Food Chemistry 194 (2016) 167–174

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Identification of monofloral honeys using HPLC–ECD and chemometrics

Jing Zhao [a], Xiaojing Du [a], Ni Cheng [a,b], Lanzhen Chen [c], Xiaofeng Xue [c,d], Jing Zhao [c,d], Liming Wu [c,*], Wei Cao [a,b,*]

[a] Institute of Analytical Science, Shaanxi Provincial Key Lab of Electroanalytical Chemistry, Northwest University, Xi'an 710069, Shaanxi, China
[b] Department of Food Science and Engineering, School of Chemical Engineering, Northwest University, Xi'an 710069, Shaanxi, China
[c] Institute of Apiculture Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China
[d] Risk Assessment Laboratory for Bee Products Quality and Safety of Ministry of Agriculture, Beijing 100093, China

ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form 31 July 2015
Accepted 4 August 2015
Available online 4 August 2015

Chemical compounds studied in this article:
Cinnamic acid (PubChem CID: 444539)
Syringic acid (PubChem CID: 10742)
p-Coumaric acid (PubChem CID: 637542)
Ferulic acid (PubChem CID: 445858)
p-Hydroxybenzoic acid (PubChem CID: 135)

Keywords:
Honey
Fingerprints
Electrochemical detection
Chemometrics
Floral origin

ABSTRACT

A total of 77 jujube, longan and chaste honey samples were collected from 18 different areas of China. Thirteen types of phenolic acids in the honey samples were analysed using high-performance liquid chromatography with electrochemical detection (HPLC–ECD). Moreover, HPLC–ECD fingerprints of the monofloral honey samples were established. From the analysis of the HPLC–ECD fingerprints, common chromatography peak information was obtained, and principal component analysis and discriminant analysis were performed using selected common chromatography peak areas as variables. By comparing with phenolic acids as variables, using a chemometric analysis which is based on the use of common chromatography peaks as variables, 36 honey samples and 41 test samples could be correctly identified according to their floral origin.

1. Introduction

Foodstuffs that are rich in both nutritional value and medicinal value are popular in today's society. Among those, honey is a notable example. Honey primarily consists of sugars but it contains more than 180 other constituents, including minerals, vitamins, enzymes, organic acids and various types of amino acids (Anklam, 1998) and polyphenols, such as flavonoids and phenolic acids (da C. Azeredo, Azeredo, de Souza, & Dutra, 2003). Honey is valuable in the treatment of heart disease, cancer, cataracts, several inflammatory diseases and possibly gastric ulcers and gastritis. Furthermore, the curative actives of honey including its antimicrobial properties, anti-inflammatory activities, and wound and burn healing properties are favourable (Andrade-Neto et al., 2004; Liu, Ye, Li, Wang, & Peng, 2013; Nasuti, Gabbianelli, Falcioni, & Cantalamessa, 2006; Postmes, Bogaard, & Hazen, 1993; Kassim, Achoui, Mustafa, Mohd, & Yusoff, 2010; Kücük et al., 2007).

In general, the taste of monofloral honey is more pure and its commodity value is higher than multiflora honey, therefore, the identification of monofloral honey deserved greater attention. Various methods have been established for determining the floral origin of monofloral honeys, such as electrical impedance spectroscopy (Scandurra, Tripodi, & Verzera, 2013), high-performance liquid chromatography (HPLC) (Martos, Ferreres, & Tomás-Barberán, 2000), HPLC–MS–MS (Truchado, Ferreres, Bortolotti, Sabatini, & Tomás-Barberán, 2008) and near-infrared spectroscopy. (Escuredoa, González-Martín, Rodríguez-Flores, & Seijoa, 2015). Appropriate floral markers are a preferable choice in the identification of monofloral honey using a variety of detection methods. Carbohydrates have been used as floral markers. Glucose is an indicator of rape (*Brassica napus*), blue curl (*Trichostema lanceolatum*) and dandelion (*Taraxacum officinale*) honeys because the amounts of glucose in these three honeys are different than in other kinds of honey (Cavia et al., 2002); Volatile compounds have also been used as floral markers. Methyl anthranilate is a potential marker

* Corresponding authors at: Department of Food Science and Engineering, School of Chemical Engineering, Northwest University, Xi'an 710069, Shaanxi, China (W. Cao).
E-mail addresses: apiswu@126.com (L. Wu), caowei@nwu.edu.cn (W. Cao).

http://dx.doi.org/10.1016/j.foodchem.2015.08.010
0308-8146/

for citrus honey (Alissandrakis, Tarantilis, Harizanis, & Polissiou, 2007), 2,6,6-trimethyl-2,4-cycloheptadien-1-one for almond tree honey and methyl salicylate for willow honey. (de la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007). Compared with other floral markers, the phenolic compounds as floral markers are more common. Gallic acid could be a favourable marker for Manuka honey (Oelschlaegel et al., 2012); Myricetin-3-O-methylether and ellagic acid could be used as floral origin markers of heather honey (Ferreres, Andrade, & Tomás-Barberán, 1996); and 2-methylbutyric acid and ophorone were detected in raspberry, rape and alder buckthorn honeys but not in heather honey (Seisonen, Kivima, & Vene, 2015).

Chromatographic fingerprinting is an efficient and widely used method in quality inspection (Sun et al., 2014). Many studies have been conducted on the chromatographic fingerprinting of honey products using HPLC–DAD (Kus et al., 2014), three-dimensional synchronous fluorescence spectroscopy (Sergiel, Pohl, Biesaga, & Mironczyk, 2014) and near-infrared spectroscopy (Woodcock, Downey, & O'Donnell, 2009). However, there are few reports regarding chromatographic fingerprinting with HPLC–ECD, even though the electrochemical detector (ECD) has better selectivity and sensitivity (Liang, Cao, Chen, Xiao, & Zheng, 2009; Wang et al., 2014). Therefore in this work, HPLC–ECD was used to establish the chromatographic fingerprints and to determine the contents of 13 phenolic acids in honey samples.

