Detection of honey adulteration by added sugars using NIR transflectance spectroscopy. Some initial studies

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Introduction

Honey is a natural product "*produced by honeybees from the nectar of blossoms or from secretions…. of plants which honeybees collect, transform, combine with substances of their own and store and leave to mature in honeycombs."*[1]. It typically comprises ~80% sugars and 17% water by weight. Fructose and glucose together account for 85 to 95% of total carbohydrate in a fructose:glucose ratio of about 1.2:1. Remaining material is accounted for chiefly by disaccharides (~6.6% w/w) and oligosaccharides (~1.5% w/w)[2]. Honey is much prized by European consumers. This demand coupled with declining European output, has made the economic adulteration of honeys an increasingly attractive proposition. One of the main types of such adulteration is the addition to honey of sweet substances such as fructose:glucose mixtures, high fructose corn syrups or beet sugar[3]. This report describes initial studies to detect the addition of fructose:glucose mixtures to artisanal honeys produced in Ireland. Importantly, the honey samples and adulterant solutions were prepared at the same soluble solids (% Brix) level.

Materials and methods

Samples

Honey samples (n=75) were obtained directly from artisanal producers from a variety of geographic locations throughout the island of Ireland. They included samples from a range of floral types and were sourced through the Irish Federation of Beekeepers' Associations and the Ulster Beekeepers Association. Prior to adulteration and spectral collection, honeys were stored for 18 hrs at 40°C to dissolve all crystalline material. They were then diluted to 70% Brix. D-glucose and D-fructose (Merck) were dissolved in distilled water to produce solutions of 70% Brix containing the following fructose:glucose ratios – 0.7:1, 1.2:1 and 2.3:1. Twenty-five of the honey samples were then adulterated with each of these solutions at three levels *i.e.* 7, 14 and 21% w/w, producing an overall total of 250 samples (25 authentic & 225 adulterated).

Spectroscopy and data analysis

Transflectance spectra (400 to 2500 nm at 2 nm intervals) were recorded on a FOSS 6500 scanning monochromator (FOSS NIRSystems, Silver Springs, MD) fitted with a sample transport module. Samples were presented in a circular camlock cell (Figure 1) fitted with a gold-plated backing disc to produce a sample thickness of 0.1mm. Sample temperature variations were minimised by executing 15 scans prior to actual spectral collection. Data analyses were performed

using The Unscrambler (v7.6; Camo, Trondheim, Norway) and Pirouette Lite Classify (v3.10; Infometrix,WA, USA). All chemometric techniques were applied to spectral data in the 400-2500, 400-750, 750-1100 and 1100-2500 nm wavelength ranges in the form of raw, first and second derivative data. In all cases, data between 1100 and 2500 nm produced the best results. Only these are reported below.

Figure 1 Circular camlock cell with gold-plated backing disc

Results and discussion

Second derivative spectra (5 nm gap size) of 25 pure honeys and the adulterant sugar solutions are shown in Figure 2. Band assignments for sugars aqueous solution have been recently summarised[4]. 1st overtone CH stretch vibrations are reported to occur at wavelengths between 1600-1800 nm, 1st overtone CH_2 stretching between 1720 and 1735, CH_2 combinations between 2310 and 2325 nm and 1st, 2nd and 3rd overtones of OH, CH and CH_2 deformations in the range 1850 to 2600 nm. Principal component analysis (PCA) revealed a degree of clustering (Figure 3) which suggested that this spectroscopic approach may have some discriminatory potential.

Figure 2. Second derivative spectra of pure honeys and adulterant sugar solutions

Identification of pure honeys was attempted initially using soft independent modelling of class analogy (SIMCA). Best results were obtained using raw spectral data. In this case, 25/25 authentic honeys were correctly identified but only 101/225 adulterated samples (45%) were classified as such. Most of these were adulterated using the 2.3:1 fructose:glucose sugar solutions. When this procedure was repeated using only authentic honeys and honeys adulterated at the 7% w/w level,

only 21/75 adulterated samples (28%) were correctly classified. Using *k*-Nearest Neighbours (*k*-NN) on this two group problem (*i.e.* authentic honeys *vs* honeys adulterated at the 7% w/w level), best results were obtained using a second derivative data pre-treatment (5 nm gap) and a value of *k* equal

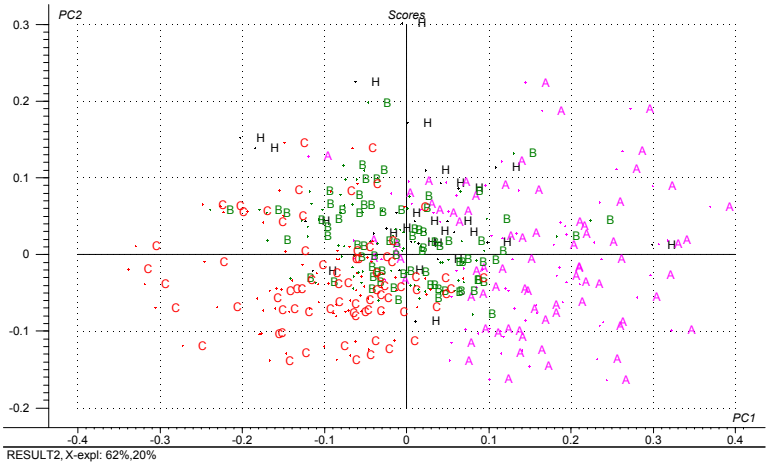

Figure 3. PCA of all samples (400-2500 nm wavelength range; H = pure honeys; A = honeys adulterated with 0.7:1 sugar solution; B = honeys adulterated with 1.2:1 sugar solution; C = honeys adulterated with 2.3:1 sugar solution)

to 4. Twenty-two of 25 pure honeys were correctly identified as were 73/75 adulterated honeys. Applying partial least squares (PLS1) regression on raw spectral data to the entire sample set (n=250) revealed that 10 out of 25 pure honeys were correctly identified as were 221 of 225 adulterated samples.

It is generally desirable in classification methods such as those mentioned that approximately equal numbers of samples are available in each category under test. *K*-nearest neighbours is particularly sensitive to number imbalances. Therefore, the work was extended by adding 50 authentic honeys to the sample set and attempting to differentiate between them and the 25 honeys adulterated at the 7% w/w level only. This gave 75 samples in each of the two (authentic & adulterated) categories. Summary results obtained are shown in Table 1.

Table 1. Results for classification of authentic and adulterated (7% w/w level) honey samples

Data treatment	Correct classification rate	
	Authentic honeys	Adulterated honeys
SIMCA	65 of 75 (87%)	23 of 75 (31%)
k-NN	59 of 75 (79%)	66 of 75 (88%)
PLS1	72 of 75 (96%)	71 of 75 (95%)

These initial findings suggest that PLS1 may be the method of choice for detection of honey samples adulterated with fructose:glucose mixtures. Further work will extend the sample collection, investigate other chemometric methods and alternative wavelength ranges.

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