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Discrimination of geographical origins of Chinese acacia honey using complex $^{13}C/^{12}C$, oligosaccharides and polyphenols

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ABSTRACT

The aim of this study was to predict the geographical origin of acacia honey of China through analysis of physicochemical parameters combination with chemometrics. Samples from six different origins were investigated on parameters of $\delta^{13}C$ value, oligosaccharides and polyphenols, using EA-IRMS/LC-IRMS, GC–MS and HPLC–MS, respectively. The results indicated that the $\delta^{13}C$ value of honey from Gansu region were lower than those of other regions. Oligosaccharides of honey from Shanxi and Shaanxi regions were both higher than other four regions. Polyphenols of honey from Shandong region was the highest and were better parameters than both $\delta^{13}C$ and oligosaccharides in discrimination of geographical origins. Partial Least Square Discriminant Analysis (PLS-DA) showed that when all 31 different parameters were combined, a correct classification rate of 94.12% could be achieved using external cross validation method. In conclusion, the method in discrimination of geographical can be used to provide reliable and useful reference information.

1. Introduction

Honey is a healthy natural and nutritious food produced by honeybee from nectar of plants. Honey contains 60–80% of carbohydrates, 17–20% of water, 0.3–0.8% of proteins, 0.2% of minerals and minor quantities of amino acids, phenols, pigments, vitamins, volatile substances, and others (Ball, 2007; Bogdanov, Jurendic, & Sieber, 2008; Khan, Anjum, Rahman, & Ansari, 2017). Studies have shown that honey has health-promoting effects that prevent gastrointestinal tract, throat from diseases, improve sleep quality, treat infectious trauma, and others (Meo, Alasiri, Mahesar, & Ansari, 2017). In general, honey can be divided into two categories: unifloral honeys and multifloral honeys. Different unifloral honeys differ in physical and chemical aspects such as color, taste, aroma, content of nutrients and so on (Escuredo, Dobre, & Fernándezgonzález, 2014; Gan, Yang, & Li, 2016). Acacia honey is light amber color, mellow flavor and is one of the most popular honey varieties in China. This is the important reason why market price of Chinese acacia honey is higher than that of other unifloral honeys. It should be mentioned that acacia honey was one of the honey varieties that were most consumed nationally and exported in China. There are certain regional characteristics of honey in terms of the content, which were given from the influence of climate, altitude and other environmental factors (Salonen, Virjamo, Tammela, Fauch, & Julkunentiitto, 2017).

As already demonstrated, there were differences in the content of and properties among different floral and geographical origins of honey. Sun, Tan, Zhang, and Zhang (2016) analyzed the content of phenolic compounds in 18 Chinese acacia honey samples by high-performance liquid chromatography with photo-diode array detection (HPLC-PDA) and solid-phase extraction (SPE) method. The study confirmed 19 polyphenols existed in acacia honey samples. But only seven polyphenol were identical in all samples. The report implied the possibility to study the identification of geographical influences on honey. Kropf, Korošec, and Bertoncelj (2010) investigated $\delta^{13}C$, $\delta^{15}N$ and physico-chemical parameters such as moisture content, pH value, free acids of black locust, lime and chestnut honey from four different

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Slovenian natural-geographical macroregions by isotope ratio mass spectrometry (IRMS) and total reflection X-ray fluorescence spectrometry (TXRF) method. This paper reported that different origins have different characteristics in terms of isotope ratio and nutrients. Juan-Borrás, Domenech, Hellebrandova, and Escriche (2014) investigated sugar and volatile composition of acacia, sunflower and tilia honeys in different countries and proved that it could be a potentially useful tool for the classification of varieties of honey. Some study also found that the content of composition and properties in honey could be determined by different methods and it was very important to choose method in detection of content (Siddiqui, Musharraf, Choudhary, & Rahman, 2017). Carbon stable isotope ratio technology was initially applied to test the adulteration of C4 syrup in honey (Doner & White, 1977). With the rapid public awareness on the provenance of honey products and brand building requirements from the industry, stable isotope technology has become one of most reliable solutions and plays an important role in the identification of honey varieties and identification of origin (Camin, Boner, & Bontempo, 2017). Schellenberg, Chmielus, and Schlicht (2010) used $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, $\delta^{36}S$ to discriminate honey from different European regions. Dinca, Ionete, Popescu, Costinel, and Radu (2014) applied complex stable isotope ratio and chemometrics to discriminate honey from different origins in Romania.

In short, previous reports on acacia honey mainly focused on nutrient composition, variety identification and adulteration (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Dong, Luo, & Xian, 2016; Tran, Duke, Abu-Mellal, & Duke, 2012). Study on the discrimination of the geographical origin of Chinese acacia honey has not been reported so far.

Therefore, a new method was used to discrimination of origins of Chinese acacia through analysis of carbon stable isotope, oligosaccharides and polyphenols combined with discriminant analysis of partial least squares in this study. The stable carbon value ($\delta^{13}C_{honey}$, $\delta^{13}C_{glucose}$, $\delta^{13}C_{protein}$ and δDiscrimination of Geographical Origins of Chinese Acacia Honey Using Complex 13C/12C, Oligosaccharides and Polyphenols$C_{fructose}$), concentration of 9 oligosaccharides and 18 polyphenols in 71 acacia honey samples (Robinia pseudoacacia L.) of different geographical origins were determinate by elemental analyzer/liquid chromatography – isotope ratio mass spectrometry (EA/LC-IRMS), gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography with mass spectrometry (HPLC–MS), respectively. Statistical treatments were then applied to these data to explore the most effective factors that could distinguish the geographical origins of acacia honeys.

