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Variation in nutritional compositions, antioxidant activity and microstructure of *Lycopus lucidus* Turcz. root at different harvest times

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ABSTRACT

The objective of this study was to investigate the variation in nutritional compositions, antioxidant activity and microstructure of *Lycopus lucidus* Turcz. root at different harvest times. *L. lucidus* Turcz. roots, harvested from two sites (S1 and S2) at three different times (T1: 19-11-2013, T2: 22-12-2013 and T3: 27-01-2014), were analyzed for nutritional compositions, antioxidant activity by DPPH, FRAP and TEAC assays and microstructure. The results revealed that the protein content in *L. lucidus* Turcz. root first decreased and then increased to a maximum at T3. The reducing sugar content had no significant differences among the three harvest dates studied. The starch content decreased drastically along with an increase of crude fat content with the harvest time delayed. The major amino acids in *L. lucidus* Turcz. root were aspartic acid and glutamate and the highest total amino acid content was found for the root harvested at T3. The most common element in *L. lucidus* Turcz. root was detected to be potassium followed by calcium, iron, magnesium, copper and manganese, and their changes were discrepant in the period of harvest. The FP and SGP possessed the highest and lowest phenolic content, respectively. The change of SEP was significantly correlated to the SGP at different harvest times. The highest TPC was found for the root harvested at T3 and the most abundant phenolic acid was chlorogenic acid. The highest and lowest DPPH radical scavenging capacity was observed for the SGP and FP, respectively. The highest and lowest FRAP and TEAC were observed for the FP and SGP, respectively. The results of correlation analysis indicated that there was significant correlation between phenolic content and FRAP and TEAC, and different antioxidant assays. The microstructure of *L. lucidus* Turcz. root also varied greatly with the harvest times.

1. Introduction

Lycopus lucidus Turcz. is a perennial member of the Lamiaceae family. In China, it is little investigated, edible and medicinal plant that grows mainly in Yunnan, Sichuan, Hebei, Liaoning, Shandong and Guizhou provinces. The aerial parts of *L. lucidus* Turcz. have been used in East Asian traditional phytomedicine as antiinflammatory, thyroid, cardiac, sedative, wound-healing and pain relieving agents, and as a tonic (Ślusarczyk, Hajnos, Skalicka-Woźniak, & Matkowski, 2009). The root, edible and medicinal part of *L. lucidus* Turcz., is white in color and its shape is similar to cordyceps. In addition, it is a rich source of nutrients such as carotenoids, carbohydrates, vitamins and minerals. According to ancient records, the *L. lucidus* Turcz. root was widely used to treat stomach-ache, oedema, traumatic injury and rheumatic arthritis in traditional Chinese medicine (Chinese materia medica compilation committee of State Administration of Traditional Chinese Medicine, 1999; State Administration of Traditional Chinese Medicine, 1985). In recent years, studies on *L. lucidus* Turcz. root have attracted more attention. The antitumor, hypolipidemic, antiaging and hypoglycemic effects of polysaccharides from *L. lucidus* Turcz. root have been studied in China (Lin, Zuo, Xiong, & Chen, 2012; Xiong, Chen, Tan, & Zuo, 2011; Xiong et al., 2012). In fact, the *L. lucidus* Turcz. root is high in nutritional value and has been widely applied as an important new resources food due to its potential biological functions. In China, there is an increasing interest in consumption of *L. lucidus* Turcz. root as a vegetable and functional food.

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The content of phytochemicals is influenced by numerous factors including harvesting time, genotype, cultivation techniques and climatic conditions that occur during the pre-harvest period, also influenced by the operations carried out during the post-harvest storage (Lee & Kader, 2000). Imeh and Khokhar (2002) have outlined various factors, including agronomic, genomic, pre- and post-harvest conditions and processing may affect the chemical composition of plant foods in general. Furthermore, they also have emphasized these factors may have a significant role in determining the phenolic composition and the bioactivity of phenolics in particular. In order to improve the nutritional value and functionality, a number of studies have focused on the effects of various factors such as cultivars, maturity stage, harvest time, storage and growing conditions on the nutritional components and phytochemicals in plant foods (Grace et al., 2014; Lin et al., 2014; Zhou, Chen, Zhang, & Blanchard, 2014; Šavikin et al., 2014). The *L. lucidus* Turcz. root can be harvested from November of the first year to January of the next year due to special biological characteristics. Dramatic variation in the nutritional components and phytochemical substances might occur because of plant development and climate change.

Nutritional components including protein, carbohydrate, fat, minerals and vitamins have significant impacts on human health. Besides nutrients, phenolic compounds are diverse groups of plant secondary metabolites and possess various health benefits such as antiproliferative, antimicrobial, antiinflammatory and antioxidant activities (Liu et al., 2010).

Antioxidant activity of polyphenols from the aerial parts of *L. lucidus* Turcz. and antioxidative constituents from aerial parts of *L. lucidus* have been evaluated (Woo & Piao, 2004; Ślusarczyk et al., 2009). However, to the best of our knowledge, there are no data on the variation in chemical compositions and bioactivity of *L. lucidus* Turcz. root at different harvest times. Therefore, in the present study, we focus on the nutritional compositions in *L. lucidus* Turcz. root in relation to the different harvest times. In addition, the antioxidant activity and the microstructure of *L. lucidus* Turcz. root collected at different times were also investigated.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Sigma–Aldrich Chemical Co. (USA). Ferulic acid, *p*-coumaric acid, caffeic acid, gallic acid, protocatechuic acid, chlorogenic acid, Folin–Ciocalteu phenol reagent, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and diammonium salt (ABTS) were purchased from Sigma Chemical Co. (USA). All other chemicals and reagents used in the experiments were of analytical grade.

2.2. Sample

Fresh *L. lucidus* Turcz. roots were harvested from Silian (S1) and Aofeng (S2) villages in Jianchuan County (Yunnan, China; latitude, 26° 53′ N; longitude, 99° 90′ E; altitude, 2200 m) on November, 19, 2013 (T1), December, 22, 2013 (T2) and January, 27, 2014 (T3). Then, the roots were homogenized and freeze-dried. The dried *L. lucidus* Turcz. root powder was to pass through a standard 60 mesh sieve and preserved at 4 °C until further extraction.

2.3. Analysis of crude protein and amino acid composition

The crude protein content was determined by AACC approved method 46-10 (N × 6.25). The analysis of amino acid composition was performed according to the method of Mu, Tan, and Xue (2009) with minor modifications. Briefly, the protein of *L. lucidus* Turcz. root was prepared by isoelectric precipitation. Subsequently, a 100 mg portion of the *L. lucidus* Turcz. root protein powder was hydrolyzed using 10 ml of 6 M HCl at 110 °C for 22 h under nitrogen atmosphere. Then, the hydrolysate was evaporated to dryness under vacuum at 60 °C. Finally, the dried sample was dissolved in 5 ml of sodium citrate buffer (pH 2.2) to yield an amino acid concentration of 50-250 nmol/ml, filtered and then injected into a Hitachi L-8900 amino acid analyzer (Hitachi, Tokyo, Japan) for separation and characterization. Tryptophan was not determined for the sample.

