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Comparison and multivariate statistical analysis of anthocyanin composition in *Lycium ruthenicum* Murray from different regions to trace geographical origins: The case of China

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ABSTRACT

Anthocyanin composition in forty-five *Lycium ruthenicum* Murray (LRM) samples grown in China was identified by high-performance liquid chromatography-electrospray ionisation-mass spectrometry (HPLC-ESI-MS) and quantified by HPLC with a diode array detector (HPLC-DAD). The results showed that the overall pattern of anthocyanin composition of LRM from different provinces was the same, while the individual and total anthocyanin concentrations, were significantly different, indicating an important impact of geographical origin on anthocyanin composition, which can be considered as credible indices for LRM classification. Principal component analysis (PCA) and linear discriminant analysis (LDA) were applied to develop discrimination models for the anthocyanin concentrations. PCA clearly separated the LRM based on its geographical origins. LDA satisfactorily categorized the samples by providing a 100% success rate based on geographical origins. The results obtained could be used to trace the geographical origin of LRM.

1. Introduction

Lycium ruthenicum Murray (LRM) is a therapeutic plant that belongs to the family Solanaceae and is known for its unique nutritional and functional properties (Peng, Liu, Lei, & Wang, 2016). Due to its characteristic sweetness, juiciness, pleasing colour, and potent antioxidant activity, the fruit of LRM has been consumed directly or used as a raw material to provide nutritional foods and drinks (Lv, Wang, Cheng, Huang, & Wang, 2013; Peng et al., 2016). In addition, since LRM is high in functional components such as anthocyanins, polysaccharides, and pectin (Peng, Liu, Shi, & Li, 2014; Peng et al., 2016; Zheng et al., 2011), its fruit has been used as a traditional medicine to treat heart diseases, abnormal menstruation and menopause, as documented in the Tibetan medical classics of "Jing Zhu Ben Cao" (Liu et al., 2013). Of these ingredients, anthocyanins possess a wide range of biological and physiological activities such as antioxidant activity, anticardiovascular disease and antitumour effects (Liobikas, Skemiene, Trumbeckaite, & Borutaite, 2016; Rahman, Ichiyanagi, Komiyama, Sato, & Konishi, 2008; Zheng et al., 2011). In addition, they are responsible for the attractive colour of the fruit (Liobikas et al., 2016). Therefore, systematically studying the LRM anthocyanin composition is important for further commercial utilization and the medical development of LRM. Zheng et al. (2011) investigated the anthocyanin composition of LRM from different areas of the Qinghai Province. However, to our knowledge, no researchers have systematically compared the anthocyanin composition in LRM from different provinces. Such studies are critical and urgent because the berries from different geographical locations within the same genus may display substantially different levels of glycosylation in the proportions of various anthocyanidins (Latti, Jaakola, Riihinen, & Kainulainen, 2010).

LRM is a natural resource in the Qinghai Province, Gansu Province, Inner Mongolia, Xinjiang Uygur Autonomous Region, Ningxia Hui Autonomous Region, and Tibet Autonomous Region. Because of the favourable climate and geographical conditions of the Qinghai-Tibetan Plateau, Qinghai is the most important region for LRM production in China (Lv et al., 2013). Active constituents of LRM from Qinghai were

Abbreviations: LRM, *Lycium ruthenicum* Murray; PCA, principal component analysis; LDA, linear discriminant analysis; DW, dried weight; TR, retention times

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reported to have a variety of biological activities (Zheng et al., 2011). Consequently, LRM from Qinghai has a higher price than those from other provinces due to higher quality level and thus it is more likely to be adulterated. Some unscrupulous farmers and traders fraudulently label their LRM as having been produced in Qinghai to take advantage of the higher price, and this severely compromises the marketability of LRM to consumers. Determining the authenticity of the geographical origin is an important issue in quality control and food safety (Amenta et al., 2015). Therefore, it is important to develop methods to enable the recognition of the geographic origin of LRM in order to protect the benefits to consumers by ensuring quality, providing adequate security controls and developing effective regulations. Up to date, no study has been conducted to identify the geographical origin of LRM in China. Currently, several analytical tools including DNA and chromatographic analyses have been used extensively to determine the geographical origin of foods (Guo, Yuan, Dou, & Yue, 2017). DNA analysis is an effective tool but it does not apply to the same species (Zhang, Liu, Li, & Zhao, 2017). Chromatographic analysis is a straightforward, robust and reproducible technique and thus is a valuable and powerful analytical tool for traceability research (Guo et al., 2017), and it was adopted in this study to determinate the geographical origins of LRM.

Anthocyanins are a class of water-soluble flavonoids that are phenolic compounds and are widely distributed in fruits (Fraige, Pereira-Filho, & Carrilho, 2014). The anthocyanin composition of the fruit is controlled by different factors such as the soil components, geographic conditions, climate and genetic differences (Li, Meng, & Li, 2016; Zheng et al., 2011), thus enabling the composition of anthocyanins to be a viable platform to reliably discriminate fruits via suitable chemometric analysis. In practice, as a major ingredient responsible for quality, anthocyanins have been successfully used as biochemical markers in the quality assessment of plant products (Fraige et al., 2014; Lee, Dossett, & Finn, 2013; Primetta, Jaakola, Ayaz, Inceer, & Riihinen, 2013) and in taxonomic studies (Li et al., 2016; Li et al., 2017). Therefore, anthocyanins were considered as chemical markers in this study to differentiate LRM from different geographical areas, which would provide valuable information about the adulteration of LRM.

