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Phenolics and abscisic acid identified in acacia honey comparing different SPE cartridges coupled with HPLC-PDA

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ABSTRACT

The major components of honey are sugar and water, but some phenolic compounds may be responsible for honey quality. We investigated the effects of four reversed-phase (RP) and four reversed-phase and anion-exchange (RP-AE) solid-phase extraction (SPE) cartridges as a pre-concentration technique for honey phenolics. Eleven acacia honey samples were collected from different apiaries of China and their levels of phenolics and abscisic acid were analyzed using high-performance liquid chromatography (HPLC). Our results reveal that RP-AE SPE cartridges are superior to RP SPE cartridges for the pre-concentration of honey phenolics. The improved pre-concentration effect of RP-AE SPE cartridges may be a novel finding of our research. The Strata-X-A cartridge may be used in the concentration of low content phenolics of complex food matrices. We identified *cis-trans*-abscisic acid and 19 phenolics occurring in acacia honey samples. Seven phenolic compounds and abscisic acid were identified in almost all samples, representing 50% of total phenolics. Abscisic acid possesses the highest average content of 146.0 μg/100 g, and pinobanksin also presented the highest average of 53.1 μg/100 g among all flavonoids. Moreover, we infer that abscisic acid could be the solid ingredient for adulteration identification and quality control of acacia honey.

1. Introduction

Honey is a natural sweetener produced by honeybees from nectar or honeydew consisting primarily of high concentrations of sugars and water (Kus et al., 2014). Amino acids, minerals and phenolics are also present in small amounts. Nonetheless, the phenolics probably are one of the most prominent constituents contributing to honey quality, especially to the color and flavor (Prasain et al., 2004). In fact, these compounds may be naturally inherent markers for the botanical origin of honey, and they can be detected for adulteration in monofloral honeys (Jasicka-Misiak et al., 2012; Kaskoniene and Venskutonis, 2010; Wang and Li, 2011). Therefore, identification and quantification of phenolic compounds in honey is of great interest. Unfortunately, these compounds are difficult to detect in honey because of their low concentrations (Sergiel et al., 2014; Tomas-Barberan et al., 2001).

In recent years, many studies have focused on the pre-concentration of phenolics from the complex honey matrix. The main approaches included using various macroporous adsorption resins (Ferreres et al., 1994; Kumazawa et al., 2012; Tomas-Barberan et al., 1992) and solid-phase extraction (SPE) (Gonzalez Paramas et al., 2006; Sadiki and Martin, 2013). Macroporous resin adsorption methods often use such resins as AB-8, XAD-2 and XAD-4. The methods involve multiple extraction procedures and organic solvents such as diethyl ether, ethyl acetate and *n*-hexane (Iurlina et al., 2009; Yao et al., 2004). One disadvantage of this method is its use of considerable amounts of honey and solvents. Another disadvantage is that some phenolic acids may not be retained on the Amberlite XAD-2 resin and the recovery of flavonoids is probably lower than expected at approximately 80% (D'arcy, 2005). Compared to the use of the macroporous resin adsorption method, SPE procedures with commercial cartridges

Abbreviations: SPE, solid phase extraction; RP, reversed phase; RP-AE, reversed phase & anion-exchange; HPLC-PDA, high performance liquid chromatography with photo-diode array detection.

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seem to be the simpler, faster, safer and less expensive method to enrich honey phenolics. Other studies evaluated various SPE cartridges for pre-concentration of phenolic acids and flavonoids from honey (Bertoncelj et al., 2011; Michalkiewicz et al., 2008). For example, when compared to the XAD-2 macroporous adsorption resins, the Oasis HLB cartridge presented a stronger absorption capacity for flavonoids of spiked honey samples (Michalkiewicz et al., 2008). The same results were obtained in a study by Pulcini et al. (2006), when the macroporous adsorption resins (XAD-2) were replaced by a Strata SPE cartridge (SDB-L).

Therefore, various solid sorbents have been used for the enrichment of phenolics, such as Bond Elut octadecyl C18, Oasis HLB and Strata-X. The C18 silica sorbent was found to be less appropriate for preconcentration of phenolics, with recoveries above 40%. In contrast, the Oasis HLB showed high recoveries of all phenolics with an average percentage of 70% (Michalkiewicz et al., 2008). The abilities of the Oasis HLB, Oasis MCX and Oasis MAX to recuperate the analyses from a standard solution containing phenolic acids were compared and the data demonstrated that the Oasis HLB resulted in the highest recoveries of 4-hydroxybenzoic acid (Munoz-Gonzalez et al., 2014). The above-mentioned SPE cartridges were reversed phase SPE (RP SPE) cartridges. Recently, several SPE cartridges with new polymeric sorbents have been developed, such as reversed phase and anion-change SPE (RP-AE SPE) cartridges. Although an improved SPE pre-concentration procedure was proposed to extract flavonoids and organic acids of honey through an RP SPE column (SDB) coupled with an RP-AE SPE column (SAX) (Pulcini et al., 2006), little research has focused on the performance of RP-AE SPE cartridges for the pre-concentration of honey phenolics.

The aim of the present study was to evaluate the effects of four RP SPE cartridges and four RP-AE SPE cartridges as a pre-concentration technique for phenolics of acacia honey, which is one of the predominant honeys found in the north of China. To this end, 11 acacia honey samples were collected from different apiaries of China, and their phenolic compounds and abscisic acid levels were analyzed by high-performance liquid chromatography with photo-diode array detection (HPLC-PDA).