2.4. Analysis of reducing sugar, starch and crude fat

Five grams of *L. lucidus* Turcz. root powder was extracted with 100 ml of distilled water for 30 min at 40 °C, and then the homogenates were centrifuged at 3000 rpm for 10 min in a centrifuge (Eppendorf, Hamburg, Germany). The supernatants after centrifugation were analyzed for reducing sugar. Reducing sugar content was determined using the method of Jemai, Bouaziz, and Sayadi (2009) with some modifications. A mixture of 1 ml of *L. lucidus* Turcz. root extracts and 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent was homogenized and boiled for 5 min, and then cooled immediately using ice cubes. The absorbance of the reaction mixtures was measured using a spectrophotometer (Hitachi, Tokyo, Japan) at 540 nm wavelength, and the amount of reducing sugar was determined using a DNS standard curve. Starch was determined according to AACC approved method 76-13. Crude fat was determined by Soxhlet extractor method. All analyses were performed in triplicate.

2.5. Analysis of main minerals

The samples were digested by dry ashing method (1.0 g of sample first carbonized on electric stove and then ashed in muffle furnace at 500 ± 50 °C for 4 h) and then dissolved in 10 ml 10% nitric acid (v/v). Finally, the content of main minerals including Cu, Mn, Ca, Mg, K, Zn and Fe were determined using a flame atomic absorption spectrophotometer (Shimadu, Tokyo, Japan).

2.6. Analysis of phenolic compounds

2.6.1. Extraction of different phenolic fractions

Four different fractions of phenolic compounds were extracted from the *L. lucidus* Turcz. roots: insoluble cell-wall-bound phenolics (ICP) and free (FP), soluble ester-bound (SEP), and soluble glycoside-bound phenolics (SGP). Extraction was based on a procedure as described previously (Santiago et al., 2007) with some minor modifications. Three grams of *L. lucidus* Turcz. root powder were extracted three times (each extraction for 10 min) with 60 ml of 80% methanol (v/v) and assisted by ultrasonic at room temperature. The mixtures were then centrifuged at 3000 rpm for 10 min (Eppendorf, Hamburg, Germany) and supernatants were collected and combined. The solvent was evaporated at 35 °C under vacuum to a final volume of approximately 10 ml. Concentrated supernatant was acidified to pH 1–2 with 6 M HCl, extracted six times with ethyl acetate. The ethyl acetate extracts were dehydrated with anhydrous sodium sulfate, filtered, and evaporated to dryness under vacuum at 35 °C. The resulting precipitates were labeled as FP. The remaining aqueous solution was divided into two parts. The fraction for SGP determination was

hydrolyzed with 10 ml of 6 M HCl for 1 h at 4 °C, whereas SEP was released by alkaline hydrolysis with 20 ml of 2 M NaOH for 4 h in the dark and under a nitrogen atmosphere. After both digestions, the pH of the solutions was adjusted to 2.0, and the phenolics were extracted six times with ethyl acetate. The ethyl acetate extracts were then combined and evaporated to dryness at 35 °C under vacuum and labeled as SGP and SEP, respectively. The solid residues from the 80% methanol extracts were treated with 20 ml of 2 M NaOH under the same conditions as the SEP. After acidification to pH 2.0 using 6 M HCl, supernatants were pooled and then extracted six times with ethyl acetate. Then, the ethyl acetate extracts were combined and evaporated to dryness under vacuum at 35 °C and labeled as ICP. FP, SGP, SEP and ICP were dissolved separately in 10 ml methanol. All of the extracts were stored at −40 °C until used.

2.6.2. Determination of phenolic content

The phenolic content was determined according to the Folin–Ciocalteu colorimetric method as described by Meda, Lamien, Romito, Millogo, and Nacoulma (2005) with slight modifications. In brief, an aliquot (0.05 mL) of the suitable diluted extracts, 2.85 mL of ultrapure water and 0.1 ml of 1.0 M Folin–Ciocalteu phenol reagent were mixed. After exactly 3 min, 2 ml of Na_2CO_3 (7.5%, w/v) was added to the mixture and mixed thoroughly. Then the reaction was kept in the dark for 90 min, after which its absorbance was read at 760 nm using a spectrophotometer (Hitachi, Tokyo, Japan). Methanol was used as the blank and gallic acid was used for calibration of standard curve (0–10 μg/ml). Phenolic content was expressed as milligram gallic acid equivalent (GAE) per gram dry weight (DW).

2.6.3. HPLC analysis of phenolic compounds

The analysis of phenolic composition was carried out using an Agilent1200 HPLC chromatograph system (Agilent, California, USA) and a reversed-phase C18 column (Agilent, ZORBAX SB-C18, 5 μm, 4.6 mm × 250 mm). The prepared phenolic extracts were filtered through a Millipore membrane (0.45 μm) before injection. The injection volume was 10 μl, the flow rate was 1 ml/min, and the column thermostat was set at 30 °C. The mobile phase consisted of solvent A (100% methanol) and solvent B (acetic acid/methanol/H_2O, 1/10/89, v/v) using the following gradient elution programme for separation: 0–1 min, 20% (A); 1–16 min, 20–38% (A); 16–18 min, 38% (A); 18–30 min, 38–60% (A); 30–35 min, 60% (A); 35–40 min, 60–20% (A); 40–43 min, 20% (A). The detecting wavelength was set at 280 nm and the duration of a single run was 43 min. The phenolic extracts and standard compounds were analyzed under the same conditions, and all of the above experiments were replicated three times. Phenolic acids were identified by comparing the retention time and the UV spectrum with those of the authentic standards including gallic acid, protocatechuic acid, ferulic acid, *p*-coumaric acid, caffeic acid and chlorogenic acid. The working calibration curves for six analytes showed good linearity over the tested range. The phenolic acid contents were calculated from their linear calibration curves under analytical conditions and expressed as microgram per gram DW.

2.7. Determination of antioxidant activity

2.7.1. DPPH radical scavenging capacity assay

The antioxidant activity was assessed by measuring the capacity of bleaching a purple colored methanol solution of DPPH radical as described by Turkoglu, Duru, Mercan, Kivrak, and Gezer (2007) with slight modifications. Briefly, the phenolic extracts were serially diluted to various concentrations in methanol, and then a 0.50 ml of the methanolic extracts were added to a 3.5 ml of 60 μM solution of DPPH in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a solvent blank. The capability to scavenge DPPH radicals was calculated according to the formula: Scavenging effect (%) = $[1 - (A_1 - As)/Ao] \times 100$, where Ao is the absorbance of the control solution (0.5 mL methanol in 3.5 mL of DPPH solution), A_1 is the absorbance in the presence of the plant extract in DPPH solution and As, which is used for error correction arising from unequal color of the sample solutions, is the absorbance of the tested sample solution without DPPH. The scavenging capacity of the phenolic extracts on DPPH radicals was determined by IC_{50} value. IC_{50} value is the effective concentration at which DPPH radicals are scavenged by 50% and was obtained through interpolation from the non-linear regression curve. The lower IC_{50} value indicates higher radical scavenging capacity and vice versa. Finally, the value of IC_{50} was expressed as microgram GAE per milliliter.