The objectives of the present study were 1) to compare the anthocyanin composition of LRM from different provinces of China; 2) to develop an adequate method for LRM classification based on its geographical origins with data obtained from the anthocyanins. Thus, chromatographic analysis was applied to detect the anthocyanin profiles, as well as the concentrations of individual anthocyanins of LRM from different provinces in China. Multivariate statistical analyses, including principal component analysis (PCA) and linear discriminant analysis (LDA), were used to distinguish the geographical origin of the LRM samples.

2. Materials and methods

2.1. Plant materials

Forty-five samples of LRM were collected from five different provinces (Fig. 1) of China in 2016. Samples of LRM from the Tibet Autonomous Region was excluded in this study were excluded due to their low yield. Three major counties for LRM production from each province were chosen as sampling areas. Three samples were randomly selected in each county. We ensured that the counties of each province have similar climatic conditions including altitude, annual mean temperature, and annual rainfall. These environmental parameters of geographical locations of LRM sampling are summarized in Fig. 1. The fully ripened fruits were hand-picked and freeze-dried with an LGJ-18C vacuum freeze drier (Sihuan, Beijing, China), crushed to a fine powder using an XS-10 pulverizer (Zhaoshen Technology Co., Ltd., Shanghai, China) and sieved through a 40-mesh sieve. The resulting powders were stored at −80 °C for further analysis.

2.2. Chemicals

Cyanidin 3-glucoside was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and acetonitrile used for high-performance liquid chromatography (HPLC) analysis were of chromatographic grade. All of the other reagents used were analytical grade.

2.3. Extraction

The anthocyanins were extracted using the method described by Zhang et al. (2007) with slight modifications. A total of 1.5 g freeze-dried powder of each sample was extracted three times with 15 mL methanol and formic acid (98:2, v/v) at room temperature in a 50-mL conical beaker with its orifice sealed by parafilm for 24 h in the dark. The clear liquid from the three extractions was combined and centrifuged at 3000*g* for 10 min at 4 °C using a centrifuge (LXJ-IIB, Shanghai Anting Scientific Instrument Factory, China) to remove the fruit residues, protein and polysaccharide sediments. The clear suspensions were collected and stored at −20 °C to determine the anthocyanin composition.

2.4. HPLC analysis

Anthocyanins in the samples were analysed by HPLC at the detection wavelength of 525 nm using the method previously described by Zheng et al. (2011) with some modifications. The HPLC analysis was carried out using an UltiMate 3000 HPLC system coupled with an ultraviolet-visible diode-array detector (HPLC-DAD), a P680 HPLC pump, and an AT-330 thermostatted column compartment (Thermo Scientific, Waltham, USA). Chromatographic separation was performed using a reverse phase C18 column (250 × 4.6 mm, 5 μm, Merck KGaA, Darmstadt, Germany) at 35 °C. After filtration through a 0.22-μm nylon membrane filter (Jinteng Laboratory Equipment Co., Ltd., Tianjin, China), an aliquot of 5 μL from an extract prepared previously was injected into the HPLC at a flow rate of 0.8 mL/min. The mobile phase was composed of solvent A (10% aqueous formic acid with 0.1% trifluoroacetic acid (liquid form, > 99.0%, Kefeng Co., Shanghai, China)) and solvent B (15% methanol in acetonitrile). Gradient elution was performed as follows: 0–3 min, 3–11.5% B; 30–40 min, 11.5% B; 40–60 min, 11.5–15.5% B; 60–70 min, 15.5–16% B; 70–80 min, 16–23% B; 80–100 min, 23–3% B.

2.5. HPLC-MS analysis

Anthocyanin identification was performed using an Agilent-6460 QQQ system on an ion trap mass spectrometer coupled with an Agilent-1260 HPLC-diode array detector-electrospray ionisation (HPLC-DAD-ESI-MS), and a high-resolution electrospray ionization time of flight-mass spectrometry (ESI-ToF-MS) (Agilent 6550 iFunnel Q-TOF) coupled with an Agilent-1260 HPLC (HPLC-ESI-ToF-MS) (Agilent Technologies, USA). The chromatographic separation conditions were the same as those used in the HPLC analysis described in *2.4*. Positive mode mass spectra of the eluent were recorded in the range of *m/z* 0–1200. Nitrogen was used as the sheath gas with a flow rate of 8 L/min. The capillary temperature was set at 350 °C, and the spray voltage was 4 kV. The rest of the MS conditions for HPLC-DAD-ESI-MS were set as follows: octopole RF amplitude, 150 Vpp; skim 1 voltage, 47.7 V; skim 2 voltage, 6.0 V; capillary exit, 127.3 V; cap exit offset, 79.6 V. The rest of the MS conditions for HPLC-ESI-ToF-MS were set as follows: Dual AJS ESI (Seg): gas temperature: 280 °C; drying gas: 13 L/min; nebulizer: 20 psig; sheath gas flow: 12 L/min; sheath gas temperature: 350 °C. Dual AJS ESI (Eept): VCap: 4 kV; nozzle voltage: 1 kV. MS TOF: fragmentor: 350 V; skimmer: 0 V; OCT TRF Vpp: 750 V.