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Using sensor and spectral analysis to classify botanical origin and determine adulteration of raw honey



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ABSTRACT

The feasibility of sensors (Electronic Nose, EN; Electronic Tongue, ET) and spectra (Near Infrared spectrum, NIR; Mid Infrared spectrum, MIR) to evaluate raw honey samples (Vitex, Jujube and Acacia) was explored. Partial least squares discriminant analysis (PLSDA) model, support vector machine (SVM) algorithms model and Interval partial least squares (iPLS) model were used to classify the botanical origin. The results indicate that spectra and sensors could classify the botanical origin of honey rapidly and accurately, since total accuracy for calibration and prediction sets was all almost 100% in EN and ET analysis by SVM model and in NIR and MIR analysis by iPLS model. Then principal components analysis (PCA) and PLSDA model were used to determine the adulterants. Total accuracy for calibration and prediction sets was all above 96% in NIR, MIR and ET by PLSDA model. The results indicate that ET is more suitable for detecting honey adulteration.

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1. Introduction

Honey is a natural food product processed by honey bees by blending the sweetened sap collected from flowers with metabolic gastric enzymes (Anjos et al., 2015).

Natural honey is a very nutritious food product, which contains water (17% in general), saccharides (main constituents in honey, 75% in general, mainly glucose and fructose), amino acids, minerals (Mg, Ca, K, Na, S, P, Fe, Mn, Co, Ni, etc.), vitamins, enzymes (invertase, catalase, amylase, etc.), phenols, organic acids, pigments, volatile oils and also over 100 varieties of aromatic substances (De la Fuente et al., 2011; Mateo and Boschreig, 1997; Ouchemoukh et al., 2010; Arvanitoyannis et al., 2005; Baroni et al., 2006).

Honey needs to be rapidly evaluated and priced after collection from a bee-house, based on botanical origin classification and adulterant determination. Honey has customarily been distinguished as unifloral and multifloral depending on the botanical origin (Lenhardt et al., 2014). In China, over 20 kinds of unifloral honey are commonly produced such as jujube, vitex, acacia, rape, and linden. In general, unifloral honey types have higher market value due to their limited production and availability. Traditionally, botanical origin was determined by sensory analysis, pollen morphology, characteristic aroma analysis and characteristic interior components analysis. These methods have shown that botanical origin classification of honey is affected by color, aroma, content of pollen, method of processing and storage, content of trace substances and so on. However, these methods are expensive and time-consuming. Although the results of sensory analysis are accurate, it is objective and needs a well trained certified taste panel with a time investment. (Arvanitoyannis et al., 2005; Aparna and Rajalakshmi, 1999; Lawal et al., 2009; Bogdanov et al., 2004; Bianchi et al., 2005: Iglesias et al., 2004: Martos et al., 2000: Gonzlez-Miret et al., 2005; Conti et al., 2007).

For unethical economic gain, honey adulteration is an obvious problem in the market. Water, sucrose, inverted sugar, hydroxymethyl cellulose, dextrin and starch are adulterants which have been regularly identified by regular physicochemical analysis (Serrano et al., 2004). High performance liquid chromatography (HPLC), isotope mass spectrometry and capillary electrophoresis



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have also been used to evaluate these. However, these methods are complicated, time and labor consuming (Morales et al., 2008; Tu et al., 2011; Luo et al., 2012). C-4 botanical glycosides like corn syrup were added to honey subsequently to avoid detection. Even though corn syrup can be detected by stable carbon isotope ratio analysis (SCIRA), it is too expensive and time consuming (Cotte et al., 2007). Moreover, SCIRA is not suitable for detection of C-3 botanical glycoside such as rice syrup.

In recent years, rapid detection techniques such as spectral technology, sensor technology and chemical kits are widely used in testing of species, habitat, grades, freshness, nutrient quality, and drug residues in agricultural products since they are time-saving, more convenient and accurate than traditional methods. Electronic nose (EN) and electronic tongue (ET) are common methods for food analysis (Cozzolino et al., 2008; Wang et al., 2009; Ghasemi-Varnamkhasti et al., 2010; Vlasov et al., 2002; Lu et al., 2014; Pan et al., 2014; Haddi et al., 2013). A sample's whole information so called fingerprint data, can be determined from EN and ET rather than qualification and quantification of some specific constituents. Then a discriminant model will be established by compiling fingerprint data and unknown samples could be determined by the model. In previous studies, based on volatile components collected by solid phase micro-extraction, botanical origin of honey has been detected by Mass spectrum-electronic nose (MS-EN) (Ampuero et al., 2004). The bp-ANN model founded by EN consisting of ten MOSFET and twelve MOS sensors could discriminate the botanical origin and geographical honey production area (Benedetti et al., 2004). αAstree-ET could classify botanical origin of honey as well, in which the accuracy of the artificial neural network model was 100% (Major et al., 2011). Also PCA and ANN models were effective in classification of honey botanical origin by using ET with metallic compound electrode (Escriche et al., 2012).