2.7.2. Ferric reducing antioxidant power (FRAP) assay

The reducing ability was determined using FRAP assay according to the method as described by Netzel, Netzel, Tian, Schwartz, and Konczak (2006) with slight modifications. Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Then 0.1 ml of the tested sample solution was mixed with 1.4 mL of FRAP reagent and 2.0 ml ultrapure water. The absorbance of the reaction mixture was measured at 593 nm after incubation for 30 min at 37 °C. A standard curve was constructed using $FeSO_4$ solution (100–1000 μM). FRAP value was expressed as micromole Fe(II) per gram DW.

2.7.3. Trolox equivalent antioxidant capacity (TEAC) assay

The Trolox equivalent antioxidant capacity (TEAC) assay was based on the method of Re et al. (1999) with slight modifications. $ABTS^{\cdot+}$ radical cation was generated by a reaction of 7 mM ABTS and 2.45 mM potassium persulphate (final concentration). The reaction mixture was allowed to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 25 μl of sample or Trolox standard to 2 ml of diluted $ABTS^{\cdot+}$ solution, absorbance at 734 nm was recorded at exactly 6 min. A standard curve was constructed using Trolox solution (100–1000 μM). Results were expressed as micromole Trolox equivalent (TE) per gram DW.

2.8. Electron microscopy scanning

Samples were mounted on bronze stubs with double-sided adhesive tape allowing surface visualization, and then coated with a layer of gold (40–50 nm) in a sputter coater to avoid charging under the electron beam. A scanning electron microscopy spectrometer (Philips-FEI Company, Amsterdam, Netherlands) was used at the operating voltage of 20 kV and the vacuum of 15 Pa. The high resolution topographic images were digitally recorded with the short dwelling times to prevent the beam induced damages.

2.9. Statistical analysis

All the experiments were performed in triplicate and the experimental data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's multiple range test were carried out to determine significant differences ($p < 0.05$) between the means by SPSS (version 13.0). Correlation

coefficient and regression analysis were determined by SPSS (version 13.0) Pearson correlation program.

3. Results and discussion

3.1. Main nutrients at different harvest times

Protein, reducing sugar, starch and crude fat contents in *L. lucidus* Turcz. root at different harvest times are shown in Table 1. The content of protein first decreased by 6.84% and 21.01% and then increased by 30.67% and 20.67% for *L. lucidus* Turcz. roots collected in S1 and S2, respectively. The amino acid composition of the protein from *L. lucidus* Turcz. root harvested at different times is presented in Table 2. As can be seen from the table, the changes of total amino acid are different between S1 and S2 *L. lucidus* Turcz. roots at different harvest times. The T3 *L. lucidus* Turcz. root possessed the highest total amino acids, followed by T2 and T1 *L. lucidus* Turcz. roots collected in S1. Whereas the T3 *L. lucidus* Turcz. root possessed the highest total amino acids, followed by T1 and T2 *L. lucidus* Turcz. roots collected in S2. Sixteen amino acids were detected of which Asp and Glu were the major amino acids. As a whole, the variations of individual amino acid content at different harvest times paralleled the changes in the content of total amino acids. The content of reducing sugar showed no significant differences between the three harvest dates studied ($p > 0.05$). The starch content decreased drastically by 77.43% and 75.08% to the lowest level in T3 *L. lucidus* Turcz. root compared with that in T1 *L. lucidus* Turcz. root collected in S1 and S2, respectively ($p < 0.05$), which indicated that the starch hydrolysis or convert into crude fiber with the harvest time delayed due to a series of chemical and enzymatic alterations. The crude fat content increased continuously by 77.08% and 53.21% to the highest concentration by the end of harvest for *L. lucidus* Turcz. root collected in S1 and S2, respectively, which revealed that the fat synthesized continually with the harvest time delayed.

It was found that the contents of protein and starch in *L. lucidus* Turcz. root collected in S1 were higher than those in *L. lucidus* Turcz. root collected in S2, whereas the content of crude fat in *L. lucidus* Turcz. root collected in S1 was lower than that in *L. lucidus* Turcz. root collected in S2. The contents of protein, starch and crude fat in *L. lucidus* Turcz. root collected from different sites at the same harvest time were differed due to the divergence in sowing time, field management, irrigation and fertilization.

The main minerals including Ca, Mg, K, Fe, Zn, Mn and Cu were analyzed for *L. lucidus* Turcz. root at different harvest times and the results are presented in Table 1. The results revealed a high variability in the content of the investigated minerals with the harvest times. The most common element in *L. lucidus* Turcz. root was found to be potassium with the content ranging from 1271.77 to 1578.38 μg/g DW in S1 *L. lucidus* Turcz. root and 1150.50 to 1432.23 μg/g DW in S2 *L. lucidus* Turcz. root, followed by calcium with the content ranging from 270.11 to 318.37 μg/g DW in S1 *L. lucidus* Turcz. root and 235.53 to 329.28 μg/g DW in S2 *L. lucidus* Turcz. root, iron with the content ranging from 149.16 to 230.46 μg/g DW in S1 *L. lucidus* Turcz. root and 209.65 to 302.41 μg/g DW in S2 *L. lucidus* Turcz. root, magnesium with the content ranging from 96.60 to 102.84 μg/g DW in S1 *L. lucidus* Turcz. root and 92.27 to 93.91 μg/g DW in S2 *L. lucidus* Turcz. root, copper with the content ranging from 23.22 to 44.76 μg/g DW in S1 *L. lucidus* Turcz. root and 15.30 to 28.62 μg/g DW in S2 *L. lucidus* Turcz. root, and manganese with the content ranging from 5.16 to 9.31 μg/g DW in S1 *L. lucidus* Turcz. root and 5.70 to 7.49 μg/g DW in S2 *L. lucidus* Turcz. root.

Minerals are essential regulators of physiological processes in humans. More than one-third of all human proteins require metal ions to function, and lacking these ions may have a significant impact on human health (Konczak & Roulle, 2011). Different mineral patterns clearly demonstrated differential capacity of the *L. lucidus* Turcz. root to absorb ions from the soil. It has been reported that individual plant may have a very different mineral content and the content varies according to the plant source, maturity, soil conditions, cultivar, weather and agricultural practices (Ekholm et al., 2007; Konczak & Roulle, 2011; Mirdehghan & Rahemi, 2007). Therefore, the significant variation in the concentration of the investigated minerals in *L. lucidus* Turcz. root was the result of interaction of various factors.