Chemometric methods are commonly used to reduce the complexity and to provide better understanding and interpretation of large data sets (Yücel & Sultanoglu, 2013). Combining the large amounts of data obtained from chromatographic fingerprints with chemometrics may be an excellent method for differentiating the floral origins of honey samples. The aim of this work was to identify three types of monofloral honey samples (Chinese jujube, longan and chaste honey samples) by establishing their chromatographic fingerprints using HPLC–ECD. The classification work was performed using chemometric methods with phenolic acids and common chromatography peaks as variables. From the analysis of phenolic acids in the honey samples, potential floral markers were identified.

2. Materials and methods

2.1. Honey sample information

Twenty-eight jujube (Z1–Z28), twenty-two longan (L1–L22) and twenty-seven chaste (J1–J27) honey samples (for a total of 77 samples) were collected from 18 different areas of China. The details of the honey samples used in this study are summarised in Supplementary Table 1. The honey samples numbered Z1–Z12, L1–L12 and J1–J12 were used as standards to conduct this test, and the others (Z13–Z28, L13–L22 and J13–J27) were used as test samples to verify the accuracy of this method. All honey samples were stored at 4 °C prior to analysis.

2.2. Sample preparation

Phenolic acids were extracted using solid-phase extraction (SPE) based on the method of Tomás-Barberán et al. (Martos et al., 2000). Honey samples (5 g) were thoroughly mixed with distilled water (25 mL) and, subsequently adjusted to pH 2.0 with concentrated HCl (6 M). The solution was then filtered through cotton to remove solid particles. Next, the filtrate was passed through a glass column (30 × 2.5 cm) containing Amberlite XAD-2 resin (30 g). The column was then washed with acidified water (100 mL) and distilled water (150 mL) successively. The phenolic compounds were retained in the column while other polar compounds and sugars were eluted. Afterwards, the phenolic compounds were eluted with methanol (150 mL) and evaporated under reduced pressure (40 °C). The residue was redissolved in HPLC-grade methanol (2 mL). The resulting methanol extracts were filtered with an organic phase nylon filter (13 mm × 0.45 μm) and stored at 4 °C for further analysis by HPLC–ECD.

2.3. Chemicals and standards

Gallic acid (GA), protocatechuic acid (Pro), *p*-hydroxybenzoic acid (p-HBA), chlorogenic acid (CA), vanillic acid (Van), caffeic acid (Caf), syringic acid (Syr), *p*-coumaric acid (p-Cou), ferulic acid (Fer), sinapic acid (Sin), ellagic acid (Ell), rosmarinic acid (Ros) and cinnamic acid (Cin) were purchased from Sigma–Aldrich (St. Louis, MO). HPLC-grade methanol used for mobile phase was obtained from Merck (Darmstadt, Germany). HPLC-grade water was obtained from a Milli-Q system (Millipore, Bedford, MA). Amberlite XAD-2 resin was from Supelco (Bellefonte, PA). Organic-phase nylon filters were purchased from Anpel (Shanghai, China).

2.4. HPLC analysis

The analysis of methanol extracts was performed using an Agilent 1100 liquid chromatography system equipped with a quaternary solvent delivery pump, an on-line vacuum degasser, a manual chromatographic valve, a thermostatic column compartment, an HP1049A programmable electrochemical detector (ECD) and a diode-array detector (DAD). The column was a Zorbax SB-C18 column (250 × 4.6 mm, 5 μm). The mobile phase was methanol (A) and 1% aqueous acetic acid (B) (*v*/*v*) with a linear gradient elution as follows: 5–15% A from 0 to 10 min, remaining at 15% A at 10–20 min, 15–17% A from 20 to 25 min, 17–30% A from 25–30 min, 30–40% A from 30 to 50 min, 40–55% A from 50 to 60 min, and 55–70% A from 60 to 70 min. The flow-rate was maintained at 1.0 mL min^{-1}, and the column was operated at 30 °C. The injection volume was 10 μL. Ten minutes were required between the injection of the two samples for re-equilibration. Online UV spectra of different phenolic acids were obtained using the DAD at 254 nm, 280 nm, 290 nm and 324 nm. The electrochemical detector was operated with a glassy carbon target electrode (diameter of 3 mm) in DC mode, with the working voltage maintained at 900 mV.

2.5. Method validation

To investigate the reliability of the analysis method, we followed the International Conference on Harmonisation (ICH) guidelines to test the method's precision, stability, repeatability and recovery rate (ICH. Guidance for industry., 1996).

To obtain calibration curves, thirteen phenolic acids at known concentrations were prepared as standard stock solution, which were then diluted to six different concentrations to obtain calibration curves for the quantitative analysis of the honey samples. Precision was tested by analysing the same honey sample (Z1) extracts six times continuously in a single day. Stability was tested by analysing one honey sample (Z1) extract every 2 h within a 24-h period at room temperature. Repeatability was tested using six sample extracts prepared from one honey sample (Z1). The recovery rate was tested using the standard addition method, in which thirteen phenolic acids standards at known concentrations were added to honey sample (Z1) extracts, and their phenolic acid contents were analysed again.

2.6. Data analysis

The Similarity Evaluation System for Chromatographic Fingerprint of TCM (2004 A) was used to analyse the