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Classification of adulterated honeys by multivariate analysis

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ABSTRACT

In this research, honey samples were adulterated with date syrup (DS) and invert sugar syrup (IS) at three concentrations (7%, 15% and 30%). 102 adulterated samples were prepared in six batches with 17 replications for each batch. For each sample, 32 parameters including color indices, rheological, physical, and chemical parameters were determined. To classify the samples, based on type and concentrations of adulterant, a multivariate analysis was applied using principal component analysis (PCA) followed by a linear discriminant analysis (LDA). Then, 21 principal components (PCs) were selected in five sets. Approximately two-thirds were identified correctly using color indices (62.75%) or rheological properties (67.65%). A power discrimination was obtained using physical properties (97.06%), and the best separations were achieved using two sets of chemical properties (set 1: lactone, diastase activity, sucrose – 100%) (set 2: free acidity, HMF, ash – 95%).

1. Introduction

Honey is a highly prized food product across the world (Chen et al., 2011; Kelly, Downey, & Fouratier, 2004), which is composed mainly of two monosaccharide sugars, *i.e.* fructose and glucose (Venir, Spaziani, & Maltini, 2010), and minor components, such as other carbohydrates (sucrose, etc.) and non-sugar components, *i.e.* proteins, enzymes, amino and organic acids, lipids, vitamins, volatile chemicals, phenolic acids, flavonoids, and minerals (Jasicka-Misiak, Poliwoda, Dereń, & Kafarski, 2012; Manzanares, García, Galdón, Rodríguez, & Romero, 2011; Tornuk et al., 2013).

According to international standards, authentic honey is a natural foodstuff that should not contain any additives or have other substances added to it, such as inexpensive sugar syrup (Kartheek, Smith, Muthu, & Manavalan, 2011).

Honey is one of the most widely used sweeteners in food industry and is used in a large number of processed food products (Corbella & Cozzolino, 2006). Its market value is much higher than other commonly utilized sweeteners, such as refined cane sugar, beet sugar, corn syrup, maple sugar, high fructose corn syrup (HFCS), and high fructose inulin syrup (HFIS) (Ghosh, Verma, Majumder, & Gupta, 2005; Kartheek et al., 2011; Paradkar & Irudayaraj, 2002; Ruiz-Matute, Rodríguez-Sánchez, Sanz, & Martínez-Castro, 2010). Hence, honey is an obvious and profitable target for adulteration with cheap industrial sweeteners, which simulate its natural carbohydrate profile and the detection of which is difficult (Frew, McComb, Croudis, Clark, & Van Hale, 2013; Sivakesava & Irudayaraj, 2001).

Detection of honey adulteration is not simple. In recent decades, research has tended to focus on instrumental analysis techniques, such as isotopic ratio (Padovan, De Jong, Rodrigues, & Marchini, 2003; Simsek, Bilsel, & Goren, 2012), chromatography (Consonni, Cagliani, & Cogliati, 2013; Tosun, 2013), nuclear magnetic resonance (NMR), and spectroscopic (Vibrational spectrometry, i.e. NIR, MIR and Raman) (Bertelli et al., 2010; Chen et al., 2011; Ruoff et al., 2006, 2007; Sivakesava & Irudayaraj, 2002). The advantages of these techniques in detecting honey adulteration have been demonstrated elsewhere (Mehryar L., 2011). However, these sophisticated tools are time-consuming, destructive, and expensive. Thus, simple, inexpensive and rapid analytical techniques are needed to be able to detect adulteration, such as addition of syrups (Kelly et al., 2004; Sun, 2008). Determination of the ratio between or among chemical constituents, as principle components, assuming these ratios are a constant, is a potential approach.

From this perspective, the addition of any amount of a substance(s) into foods will modify the ratio of constituents or create an irregularity in composition (Cordella, Faucon, Cabrol-Bass, & Sbirrazzuoli, 2003; Cordella et al., 2002b). This view is associated mostly with large sets of data and needs multivariate statistical analysis to be useful. For this approach, pattern classification procedures can be applied to compare similarities or differences in a large dataset (Cordella, Moussa, Martel, Sbirrazzuoli, & Lizzani-Cuvelier, 2002b). Over the past decade, we have seen rapid developments in multivariate statistical analysis in food product

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authentication, discrimination, and classification (Corbella & Cozzolino, 2006; Gutiérrez & Quintana, 2011; Manzanares et al., 2011).

Since honey composition is complex, its authentication needs a statistical approach capable of interpreting patterns in multivariate data. Principal component analysis (PCA) (Corbella & Cozzolino, 2006; Sivakesava & Irudayaraj, 2002; Wei, Wang, & Wang, 2010; Özbalci, Boyaci, Topcu, Kadılar, & Tamer, 2013), linear discriminant analysis (LDA) (Corbella & Cozzolino, 2006; Sivakesava & Irudayaraj, 2002), canonical variate analysis (CVA), cluster analysis (CA) (Wei et al., 2010), artificial neural networks (ANN) (Özbalci et al., 2013), *k-nearest neighbors* (*KNN*) (Kelly et al., 2004), and partial least squares (PLS) (Kelly et al., 2004; Ruoff et al., 2006, 2007; Sivakesava & Irudayaraj, 2002; Wei et al., 2010; Özbalci et al., 2013) are the most commonly used multivariate analysis techniques in foods authentication (Cordella et al., 2002; Paradkar & Irudayaraj, 2002b). Thus, multivariate analysis of physicochemical and rheological data might be useful for detecting pure and adulterated honeys.

Considering the lack of previous studies using physicochemical and rheological properties to detect honey adulteration with sugar syrups, the aims of this study were to (1) characterize principal components of physicochemical and (2) identify and classify different types and concentrations of adulteration using LDA.