The changes of protein, starch, crude fat and minerals may significantly affect the nutritive value and processing of *L. lucidus*

Table 1
Contents of protein, reducing sugar, starch, crude fat, main minerals and phenolics in *L. lucidus* Turcz. root at different harvest times.

Site	S1			S2		
Harvest time	T1	T2	T3	T1	T2	T3
Parameters						
Protein (% DW)	11.55 ± 1.02a	10.76 ± 1.33a	14.06 ± 2.05b	8.33 ± 0.98a	6.58 ± 0.44a	7.94 ± 1.22a
Reducing sugar (% DW)	1.73 ± 0.03a	1.73 ± 0.02a	1.80 ± 0.20a	1.71 ± 0.05a	1.67 ± 0.10a	1.74 ± 0.01a
Starch (% DW)	46.07 ± 0.38a	22.77 ± 0.06b	10.40 ± 0.11c	39.61 ± 0.13a	19.67 ± 0.34b	9.87 ± 0.47c
Crude fat (% DW)	1.44 ± 0.09a	2.11 ± 0.21a	2.55 ± 0.10b	2.65 ± 0.21a	3.44 ± 0.65a	4.06 ± 1.26a
Main minerals (μg/g DW)						
Ca	287.23 ± 4.98b	270.11 ± 0.53b	318.37 ± 10.69a	292.06 ± 16.45ab	329.28 ± 9.35a	235.53 ± 16.72b
Mg	96.60 ± 0.02c	98.41 ± 0.23b	102.84 ± 0.15a	93.91 ± 0.75a	93.03 ± 0.23a	92.27 ± 1.39a
K	1578.38 ± 87.09a	1271.77 ± 64.82b	1352.31 ± 33.31ab	1209.13 ± 89.56a	1150.50 ± 11.48a	1432.23 ± 39.52a
Fe	149.16 ± 24.46b	230.00 ± 15.87a	230.46 ± 10.92a	302.41 ± 7.80a	255.10 ± 3.27b	209.65 ± 47.27c
Zn	nd	nd	nd	nd	nd	nd
Mn	9.31 ± 0.40a	8.66 ± 1.63a	5.16 ± 1.87b	7.49 ± 0.40a	5.70 ± 1.12a	6.18 ± 0.25a
Cu	23.34 ± 2.78b	44.76 ± 6.11a	23.22 ± 1.42b	28.62 ± 0.28a	19.28 ± 2.15b	15.30 ± 0.66b
Phenolic content (mg GAE/g DW)						
FP	8.94 ± 0.02a	11.50 ± 2.11a	12.38 ± 1.86a	13.17 ± 0.37a	11.71 ± 0.36b	8.63 ± 0.21c
SEP	3.09 ± 0.06b	3.51 ± 0.09ab	4.13 ± 0.27a	3.36 ± 0.24b	3.39 ± 0.12b	6.82 ± 0.63a
SGP	0.60 ± 0.04b	0.88 ± 0.02a	0.92 ± 0.02a	0.47 ± 0.01b	0.47 ± 0.01b	1.74 ± 0.03a
ICP	2.43 ± 0.16b	3.67 ± 0.05a	2.83 ± 0.08b	2.43 ± 0.20a	3.36 ± 0.08a	2.92 ± 0.37a
TPC	15.06 ± 0.07a	19.56 ± 2.09a	20.28 ± 2.19a	19.43 ± 0.34ab	18.94 ± 0.12b	20.10 ± 0.02a

S1 and S2 refer to the different farming sites. T1, T2 and T3 refer to the different harvest times. nd, not detected. FP, free phenolics; SEP, soluble ester-bound phenolics; SGP, soluble glycoside-bound phenolics; ICP, insoluble cell-wall-bound phenolics; TPC, total phenolic content. Data are expressed as mean values of three independent replicates ± SD. Values within a row with different letters for the same farming site at different harvest times are significantly different at $p < 0.05$.

Table 2
Amino acid composition (g/100 g DW) of the protein from *L. lucidus* Turcz. root at different harvest times.

Amino acid	S1			S2		
	T1	T2	T3	T1	T2	T3
Asp	1.90 ± 0.28	1.96 ± 0.33	3.17 ± 0.64	1.13 ± 0.48	1.11 ± 0.87	1.33 ± 0.77
Thr[a]	0.37 ± 0.04	0.42 ± 0.02	0.52 ± 0.06	0.31 ± 0.05	0.31 ± 0.04	0.33 ± 0.06
Ser	0.38 ± 0.06	0.43 ± 0.04	0.51 ± 0.04	0.32 ± 0.02	0.33 ± 0.03	0.35 ± 0.07
Glu	1.85 ± 0.32	2.06 ± 0.87	3.46 ± 0.68	1.49 ± 0.88	1.25 ± 0.05	1.66 ± 0.53
Gly	0.27 ± 0.02	0.29 ± 0.03	0.35 ± 0.09	0.23 ± 0.02	0.25 ± 0.01	0.25 ± 0.01
Ala	0.63 ± 0.06	0.68 ± 0.04	0.71 ± 0.06	0.56 ± 0.08	0.60 ± 0.03	0.59 ± 0.04
Cys	nd	nd	nd	nd	nd	nd
Val[a]	0.37 ± 0.02	0.41 ± 0.05	0.44 ± 0.03	0.29 ± 0.06	0.32 ± 0.02	0.31 ± 0.02
Met[a]	0.58 ± 0.08	0.56 ± 0.03	0.24 ± 0.01	0.14 ± 0.03	0.27 ± 0.01	0.17 ± 0.01
Ile[a]	0.34 ± 0.04	0.41 ± 0.03	0.26 ± 0.03	0.16 ± 0.02	0.17 ± 0.01	0.18 ± 0.01
Leu[a]	0.37 ± 0.05	0.42 ± 0.01	0.39 ± 0.04	0.24 ± 0.03	0.23 ± 0.02	0.25 ± 0.03
Tyr	0.53 ± 0.01	0.54 ± 0.06	0.51 ± 0.05	0.41 ± 0.07	0.37 ± 0.02	0.44 ± 0.06
Phe[a]	0.60 ± 0.06	0.64 ± 0.07	0.62 ± 0.07	0.48 ± 0.05	0.36 ± 0.04	0.46 ± 0.05
Lys[a]	0.49 ± 0.03	0.53 ± 0.07	0.51 ± 0.08	0.42 ± 0.07	0.19 ± 0.03	0.40 ± 0.08
His	0.36 ± 0.04	0.38 ± 0.02	0.40 ± 0.09	0.28 ± 0.04	0.08 ± 0.01	0.23 ± 0.05
Arg	0.44 ± 0.06	0.51 ± 0.06	0.59 ± 0.04	0.33 ± 0.04	0.23 ± 0.01	0.25 ± 0.04
Pro	0.12 ± 0.03	0.23 ± 0.01	0.22 ± 0.06	0.17 ± 0.03	0.18 ± 0.03	0.18 ± 0.01
Total	9.58 ± 0.98b	10.48 ± 1.22b	12.90 ± 1.78a	6.97 ± 0.93b	6.25 ± 0.63b	7.40 ± 0.81a

S1 and S2 refer to the different farming sites. T1, T2 and T3 refer to the different harvest times. nd, not detected. Data are expressed as mean values of three independent replicates ± SD. Values within a row with different letters for the same farming site at different harvest times are significantly different at $p < 0.05$.
[a] Essential amino acids.