2. Materials and methods

2.1. Honey samples

In this paper, acacia honey samples were collected from six geographical regions of China: namely Gansu, Henan, Liaoning, Shandong, Shaanxi, and Shanxi. The sampling areas are the most important honey-producing areas in China. Liaoning and Shandong are located in coastal regions. While Gansu, Shanxi and Shaanxi belong to the northern of China, Henan belongs to the plain region, which is at the junction with Shandong, Shanxi and Shaanxi. There are significant differences for these sampling regions in terms of geochemical characteristics. A total of 71 acacia honey samples were collected directly from different beekeepers during harvest period: 10 samples were collected in Gansu, 12 in Henan, 10 in Liaoning, 15 in Shandong, 10 in Shanxi, and 14 in Shanxi. The samples were stored at 4 °C until analysis.

2.2. Reagents and solutions

All chemicals used in this study were of analytical reagent grade unless otherwise stated. Deionized water (18.2 MΩ/cm) was obtained from a Milli-Q Plus system (Millipore, Bedford, MA). Orthophosphoric acid, sodium peroxodisulfate, ethanol, hexane, pyridine and HMDS + TMS + pyridine, gallic acid, protocatechuic, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic, rutin, benzoic acid, myricetin, morin, quercetin, naringenin, apigenin and kaempferol, chrysin, pinocembrin, galangin and caffeic acid phenethyl ester (CAPE) were supplied by Sigma-Aldrich (Munich, Germany). Maltulose, turanose, palatinose, isomaltose, kestose, erlose, melezitose, maltotriose, panose and β-phenyl glucoside were obtained from Accelerating Scientific and Industrial Development thereby Serving Humanity Ltd (Beijing, China). Tungstic acid, sulfuric acid, hydrochloric acid, acetonitrile and formic acid were purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

2.3. Carbon isotope ratios measurement

Carbon isotope ratios were reported in $\delta^{13}C$ notation in units of parts per thousand (‰) relative to the accepted international standards, the Pee Dee Belemnite (PDB). The delta values were calculated as$\delta x = (R_{sample}/R_{reference} - 1) \times 100$. According to the equation, R_{sample} represent the result of the isotopic ratio mass spectrometer measured for sample and $R_{reference}$ is that of an international standard. In the study, R_{sample} represents $\delta^{13}C_{honey}$, $\delta^{13}C_{protein}$, $\delta^{13}C_{glucose}$ and $\delta^{13}C_{fructose}$, respectively.

a. The values of $\delta^{13}C_{honey}$ and $\delta^{13}C_{protein}$ were obtained through the AOAC Official Method 998.12 (1999). This method is based on the determination of $^{13}C/^{12}C$ ratio in C atom of CO_2 gas produced as a result of combustion of acacia honey and precipitated protein by means of a mass spectroscopy device. An IRMS (Thermo Electron Corporation, USA) device has been used for the analysis with 2 parallels.

b. For $^{13}C/^{12}C$ determination of monosaccharide are in accordance with the reference methods (Elflein & Raezke, 2008). The honey samples were diluted with deionized water, filtered through 0.45 μm filters, placed into glass vials, and analyzed by LC- IRMS (Thermo Electron Corporation, USA).

2.4. Oligosaccharide compound measurement

The oligosaccharide extraction was carried out according to the reference method (Guadalupe, Martínez-Pinilla, & Garrido, 2012; Fernandez, Obel, Scheller, & Roepstorff, 2004). In brief, 0.5 g honey sample was completely dissolved in 25 mL 80% Aqueous-Ethanol solution. Next, the solution was centrifuged at 10,000 rpm for 10 min. Then, 100 μL supernatant were concentrated under vacuum at 60 °C in a rotary evaporator. The concentrated samples was added 20 μL β-phenyl glucoside (0.25 mg/mL) and 200 μL 2% hydroxylamine hydrochloride (dissolved in pyridine) with reacted at 70 °C for 30 min. Then 300 μL HMDS + TMS + pyridine (7:3:1) was added and reacted at 70 °C for 40 min. After this, the solution was centrifuged at 8000 rpm for 5 min. 200 μL supernatant was five times diluted with hexane on the glass vials, and analyzed by GC–MS (Shimadzu, Japan).

2.5. Polyphenol compound measurement

The polyphenols extraction was performed as described by the reference method (Baltrušaitytė, Venskutonis, & Čeksterytė, 2007; Oroian & Ropciuc, 2017; Wen, Zhang, & Li, 2017). Oasis HLB solid phase extraction column was activated sequentially by methanol and hydrochloric acid (pH = 2). Oasis HLB solid phase extraction column were used for purification and concentration of polyphenols. 5.0 g of samples was completely dissolved in 30 mL hydrochloric acid aqueous solution (pH = 4.9). Then, the subsequently was centrifuged at 10,000 rpm for 10 min. Afterwards, 10 mL supernatant was slowly filtered through the column packed as previously described. The column was washed with 10 mL of distilled water to remove all sugars and other polar compounds of honey. The phenolic compounds were eluted from the