2. Materials and methods

2.1. Reagents, chemicals and materials

Standards of alpinetin (C8249, ≥98.0%), benzoic acid (242381, ≥99.5%), caffeic acid (C0625, ≥98.0%), cinnamic acid (C80857, ≥99.0%), chrysin (C80105, ≥97.0%), ferulic acid (46278, ≥99.0%), galangin (282200, ≥95.0%), isoferulic acid (103012, ≥97.0%), kaempferol (60010, ≥97.0%), phenethyl caffeate (C8221, ≥97.0%), pinobanksin (68530, ≥95.0%), pinocembrin (P5239, ≥95.0%), *p*-coumaric acid (C9008, ≥98.0%), rutin (380709, ≥99.0%), vanillic acid (94770, ≥97.0%), 3,4-dimethoxycinnamic acid (D133809, ≥99.0%), and 2-*cis*,4-*trans*-abscisic acid (862169, ≥98.0%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); benzyl caffeate (107843-77-6, ≥98.0%) was obtained from Funakoshi Company (Tokyo, Japan); pinobanksin-3-*O*-acetate (52117, ≥97.0%) was obtained from BioBioPha Co., Ltd. (Kunming, China); and 5-methoxy pinobanksin (≥95.0%) was prepared in-house (Sun et al., 2015).

Methanol (analytical grade and HPLC grade) was obtained from Thermo Fisher Scientific Inc. (Fair Lawn, NJ, USA). Formic acid (analytical grade) and acetic acid (HPLC grade) were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Ultrapure water was purified using a Milli-Q-Integral System (Millipore, Billerica, MA, USA).

The cartridges were as follows: Strata-X (60 mg/3 mL) and Strata-X-A (60 mg/3 mL) from Phenomenex Inc. (Torrance, CA, USA), Oasis HLB (60 mg/3 mL) and Oasis MAX (60 mg/3 mL) from Waters Corporation (Milford, MA, USA), WondaSep Pharma (60 mg/3 mL), WondaSep MAX (60 mg/3 mL) from Shimadzu Corporation (Tokyo, Japan), BRP and P-SAX from Welch Materials, Inc. (Shanghai, China). A 24-port Visiprep™ solid-phase extraction vacuum manifold from Sigma-Aldrich-Supelco (St. Louis, MO, USA) was used for all pre-concentration procedures.

2.2. Acacia honey samples

A total of 11 acacia (*Robinia pseudoacacia* L.) honey samples were obtained from 11 different apiaries during May 2014. These apiaries were located in Beijing (115°42′–117°24′*E*, 39°24′–41°36′*N*, samples A, B and C), Gansu province (92°13′–108°46′*E*, 32°31′–42°57′*N*, samples D, E, F and G), Shanxi province (110°14′–114°33′*E*, 34°34′–40°44′*N*, samples H and I), and Hebei province (113°27′–119°50′′*E*, 36°05′–42°40′*N*, samples J and K) (see Fig. S1 in Supplementary material in the online version at DOI: http://dx.doi.org/10.1016/j.jfca.2016.08.006). According to the locations of their hives and the floral sources available in the bee forage area, the botanical origin of these samples was declared and recorded by professional beekeepers. After acquisition, pollen analysis was accomplished with a microscope and the honey samples were stored at 4 °C in the dark until used.

2.3. Pre-concentration procedures

Honey samples were pre-concentrated with four RP SPE cartridges including the Strata-X, Pharma, Oasis HLB and BRP, or four RP-AE SPE cartridges, including the Strata-X-A, Oasis MAX, P-SAX and WondaSep MAX.

The SPE method was carried out according to the previous study (Dimitrova et al., 2007) with minor modifications. Honey samples of 30 g were mixed with 120 mL of ultrapure water, and then the solution was adjusted to pH = 2 with concentrated HCl for the RP SPE cartridges or adjusted to pH = 7 with 5% ammonium (v/v) for the RP-AE SPE cartridges. The fluid samples were centrifuged at 8000 × *g* for 10 min to remove the solid particles. The supernatants were loaded onto the previously conditioned cartridges (under these conditions: 3 mL of methanol, equilibrated as follows: 3 mL of acidified ultrapure water (pH = 2) for the RP SPE cartridges, or 3 mL of ultrapure water (pH = 7) for the RP-AE SPE cartridges). After loading, these cartridges were washed with 4 mL of acidified ultrapure water (pH = 2) for the RP SPE cartridges to remove sugars and other polar compounds of honey that were not absorbed on the sorbents, or washed with 4 mL of ultrapure water (pH = 7) for the RP-AE SPE cartridges. Then, phenolic compounds retained on the cartridges were eluted with 5 mL of formic acid: methanol (1:9, v/v). The eluate was dried at 50 °C to a constant weight in a vacuum-drying oven (KL512, Beijing Kanglin Technology Co., LTD, Beijing, China) and then redissolved in 2 mL of methanol with 2% acetic acid. All solutions were filtered through a 0.22-μm filter (Millipore, Carrigtowhill, Cork, Ireland) prior to HPLC injection.

2.4. Recoveries of phenolics using model honey with caffeic acid-rutin-glucose solution

For accurately evaluating the recoveries of phenolics, the model honey was prepared to simulate honey consisting of 20% glucose, rutin (1 μg/mL) and caffeic acid (0.5 μg/mL). Half of the model honey was adjusted to pH = 2, and the other half was adjusted to pH = 7. According to the pre-concentration procedures, the model honey solution was subjected to different types of SPE cartridges. The concentrations of rutin and caffeic acid were evaluated based on the peak area at a wavelength of 280 nm using external