Turcz. root. Therefore, the *L. lucidus* Turcz. root should be harvested at a proper time according to the requirement of nutrition and processing.

3.2. Phenolic compounds at different harvest times

3.2.1. Colourimetric determination of phenolic extracts

The levels of four different fractions of phenolics (FP, SGP, SEP and ICP) and total phenolics were incoordinately influenced by harvest times (Table 1). The FP content increased by 38.48% to the highest level at T3 for the *L. lucidus* Turcz. root collected in S1. In contrast, it decreased by 34.47% to the lowest level at T3 for the *L. lucidus* Turcz. root collected in S2. The contents of SEP and SGP increased continuously by 33.66% and 53.33% by the end of harvest in *L. lucidus* Turcz. root collected in S1, respectively. However, no variation was detected in the contents of SEP and SGP at the first two harvest times. A drastic increase was observed at the third harvest time for *L. lucidus* Turcz. root collected in S2. Disimilar to the changes observed in the contents of FP, SEP and SGP, the ICP content first increased by 51.02% and 38.27% and then decreased by 22.89% and 13.10% in *L. lucidus* Turcz. root collected in S1 and S2, respectively. The TPC increased continuously by 34.66% and reached a maximum at T3 in *L. lucidus* Turcz. root collected in S1, while it first decreased by 2.52% and then increased by 6.12% to the highest level at T3 in *L. lucidus* Turcz. root collected in S2. The correlation analysis results indicated that the change of SEP was significantly correlated ($p < 0.01$) to the SGP (Table 5), which may imply that the SEP and the SGP might possess same metabolic pathway during different harvest times.

It was interesting to note that the decline of FP content coincided with a rise in SEP and SGP content with the harvest time delayed in S2 *L. lucidus* Turcz. root, although not to the same extent, suggesting that the increased soluble bound phenolics might come from the combination of FP with polysaccharide, protein, lignin and so on.

It is known that phenolics are an ecologically significant class of secondary metabolites expressed in all higher plants and its content in plant materials varies depending on several factors, such as the effect of soil, temperature, cultural practices and developmental stage. Variation of phenolic concentration in *L. lucidus* Turcz. root affirms the influence of soil, cultural practices and developmental stage on production and release of these metabolites. The variations of phenolic content in plant materials are closely related to different biosynthesis pathways of phenolic compounds (Hudec et al., 2007). The key enzyme for the phenolic metabolism is phenylalanine ammonia-lyase (PAL), it catalyzes the reductive deamination of phenylalanine to form cinnamic acid (Morello, Romero, Ramo, & Motilva, 2005), and its activity was found to vary greatly with the stage of plant development. It has been reported that various stresses such as irradiation, nutrient deficiencies, wounding, lower temperature exposure, herbicide treatment and viral, fungal and insect attacks can increase either PAL synthesis or PAL activity in various plants (Morello et al., 2005). However, it has also been reported that some stress treatments delay the increase in wound-induced PAL activity (Campos-Vargas, Nonogaki, Suslow, & Salveit, 2005). In view of this, further investigations will be required to understand the mechanism of complex changes in the content of different phenolics in *L. lucidus* Turcz. root at different harvest times.

In addition, the data showed that the *L. lucidus* Turcz. root contains higher amount of FP, which were not in agreement with previous reports (Gao et al., 2011; Santiago et al., 2007; Xu et al., 2010) that a majority of phenolic compounds are present in some plants in bound forms. This discrepancy in the composition of phenolic compounds can be due to the different plant species and varieties.

3.2.2. HPLC analysis of phenolic extracts at different harvest times

In *L. lucidus* Turcz. root phenolic extracts, a total of six individual phenolic compounds were identified. The variation of amounts of different phenolic compounds in different phenolic fractions at different harvest times are shown in Table 3. Results revealed that the content of the investigated phenolic compounds varied greatly with the harvest times. Beyond our expectation, the investigated phenolic compounds were not found in the SGP extract, suggesting that the major phenolic compounds might be flavonoids in this phenolic fraction. In the FP fraction, a total of four, four and three individual phenolic acids were detected in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S1, respectively. While a total of three, four and five individual phenolic acids were detected in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S2, respectively. Chlorogenic acid and *p*-coumaric acid were the major phenolic acids in T1 *L. lucidus* Turcz. root collected in both S1 and S2. Chlorogenic acid, *p*-

Table 3
Phenolic acid content (μg/g DW) of phenolic extracts from *L. lucidus* Turcz. root at different harvest times.

Site	S1			S2		
Harvest time	T1	T2	T3	T1	T2	T3
FP						
Gallic acid	6.29 ± 0.19b	5.71 ± 0.26b	9.8 ± 0.76a	8.13 ± 0.15b	12.16 ± 0.20a	7.72 ± 0.13b
Protocatechuic acid	5.56 ± 0.21c	20.89 ± 1.30b	26.17 ± 1.18a	nd	22.81 ± 0.91b	29.04 ± 0.56a
Chlorogenic acid	51.54 ± 2.48b	63.07 ± 4.03a	67.53 ± 2.36a	56.34 ± 0.92b	48.86 ± 1.34c	66.85 ± 0.74a
Caffeic acid	nd	nd	nd	nd	nd	nd
p-Coumaric acid	26.35 ± 1.9b	33.20 ± 2.75a	nd	14.39 ± 0.26b	48.50 ± 2.09a	47.19 ± 1.69a
Ferulic acid	nd	nd	nd	nd	nd	140.98 ± 6.14
SEP						
Gallic acid	22.23 ± 1.75a	18.66 ± 1.26a	23.33 ± 2.20a	21.28 ± 0.82a	21.38 ± 0.47a	18.13 ± 1.52a
Protocatechuic acid	8.74 ± 0.12a	nd	7.78 ± 0.33a	6.40 ± 0.55a	nd	8.25 ± 0.30a
Chlorogenic acid	64.15 ± 1.76a	55.40 ± 2.26a	45.00 ± 2.43b	33.91 ± 1.22a	37.44 ± 0.65a	33.42 ± 2.33a
Caffeic acid	14.59 ± 1.83a	nd	12.62 ± 0.90a	nd	11.95 ± 0.86	nd
p-Coumaric acid	20.28 ± 0.92a	nd	19.10 ± 0.61a	nd	nd	nd
Ferulic acid	nd	nd	nd	nd	nd	nd
ICP						
Gallic acid	2.12 ± 0.04c	4.80 ± 0.17b	6.49 ± 0.05a	3.53 ± 0.14b	13.03 ± 1.22a	5.39 ± 0.21b
Protocatechuic acid	12.55 ± 0.74b	5.82 ± 0.14c	91.88 ± 2.51a	16.05 ± 1.03c	27.51 ± 0.69a	23.62 ± 0.89b
Chlorogenic acid	78.93 ± 1.79a	51.98 ± 2.64b	nd	143.32 ± 3.28c	243.72 ± 4.22b	319.82 ± 9.99a
Caffeic acid	nd	nd	nd	nd	13.47 ± 0.61b	16.56 ± 1.87a
p-Coumaric acid	nd	nd	nd	nd	27.82 ± 0.76b	37.62 ± 1.08a
Ferulic acid	nd	nd	nd	4.36 ± 0.22c	43.97 ± 1.35a	32.68 ± 2.28b

