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

Yue-hong Lu [a], Ju-hua Huang [b], Yun-cheng Li [b], Ting-ting Ma [c], Peng Sang [b], Wen-jing Wang [b], Chun-yan Gao [b,*]

[a] College of Agronomy and Biological Science, Dali University, Dali 671003, China
[b] College of Public Health, Dali University, Dali 671000, China
[c] College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China

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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.

* Corresponding author. Tel.: +86 872 2257382; fax: +86 872 2257367.
E-mail address: gcyxixi@163.com (C.-y. Gao).

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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