Identification of honey authenticity by near infrared spectroscopy

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Introduction

Honey is well known for its nutrient and therapeutic effect. Several food industries use honey as an ingredient. The honey trade as imported and exported products is world-wide. Sugars (saccharides) represent the main components of honey. Besides the two main constituents, the monosaccharides glucose and fructose, there are the minor components consisting of about 25 oligosaccharides (disaccharides, trisaccharides, tetrasaccharides). The knowledge of the carbohydrate composition of honey is useful in judging its authenticity. Limited availability, and the increased price of honey have provided major incentives for falsification with other carbohydrate materials.[1] The traditional adulteration in Thailand uses sucrose from table sugar. There is no simple method to confirm whether it is authentic (true) honey. The most effective way is to use high performance liquid chromatography (HPLC) to quantify the content of glucose, fructose, sucrose and moisture in honey. Near infrared spectroscopy was also used in confirmation of honey claimed provenance and best PLS discriminant models developed using full cross-validation, a variable selection algorithm and a 2nd derivative data pre-treatment, gave correct classification results of 90.0 % and 90.3 % for the Corsican and non-Corsican honey samples respectively.[2] Anklam[1] reviewed the analytical methods to determine the geographical and botanical origin of honey. He also reported that several authentic Ligurian (Italian) honey samples were studied with respect to their sugar composition, by GC.[3] The aim of their study was to detect the addition of syrups to honey. The maltose/isomaltose ratio was shown to be unsuitable for the detection of adulteration with syrups. However, the determination of the sucrose and erlose content was shown to have a potential for this purpose. The addition of sucrose in concentrations of less than 5 %[4] or the distinction between authentic honey from honey, produced by artificially fed bees, has been detected by HPLC.[5] Authentic honey contains fructose around 38.19 %, glucose around 31.21 %, sucrose around 1.31 % and moisture around 17.20 %. Significantly higher content of sucrose or lower content of fructose and glucose than these values indicates adulterated honey. In this study near infrared spectroscopy was proposed to identify honey authenticity and showed its effectiveness in classifying true honey from the adulterated honey.

Materials and methods

Ninety samples of honey were collected around the country (Thailand). They were classified to be true and adulterated honey by determination of sugar composition by HPLC (SpectraSYSTEM RI-150, USA). The numbers of true and adulterated honeys were 40 and 50 samples, respectively. True honey contained fructose, glucose and sucrose at 33.7–49.5 %, 24.4–39.8, and 1.8–11.3, respectively while the adulterated (adulterated) honey contained 0.6–27.5 % fructose, 2.4–37.5 glucose, and 1.5–44.6 sucrose, respectively. The authentic honey had a moisture content of 15.2–23.8 % and the adulterated honey had 15.2–24.8 % moisture.

Determination of moisture content

The moisture content of honey was determined by the refractometer method (Atago, PAL-2, Japan) as described in AOAC.[6]

High performance liquid chromatography (HPLC) analysis

Chromatographic analyses for determination of fructose, glucose and sucrose, were carried out in a HPLC (SpectraSYSTEM RI-150, USA). The chromatographic separation of sugars was achieved in an amine-bonded phase column (Luna 5u NH_2 250×4.6 mm), using acetonitrile/water (80:20 by volume) as mobile phase, at a flow rate of 3.0 mL min^{-1} and refractive index detection. The