S1 and S2 refer to the different farming sites. T1, T2 and T3 refer to the different harvest times. nd, not detected. FP, free phenolics; SEP, soluble ester-bound phenolics; ICP, insoluble cell-wall-bound phenolics. Data are expressed as mean values of three independent replicates ± SD. Values within a row with different letters for the same farming site at different harvest times are significantly different at $p < 0.05$.

coumaric acid, and protocatechuic acid were the major phenolic acids in T2 *L. lucidus* Turcz. root collected in both S1 and S2. Chlorogenic acid and protocatechuic acid were the major phenolic acids in T3 *L. lucidus* Turcz. root collected in S1, whereas ferulic acid, chlorogenic acid and *p*-coumaric acid were the major phenolic acids in T3 *L. lucidus* Turcz. root collected in S2. In the SEP fraction, a total of five, two and five individual phenolic acids were identified in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S1, respectively. While a total of three, three and three individual phenolic acids were identified in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S2, respectively. Chlorogenic acid and gallic acid were the abundant phenolic acids in both S1 and S2 *L. lucidus* Turcz. roots gathered at three different times. In the ICP fraction, a total of three, three and two individual phenolic acids were found in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S1, respectively. While a total of four, six and six individual phenolic acids were found in T1, T2 and T3 *L. lucidus* Turcz. roots collected in S2, respectively. Chlorogenic acid and protocatechuic acid were the predominant phenolic acids in T1 *L. lucidus* Turcz. root collected in both S1 and S2. Chlorogenic acid was the predominant phenolic acid in T2 *L. lucidus* Turcz. root collected in S1, whereas chlorogenic acid and ferulic acid were the predominant phenolic acids in T2 *L. lucidus* Turcz. root collected in S2. Protocatechuic acid was the predominant phenolic acid in T3 *L. lucidus* Turcz. root collected in S1, while chlorogenic acid was the predominant phenolic acid in T3 *L. lucidus* Turcz. root collected in S2.

In the present study, the obtained results showed that chlorogenic acid was the predominant phenolic acid among the investigated phenolic compounds in *L. lucidus* Turcz. root. However, rosmarinic acid was the most abundant phenolic acid in the aerial parts of *L. lucidus* Turcz. and *L. lucidus* (Woo & Piao, 2004; Ślusarczyk et al., 2009). Phenolic compounds in *L. lucidus* Turcz. root have not been identified completely. Therefore, it is difficult to compare the phenolic composition between the root and the aerial parts of *L. lucidus* Turcz., and it is necessary to identify more phenolic compounds in the root.

Variations in the level of the individual phenolic acid indicated that different phenolic acids possessed different metabolic pathway, some were decomposed and some were synthesized during the development of *L. lucidus* Turcz. root growing processes. It is well known that cultivar, climate, soil, water availability, cultural practices, development stage and degree of maturity have a significant effect on phenolic composition of plant materials. Significant changes in the phenolic composition of different phenolic fractions from *L. lucidus* Turcz. root at different harvest times could be due to the interaction of various factors.

3.3. Antioxidant activity at different harvest times

The antioxidant activity is influenced by many factors, which cannot be fully described with a single antioxidant assay. Thus, utilizing multiple assays to evaluate antioxidant activity tend to be necessary and may provide exclusive information on their multiple abilities to scavenge different radicals. In the present study, DPPH radical scavenging capacity, FRAP and TEAC assays were employed to test the antioxidant activity of phenolics extracted from *L. lucidus* Turcz. root and the results are shown in Table 4. The scavenging ability assayed herein on DPPH radicals were expressed as IC_{50} values (μg GAE/ml) for comparison. The IC_{50} values of phenolic extracts from *L. lucidus* Turcz. root ranged from 0.45 (SGP from T2 root collected in S2) to 27.49 GAE/ml (FP from T3 root collected in S2). For each phenolic extract, there was difference in DPPH radical scavenging activity among different harvest times. Regarding the FP, the T3 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T2 and T1 *L. lucidus* Turcz. roots collected in both S1 and S2. Regarding the SEP, the T1 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T2 and T3 *L. lucidus* Turcz. roots collected in S1, whereas the T2 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T3 and T1 *L. lucidus* Turcz. roots collected in S2. Regarding the SGP, the T3 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T2 and T1 *L. lucidus* Turcz. roots collected in S1, whereas the T1 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T3 and T2 *L. lucidus* Turcz. roots collected in S2. Regarding the ICP, the T2 *L. lucidus* Turcz. root possessed the highest IC_{50} values, followed by T3 and T1 *L. lucidus* Turcz. roots collected in S1, whereas the T1

Table 4
DPPH radical scavenging capacity, FRAP and TEAC of phenolic extracts from *L. lucidus* Turcz. root at different harvest times.

