'K-1') (Figure S4). The goodness of fit ($R_2X = 0.736$; $R_2Y = 0.999$), predictability ($Q_2 = 0.995$), *p*-value (6.19×10^{-4}), and cross-validation determined using the permutation test (n = 5) indicated that the PLS-DA model was statistically acceptable. In the PLS-DA score plot, 'Yunpoong' and 'K-1' were separated by t1 and t2. The VIP and *p*-values of individual metabolites were analyzed to find secondary metabolites that contributed to the difference between ginseng cultivars (Figure S2 and Table S2). In total, 32 metabolites were detected. The boxplots and heatmap showed differences in secondary metabolites is different for each cultivar of ginseng and that 'Yunpoong' had higher contents of all secondary metabolites than 'K-1'.



Figure 2. Box plots for secondary metabolites in 'Yunpoong' and 'K-1' cultivars.

Eom et al. [40] reported that ginseng metabolites are related to properties such as anti-aging, anti-diabetic, anti-cancer, and anti-inflammatory. Previously, 21 and 34 metabolites detected using GC/MS were identified and reported in Asian and Western ginseng, respectively [56]. However, in our results, only 18 metabolites were detected through GC/MS.

3.5. Annual Net Production

Since year-round production is possible in plant factories, it is necessary to compare net production with actual production for efficiency. The annual net production of 'Yunpoong' showed a higher total yield and ginsenoside production per year than 'K-1' (Table 6). Through analyzing the growth of ginseng and the net production of ginsenosides at 3, 4, and 5 WAT, it was confirmed that the annual net production and the content of ginsenosides decreased as the cultivation period increased for both cultivars.

		Annual Net Production						
Cultivar	WAI	Yield (g m ² Year)	Ginsenoside (mg m ² Year)					
	3	$1.72\pm0.06~\mathrm{a}$	$102.12 \pm 1.01~{ m a}$					
Yunpoong	4	$1.22\pm0.08~\mathrm{b}$	$80.28\pm0.22\mathrm{b}$					
1 0	5	$0.96\pm0.05~c$	$69.99\pm0.64~\mathrm{c}$					
	3	$1.03\pm0.06~\mathrm{a}$	71.26 ± 0.16 a					
K-1	4	$0.94\pm0.05~\mathrm{a}$	71.02 ± 0.68 a					
	5	$0.71\pm0.03~\mathrm{b}$	$60.18\pm0.12\mathrm{b}$					
Significance								
Cultivar (C)		< 0.0001	< 0.0001					
Week (W)		< 0.0001	< 0.0001					
$C \times W$		0.0005	<0.0001					

Table 6. Annual net production of 'Yunpoong' and 'K-1' at 3, 4, and 5 weeks after transplanting.

WAT means the weeks after transplanting. Different letters within columns indicate significant differences (p < 0.05), based on Duncan's test.

Stable production of medicinal crops can be obtained in vertical farms [25]. Park et al. [57] showed that the yield of ginseng grown in vertical farms was higher than in open fields. In this analysis of the efficiency for the net production in plant factories, the efficient cultivation period may be 3 and 4 WAT for 'Yunpoong' and 'K-1', respectively.

4. Conclusions

Ginseng sprouts are widely used as a medicinal crop. According to ginseng cultivars, growth and quality (for example, ginsenosides) differ. This study compared two ginseng cultivars grown in a plant factory. Growth characteristics showed higher values for growth parameters at 3 and 4 WAT in 'Yunpoong' and 'K-1', respectively. Moreover, the 'Yunpoong' cultivar yield was higher than that of the 'K-1' cultivar. Biological compounds and antioxidant properties were the highest at 5 and 4 WAT in 'Yunpoong' and 'K-1', respectively. The highest ginsenoside content was detected at 5 WAT in both 'Yunpoong' and 'K-1' cultivars. 'Yunpoong' had a higher content than 'K-1' for most primary and secondary metabolites. Yunpoong at 3 WAT and K-1 at 3 and 4 WAT showed the highest yield and ginsenoside content in the annual net production. In conclusion, the cultivar and harvesting period might be important factors for the production, yield, and quality of ginseng. Moreover, it is essential to determine a vertical farm's annual net production efficiency for the optimum cultivation time.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/horticulturae9050583/s1, Figure S1: Standard chromatograms analyzed using GC/MS; Figure S2: Standard chromatogram analyzed using UPLC/Q-TOF-MS; Figure S3: Partial least-squares discriminant analysis and Permutation in 'Yunpoong' and 'K-1' of primary metabolites; Figure S4: Partial least-squares discriminant analysis and Permutation in 'Yunpoong' and 'K-1' of secondary metabolites. Table S1: Identification of significant primary metabolites contributing to the separation among sample groups; Table S2: Identification of major secondary metabolites contributing to the separation among sample groups.

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