2. Materials and methods

2.1. Samples

Pure honey was purchased directly from a beekeeper in Urmia, West Azerbaijan province (Iran), and date syrup (DS) from Shahd Bab Pars Co. (Tabriz, Iran). Invert sugar syrup (IS) was produced by acid hydrolysis of sugar. To make adulterated samples, pure honey was mixed with different concentrations of date and invert sugar syrups, *i.e.* 7%, 15%, and 30%. These concentrations are typical, and do not raise the qualitative and quantitative results of honey above international standard threshold (Cabanero, Recio, & Rupérez, 2006; Paradkar & Irudayaraj, 2002). Pure and adulterated honeys were placed in an oven at 40 °C for promote mixing.

To analyze the types and levels adulteration, 102 adulterated samples were prepared in six batches with 17 replications of each batch. Physicochemical and rheological variables (PCs) were collected in five sets (color indices, rheological properties, physical properties, chemical properties I, and chemical properties II). Each dataset included: color indices (L^*, a^*, b^*, C^*, h^*, ΔE^*), rheological properties ($t_{Stringiness}$, adhesiveness, F_{max}, surface stickiness, stringiness, $t_{Start\text{-}Stringiness}$), physical properties (viscosity, electrical conductivity, a_w), chemical properties I (lactone, diastase activity, sucrose), and chemical properties II (free acidity, HMF, ash). The first (B1), second (B2) and third (B3) batches of honey were prepared at (W/W) 7%, 15%, and 30% IS, respectively, and the fourth (B4), fifth (B5) and sixth (B6) batches at (W/W) 7%, 15%, and 30% DS, respectively. Each sample was homogenized mechanically for 30 min at 40 °C, and kept at the same temperature for 2 h in water bath to dissolve any crystals.

2.2. Physicochemical analysis

All physicochemical parameters were determined according to official methods of analysis (AOAC, 2002) and harmonized methods of the international honey commission (Bogdanov, Martin, & Lullmann, 2002). All measurements were carried out in triplicate.

Moisture content was measured using an Abbe-type refractometer (NAR-3T, Atago Co., Ltd., Tokyo, Japan) at 20 °C according to AOAC 969.38 and the corresponding moisture percent obtained from table 969.38 (AOAC, 2002). Diastase activity was determined according to AOAC 958.09 using a spectrophotometer (80-2088-64, Pharmacia LKB Biochrom, Cambridge, UK). Hydroxymethylfurfural (HMF) content was determined using the White method, described in the International Honey Commission's harmonized methods (Bogdanov et al., 2002). Water activity (a_w) was measured using a water activity meter (ms1, Novasina, Lahen, Switzerland). Free, lactone and total acidity were determined as indicated by AOAC 962.19, and pH measured using a pH meter (781-pH/Ion meter, Metrohm, Herisau, Switzerland) in a 10% (W/V) solution of honey prepared with double distilled water. Electrical conductivity was determined with a conductivity meter (Ohm-644, Metrohm AG Herisau, Switzerland) as an aqueous solution (20 g dry matter in 100 ml double distilled water) at 20 °C. The ash content was determined using an electric muffle furnace (SEF-101, SHIN SAENG SCIENTIFIC CO. LTD, Paju, South Korea). Reducing and total sugars, apparent sucrose, and fructose to glucose ratio were measured according to the International Honey Commission's harmonized methods, as was insoluble matter.

Color indices (a^*, b^* and L^*) were measured using a colorimeter (Chroma Meter CR 410, Konica Minolta, Tokyo, Japan), and the hue angle (h^*), chroma (C^*) and total color difference ($\Delta E*$) calculated as:

$$h^* = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C^* = \left[(a^*)^2 + (b^*)^2\right]^{1/2} \quad (2)$$

$$\Delta E^* = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2} \quad (3)$$

2.3. Thermal analysis

The glass transition temperature (T_g) was determined using a differential scanning colorimetry (DSC – 822e Mettler-Toledo, Inc., Columbus, OH, USA). The DSC was connected to a liquid nitrogen source for temperature control. Samples were weighed (with an uncertainty of ±0.01 mg and Sartorius Cubis® analytical balance MSA224S-000-DI, Göttingen, Germany) into 40 mL aluminum pans with an empty pan as the reference. To achieve the full thermal response of adulterated honeys, thermal scans were carried out in a temperature range from −65 °C to 225 °C with a scan rate of 20 °C per minute (Cordella, Faucon, Cabrol-Bass, & Sbirrazzuoli, 2003; Cordella et al., 2002a).

2.4. Rheological analysis

Viscosity of samples was determined using a rotating viscometer (DV-II+Pro No. M/03-165-b0707, Brookfield Engineering, MA, USA) equipped with type LV-64 spindle, in 250 mL glass jars at 20 °C and 10 rpm.

Surface stickiness, stringiness, and texture profile analysis (TPA) were measured using a texture analyzer (TA.XT. plus, Stable Micro Systems Ltd, Godalming, Surrey, UK). To determine the surface stickiness and stringiness, using a 25 mm diameter cylindrical probe (P/25), a force of 6 g was exerted on the surface of samples and maintained for two seconds. The probe was drawn back from the sample at 8 mm/s and stopped at a distance of 170 mm above the sample surface. The maximum force (Fmax) required to separate the probe from the sample was recorded as surface stickiness, and stringiness was recorded as the distance the probe moved away from the sample surface before the force dropped to 2.5 g; the corresponding time for this distance was registered as $t_{Stringiness}$. When the probe was withdrawn, a string of honey