Site	Harvest time	FP	SEP	SGP	ICP
DPPH radical scavenging capacity IC_{50} value (μg GAE/mL)					
S1	T1	9.49 ± 0.15b	7.66 ± 0.16a	0.92 ± 0.01b	7.03 ± 0.13b
	T2	18.63 ± 0.32a	6.78 ± 0.09b	1.01 ± 0.04b	7.87 ± 0.03a
	T3	20.01 ± 0.57a	5.91 ± 0.26c	1.67 ± 0.04a	7.39 ± 0.22ab
S2	T1	21.27 ± 0.15b	6.97 ± 0.13b	0.88 ± 0.01a	8.24 ± 1.17a
	T2	21.72 ± 0.70b	9.20 ± 0.51a	0.45 ± 0.12b	7.29 ± 0.29a
	T3	27.49 ± 0.23a	7.78 ± 0.23b	0.47 ± 0.02b	7.55 ± 0.88a
FRAP value (μmol Fe(II)/g DW)					
S1	T1	336.77 ± 20.52b	96.89 ± 1.10c	18.47 ± 0.39c	61.84 ± 1.34c
	T2	349.99 ± 12.62b	104.61 ± 0.16b	21.62 ± 0.78b	112.12 ± 3.41a
	T3	409.14 ± 0.79a	119.44 ± 2.82a	30.18 ± 0.39a	79.77 ± 1.09b
S2	T1	435.17 ± 26.77a	102.22 ± 0.47a	9.06 ± 0.78b	76.91 ± 3.16b
	T2	410.93 ± 16.57a	102.98 ± 1.88a	9.97 ± 1.10b	122.92 ± 2.07a
	T3	310.52 ± 1.58b	194.72 ± 3.89b	63.92 ± 3.76a	83.45 ± 1.70b
TEAC value (μmol TE/g DW)					
S1	T1	57.45 ± 1.19c	29.11 ± 1.06b	2.54 ± 0.12b	20.05 ± 1.71b
	T2	93.57 ± 1.19b	30.18 ± 0.71b	3.41 ± 0.15ab	28.12 ± 0.29a
	T3	104.36 ± 2.37a	34.88 ± 0.82a	3.74 ± 0.38a	23.67 ± 0.06b
S2	T1	126.57 ± 4.15a	29.97 ± 0.94b	2.07 ± 0.28b	19.76 ± 0.53b
	T2	94.02 ± 0.59b	24.53 ± 0.24c	1.84 ± 0.14b	27.59 ± 0.71a
	T3	68.67 ± 1.78c	48.84 ± 0.59a	6.71 ± 0.06a	24.05 ± 2.94ab

S1 and S2 refer to the different farming sites. T1, T2 and T3 refer to the different harvest times. FP, free phenolics; SEP, soluble ester-bound phenolics; SGP, soluble glycoside-bound phenolics; ICP, insoluble cell-wall-bound phenolics. Data are expressed as mean values of three independent replicates ± SD. Values within a column with different letters for the same farming site at different harvest times are significantly different at $p < 0.05$.

L. lucidus Turcz. root possessed the highest IC_{50} values, followed by T3 and T2 *L. lucidus* Turcz. roots collected in S2. Obviously, the DPPH radical scavenging activity of FP, SEP, SGP and ICP varied greatly with the harvest times. Unexpectedly, the highest DPPH radical scavenging capacity was found for the SGP with the lowest phenolic content, and the lowest was found for the FP with the highest phenolic content. These results may imply that not only phenolic content but also the type of phenolic constituent present in phenolic extracts may be responsible for the DPPH radical scavenging capacity. Furthermore, the phenolics extracted from *L. lucidus* Turcz. root exhibited higher DPPH radical scavenging capacity than that of BHT (IC_{50} value 110.75 ± 14.83 mg/mL).

In the FRAP assay, the antioxidant activity is measured based on the ability to reduce ferric (III) ions to ferrous (II) ions. The FRAP values of phenolic extracts from *L. lucidus* Turcz. root ranged from 9.06 (SGP from T1 root collected in S2) to 435.17 μmol Fe(II)/g DW (FP from T1 root collected in S2). For each phenolic extract, difference in FRAP value among different harvest times was observed. For the FP, the T3 *L. lucidus* Turcz. root had the highest FRAP values, followed by T2 and T1 *L. lucidus* Turcz. roots collected in S1, whereas the T1 *L. lucidus* Turcz. root had the highest FRAP values, followed by T2 and T3 *L. lucidus* Turcz. roots collected in S2. For the SEP and SGP, the T3 *L. lucidus* Turcz. root had the highest FRAP values, followed by T2 and T1 *L. lucidus* Turcz. roots collected in both S1 and S2. For the ICP, the T2 *L. lucidus* Turcz. root had the highest FRAP values, followed by T3 and T1 *L. lucidus* Turcz. roots collected in both S1 and S2. In addition, the data demonstrated that the highest and lowest ferric reducing ability was observed for the FP and SGP regardless of the harvest times, respectively, which were opposed to the results determined by DPPH radical scavenging activity assay because of different chemistry and reaction conditions. Furthermore, the ferric reducing ability of phenolics extracted from *L. lucidus* Turcz. root was much lower than that of BHT (6.3×10^3 μmol Fe(II)/g).

The TEAC assay, based on ABTS oxidation, has been widely utilized for quantification of antioxidant activity of plant extracts. The TEAC values of phenolic extracts from *L. lucidus* Turcz. root ranged from 1.84 (SGP from T2 root collected in S2) to 126.57 μmol TE/g DW (FP from T1 root collected in S2). As a whole, the trend of change in TEAC value at different harvest times was similar to FRAP value for each phenolic extract, but the extent of change was not in keeping with the former. Similar to the results of FRAP assay, the highest and lowest Trolox equivalent antioxidant capacity was observed for the FP and SGP regardless of the harvest times, respectively. Additionally, the TEAC value of phenolics extracted from *L. lucidus* Turcz. root was lower than that of BHT (784.50 ± 2 2.98 μmol TE/g).

To further investigate the influences of phenolic content on the antioxidant activity of extracts from *L. lucidus* Turcz. root, a correlation coefficient was established by a linear regression analysis between the phenolic content and their activity in each assays, and correlation coefficients (R) are shown in Table 5. The data demonstrated that no significant correlation was found between phenolic content and DPPH radical scavenging activity, whereas significant correlation was found between phenolic content and antioxidant activities (FRAP and TEAC) for each phenolic extract. These results suggested that non phenolic constituents present in the phenolic extracts may be also responsible for a

Table 5
Correlation analysis of phenolics and antioxidant activity.

	SEP	SGP	ICP	TPC	DPPH	FRAP	TEAC
FP	−0.571	−0.579	0.109	0.471	0.134	0.917*	0.960**
SEP		0.956**	0.030	0.469	0.016	0.999**	0.961**
SGP			0.132	0.415	−0.142	0.990**	0.998**
ICP				0.416	0.051	0.905*	0.983**
DPPH						0.892**	0.897**
FRAP							0.974**

FP, free phenolics; SEP, soluble ester-bound phenolics; SGP, soluble glycoside-bound phenolics; ICP, insoluble cell-wall-bound phenolics. TPC, total phenolic content.

* Significant correlation at $p < 0.05$.