NIR and MIR are extensively performed in agricultural products, food and medicine analysis (Magwaza et al., 2011; Cen and He, 2007; Balabin and Smirnov, 2011). Much research has revealed that NIR could classify the botanical origin of honey by using PCA analysis, canonical variate analysis, PLS discriminant analysis and linear discriminant analysis (Davies et al., 2002; Ruoff et al., 2007). Some reports showed that NIR was also an effective method to discriminate the adulterant of honey. Irish honey adulterated with beet syrup and high fructose syrup has been correctly discriminated and adulterant ratio could be predicted by using PLS analysis (Kelly et al., 2006a). Honey adulterated with different ratio of fructose/glucose was also determined by PLSR, K-NN and SIMCA models (Downey et al., 2003). MIR could discriminate adulterant of honey as well. Honey with glucose, fructose, sucrose and corn syrup added was detected by MIR and the discriminant accuracy could reach 90% by using LDA model (Irudayaraj et al., 2003). In another study, Irish artificial honey adulterated with fully inverted beet syrup, high-fructose corn syrup, partially invert cane syrup, dextrose syrup and beet sucrose was evaluated by MIR and well classified by using SIMCA and PLSAD models (Kelly et al., 2006b).

Compared with previous studies, this work focused on evaluating the performance of sensors (EN and ET) and spectra (NIR and MIR) in botanical origin classification and adulterant determination of raw honey. All honey samples used in this research were raw materials obtained directly from a bee-house and without any processing. Different types of discriminant analysis models were built. Sensors (ET and EN) and spectra (NIR and MIR) showed different accuracy and performance in botanical origin classification and adulterants determination.

2. Materials and methods

2.1. Sample preparation

Three types of honey samples, vitex, jujube and acacia, were provided by Beijing Baihua Apiculture Technology Development Company. Sample information is described in Table 1. Each sample, sourced from a different bee-house was filtered by 60 mesh screen in order to eliminate impurities. Samples were stored in the dark at 4 °C until analysis.

In botanical origin classification, 105 samples were divided into two sets with calibration (79) and prediction (26) as shown in Table 2, respectively. Then 35 pure honey samples (including vitex, jujube and acaica) were chosen randomly to prepare adulterant samples by adding two varieties of syrup (rice syrup and corn syrup). Both syrups were purchased from Cargill Investments (China) Co., Ltd. The physicochemical properties of syrups and honey samples were shown in Table 3. Adulterant samples were prepared by mixing pure honey solution with syrup in different concentrations (5%, 10%, 20% and 40%). The samples, 259 in total, included 105 pure honey samples and 154 adulterated samples. The division of sets was described as follow in Table 4.

2.2. Physical and chemical analysis of honey and syrup

The conductivity was measured by a Orion 5-Star Benchtop Meter (Thermo Fisher Scientific, Waltham, USA). The pH values were tested by a PB-10 pH meter (Sartorius Corp., Bohemia, NY, USA). Proline was evaluated by spectrophotometry. Saccharides, fructose, gluctose, cane sugar and maltose were determined by HPLC equipped with a YMC-Pack Polyamine II (250 mm \times 4.6 mm, 5 μ m) column. All of the physiochemical properties above were conducted by methods modified from previous studies (Bogdanov et al., 2004; Bogdanov, 2002).

2.3. Electronic nose

The EN system used for this study was a Heracles (Alpha Mos, Toulouse, France) which was equipped with a highly selective and sensitive specialty gas chromatograph. Volatile constituents of honey samples were collected by way of static headspace. Separation columns used in this work were DB5 and DB1701, respectively. Retention time and peak area were detected by hydrogen flame ionization detector (FIDs). Three grams of honey sample was weighed into a septa-sealed screw-cap bottle and equilibrated for 10 min at 65 °C (500 rpm). The parameters of EN were as follows: (1) Injector: temperature 180 °C, inject volume 3000 μ L. (2) Temperature program: initial 40 °C, final 200 °C, heating rate 2.0 °C/s. (3) Trap temperature: 250 °C, purge time 15 s (4) Temperature of FID 220 °C. (5) Acquisition time: 84 s (Yüzay and Selke, 2007). All determinations reported were conducted in triplicate.

2.4. Electronic tongue

The sensor array of the α -Astree ET (Alpha Mos, Toulouse, France) was comprised of seven potentiometric chemical sensors

Table 1Summaries of all the honey samples.

Variety	Place of origin (different province of China)	Quantity
Vitex Jujube Acacia	Liao Ning, Hei Bei, Bei jing, Shan Xi Liao Ning, Hei Bei, Shan Dong, Shan Xi Shan Xi	41 27 37

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