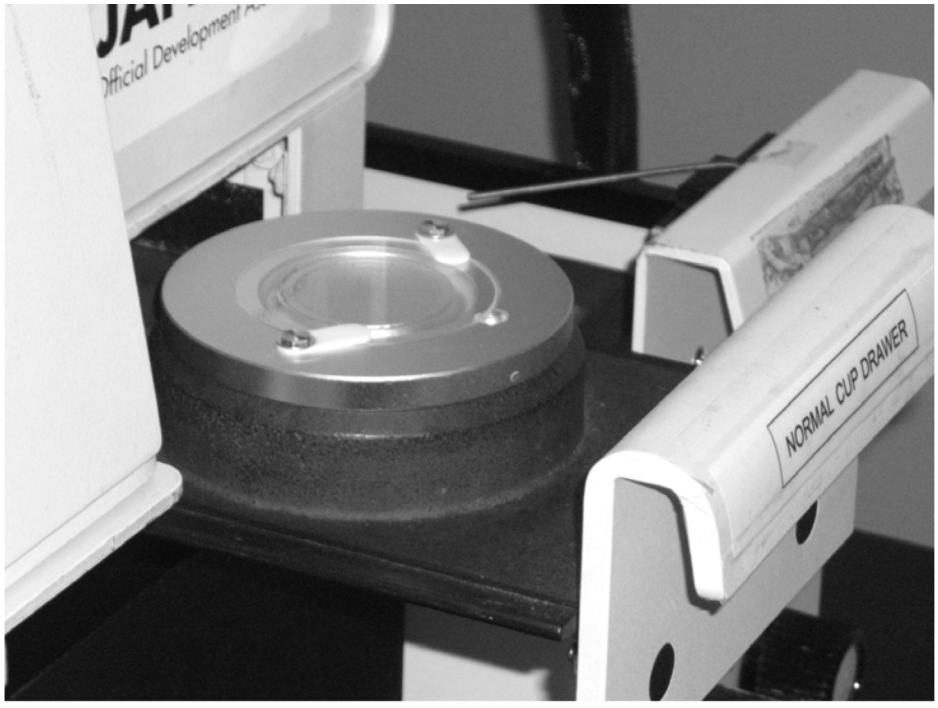

Figure 1. NIR measurement of honey.

sample preparation involved dissolution of 1 g of honey in 10 mL of deionized water and 10 mL of acetonitrile, followed by filtration through a syringe filter of 0.45 μm.

Near infrared spectral collection

Before scanning, honey samples (1.5 mL approx.) were subjected to microcentrifuging (Spectrafuge, Labnet, USA) with 6.3 cm centrifuging radius at 8000 rpm (4515.8 g approx.) for 10 min or more to remove bubbles. The NIR measurements were performed with an InfraAlyzer 500 (Bran+Luebe Gmbh, Germany) over the wavelength region of 1100–2500 nm at 2 nm intervals, in diffuse transflectance mode, using the British cup at 25 °C (Figure 1). Each sample was divided into two subsamples and subjected to NIR scanning.

Data analysis

Data analysis was carried out using "The Unscrambler V 9.8" software (CAMO, Oslo, Norway). The spectra were pretreated by full multiplicative scatter correction (MSC), first and second

Table 1. Result of PCA and SIMCA in identify groups of honey.

Type of spectra	Honey groups	No. of honey samples for calibration	No. of honey samples for prediction	% correct classification by PCA		% Overall correct classification by SIMCA
				Adulterated model	True model	
Raw spectra	False	70	26	69.3	92.3	61.5
	Real	58	22	4.5	81.8	13.63
	Total	128	48	39.5	87.5	39.58
1st Der (Seg:10 nm)	False	70	26	77	84.6	61.5
	Real	58	22	0	95.5	0
	Total	128	48	41.6	89.6	33.3
1st Der (Seg: 20 nm)	False	70	26	69	84.6	53.8
	Real	58	22	9	95.5	4.54
	Total	128	48	41.6	89.6	31.25
2nd Der (Seg: 10 nm)	False	70	26	76.9	92.3	69.2
	Real	58	22	9	86.4	1
	Total	128	48	45.8	89.6	39.58
2nd Der (Seg: 20 nm)	False	70	26	80.8	88.5	69.2
	Real	58	22	0	**95.5**	0
	Total	128	48	43.75	91.6	37.5
MSC	False	70	26	19.2	**96.2**	3.84
	Real	58	22	77	27.3	0
	Total	128	48	45.8	64.6	2.08

Der: Derivative; Seg: Segment; MSC: Multiplicative scattering correction

derivative with left and right averaging of 10 nm and 20 nm. Principle Component Analysis (PCA) and Soft independent modeling of class analogy (SIMCA) was also used to identify true and adulterated groups of honey. The samples were assigned to calibration set and prediction set, with the ratio of 5:2.

Results and discussion

Table 1 shows the relative efficiency of PCA and SIMCA in identifying the two groups of honeys. The PCA model developed by true honey spectra showed better classification performance than the model developed from adulterated honey spectra. The best classification was obtained from the PCA calibration model developed by spectra of true samples pretreated by 2nd derivative with segment of 20 nm. It provided the highest percentage of correctness of 95.5 %. The best PCA model from real spectra pretreated by MSC could identify the false group with the highest percentage of correctness of 96.2 %. If the two PCA models are applied in combination, the overall correctness for identification of true and adulterated honeys would be more than 95.5 %. It was observed that the percentage of overall correct classification by SIMCA was clearly lower than prediction by individual PCA models.

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