** Significant correlation at $p < 0.01$.

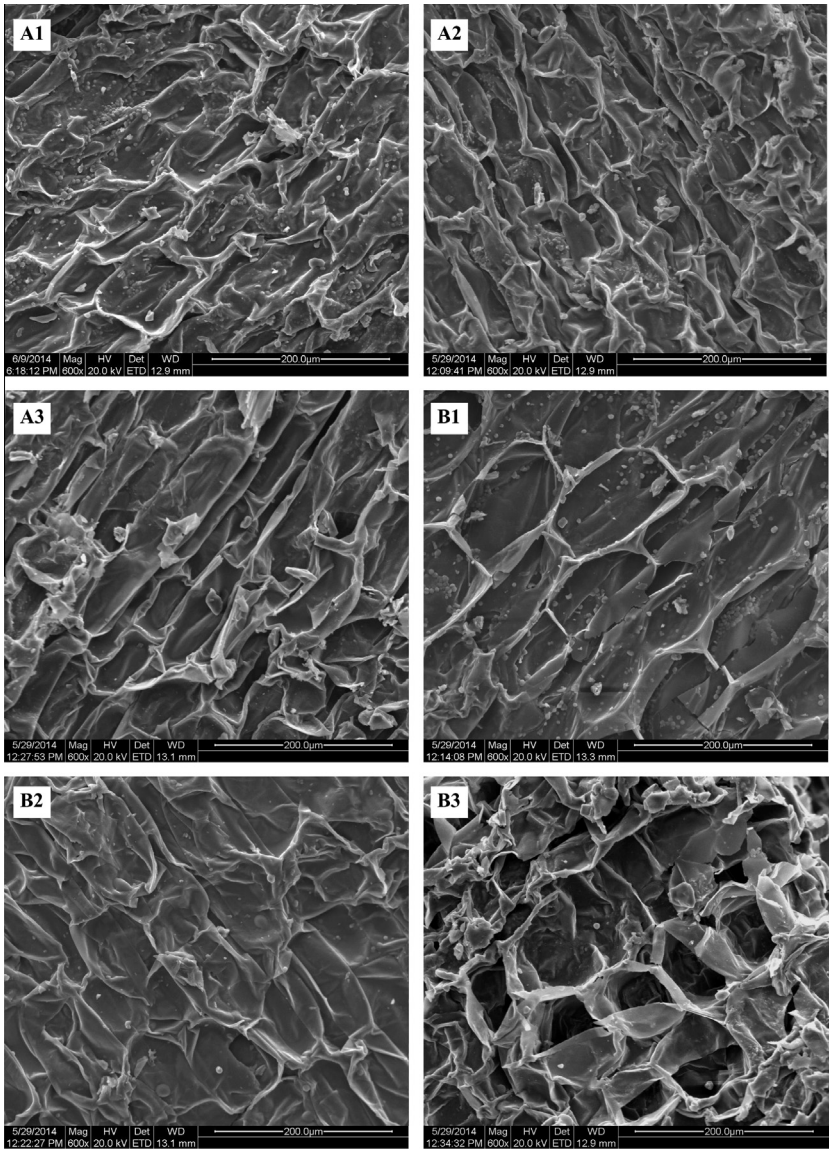

Fig. 1. Scanning electron micrographs of *L. lucidus* Turcz. root at different harvest times. A1–A3, T1–T3 *L. lucidus* Turcz. root harvested from S1, respectively. B1–B3, T1–T3 *L. lucidus* Turcz. root harvested from S2, respectively.

large proportion of the DPPH radical scavenging capacity, and the phenolic compounds make significant contributions to the antioxidant activity determined by FRAP and TEAC. Some studies reported that there was a high correlation between phenolic content and antioxidant activity of some plant products (Du, Li, Ma, & Liang, 2009; Sahreen, Khan, & Khan, 2010; Xu et al., 2010). In other reports, no correlation was observed between phenolic content and DPPH radical scavenging activity (Gao et al., 2011; Nayaka, Sathisha, & Dharmesh, 2010). These differences in correlation between phenolic content and antioxidant activity could be attributed to the divergence of materials, assessment methods, solvent extraction systems, and complicated extracts containing two or more antioxidant substances and so on.

The correlation coefficient was also determined between different antioxidant activity assays. Significant correlations were found among DPPH radical scavenging capacity, FRAP and TEAC assays ($p < 0.01$). The significant correlations between the antioxidant activities are expected because these assays share the same principle (electron transfer reaction) (Gao et al., 2011).

3.4. Microstructure of L. lucidus Turcz. root at different harvest times

In order to investigate the relation of chemical compositions to internal tissue morphous of *L. lucidus* Turcz. root at different harvest times, the microstructure was observed with scanning electron microscopy (SEM). The scanning electron micrographs of *L. lucidus* Turcz. root harvested at three different times are presented in Fig. 1. As can be seen from the figure, the microstructure of *L. lucidus* Turcz. root exhibited a regular and neat honeycomb structure, and varied significantly with the harvest times. A great number of globules were observed in *L. lucidus* Turcz. root collected at T1 (Fig. 1, A1 and B1), whereas the globules were not seen in *L. lucidus* Turcz. root collected at T3 (Fig. 1, A3 and B3). A possible explanation for this phenomenon is that, the globules are starch particles and the starch converted into other compounds via some metabolic pathways in the period of harvest. Therefore, the globules disappeared in the micrograph of *L. lucidus* Turcz. root harvested at T3, which was consistent with the above analytical results of starch.

4. Conclusions

Variation in nutritional compositions, antioxidant activity and microstructure of *L. lucidus* Turcz. root collected from two sites during different harvest times were investigated to provide useful information regarding quality changes during development. The results obtained showed that the main nutrients, phenolics, antioxidant activity and microstructure of *L. lucidus* Turcz. root varied remarkably with the harvest times. The protein content in *L. lucidus* Turcz. root first decreased and then increased to maximum at T3. The reducing sugar content had no significant differences between the three harvest dates studied ($p > 0.05$). The starch content decreased drastically ($p < 0.05$) along with an increase of crude fat content with the harvest time delayed. The major amino acids in *L. lucidus* Turcz. root were Asp and Glu and the highest total amino acid content was found for the root harvested at T3. The most common element in *L. lucidus* Turcz. root was found to be potassium, followed by calcium, iron, magnesium, copper and manganese, and their changes were differed in the period of harvest. The FP and SGP possessed the highest and lowest phenolic content, respectively, and the root harvested at T3 possessed the highest TPC. The change of SEP was significantly ($p < 0.01$) correlated to the SGP at different harvest times. Significant variation in the content of the investigated individual phenolic acid in different phenolic fractions at different harvest times was observed, and the most abundant phenolic acid was chlorogenic acid in *L. lucidus* Turcz. root.

In addition, different phenolic extracts from *L. lucidus* Turcz. root showed various antioxidant activity in different systems. The highest and lowest DPPH radical scavenging activity was found for the SGP and FP, respectively. However, the highest and lowest FRAP and TEAC were found for the FP and SGP, respectively. Results of correlation analysis revealed that there was significant correlation between phenolic content and antioxidant activity (FRAP and TEAC), and different antioxidant assays. The microstructure of *L. lucidus* Turcz. root also varied greatly with the harvest times. Differences in these parameters indicate harvest time have significant influences on functional properties of *L. lucidus* Turcz. root. Therefore, the choice of the harvest time of *L. lucidus* Turcz. root is one of the important factors that should be considered while consuming *L. lucidus* Turcz. root as a functional food. Consequently, further studies need to be performed on the differences of other bioactive compounds among different harvest times.

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