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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

Table 1
Results of the PCA.

Discriminant Function	Eigenvalues	Variance (%)	Cumulative variance (%)
Color indices			
Canonical 1	2.864	73.828	73.828
Canonical 2	0.775	19.988	93.816
Canonical 3	0.185	4.775	98.591
Canonical 4	0.042	1.085	99.676
Canonical 5	0.013	0.324	100.000
Canonical 6	0.000	0.000	100.000
Rheological properties			
Canonical 1	5.009	75.580	75.580
Canonical 2	1.394	21.032	96.612
Canonical 3	0.193	2.915	99.526
Canonical 4	0.025	0.381	99.907
Canonical 5	0.006	0.093	100.000
Canonical 6	0.000	0.000	100.000
Physical properties			
Canonical 1	14.887	65.580	65.580
Canonical 2	7.306	32.186	97.765
Canonical 3	0.507	2.235	100.000
Chemical properties (I)			
Canonical 1	19.777	66.462	66.462
Canonical 2	9.910	33.305	99.767
Canonical 3	0.069	0.233	100.000
Chemical properties (II)			
Canonical 1	94.523	77.432	77.432
Canonical 2	24.263	19.876	97.308
Canonical 3	3.286	2.692	100.000

formed between the surface of honey and the probe. The time from withdrawal until the string tore was recorded as $t_{Start\text{-}Stringiness}$. $t_{Start\text{-}Stringiness}$ and $t_{Stringiness}$ were noted using a chronometer and texture exponent software, respectively.

To determine the adhesion-cohesion characteristic of the samples, a double penetration test was developed using the texture analyzer. For this purpose, a cylinder stainless probe (P/6; 6 mm DIA) was used with a penetration distance of 0.5 mm. The time between 'bites' was set at 2 s, and test-speed and post-test speed were adjusted to 5 mm/s. Force–time curves were used to determine adhesiveness (N.s). All the containers were identical and rheological measurements were performed at 22 °C.

2.5. Multivariate statistical analysis

A discrimination procedure was applied using principal component analysis (PCA) followed by linear discriminant analysis (LDA) for differentiated batches, taking into account the type and concentration of the adulterant. PCA was conducted to reduce the dimensions of the original data to a smaller number of component sets by examining the relationship between measured parameters. This is a statistical procedure that has been applied widely to transform a set of possibly correlated data to a set of orthogonal components, which are called principal components (PCs) (Raykov & Marcoulides, 2008; Sun, 2008; Varmuza & Filzmoser, 2009). LDA was used to evaluate the potential of physicochemical and rheological properties to classify models and separate batches. This method is a supervised pattern recognition technique that is based on discriminant canonicals in which the center of the matrix variance and covariance of each batch is calculated. In this method, the variance is maximized between categories and minimized within categories (Raykov & Marcoulides, 2008; Sun, 2008; Varmuza & Filzmoser, 2009). The multivariate statistical analysis was performed using SAS version 10.0 software package (SAS Institute, Cary, NC, USA).

Table 2
PCs model for five sets of parameters.

Discriminant Function	Models					
Color indices						
	L^*	a^*	b^*	C^*	h^*	ΔE^*
Canonical 1	97.558	22.120	−11.730	−6.319	−0.063	−99.885
Canonical 2	56.940	−2.068	−99.102	99.726	0.310	−55.702
Canonical 3	−461.192	−45.645	73.635	−17.818	−0.212	465.701
Canonical 4	−490.895	−49.600	216.087	−125.806	−0.783	499.075
Canonical 5	80.310	50.417	7.966	−32.628	0.512	−80.387
Canonical 6	−12.946	33.124	−21.154	15.898	0.583	12.683
Rheological properties						
	$t_{Stringiness}$ (S)	Adhesiveness (N.s)	F_{max} (N)	Surface Stickiness (N)	Stringiness (mm)	$t_{Start\text{-}Stringiness}$ (S)
Canonical 1	−1.026	−7106.479	216.117	3.992	0.620	0.676
Canonical 2	−0.123	3121.664	58.671	−65.955	1.021	0.953
Canonical 3	0.630	−13744.500	−293.685	14.322	0.268	0.289
Canonical 4	0.078	4566.202	−509.108	49.309	−0.260	−0.006
Canonical 5	0.139	−2416.353	920.498	−4.085	−0.065	0.440
Canonical 6	0.141	−5134.072	809.122	39.278	−0.062	−0.311
Physical properties						
	Viscosity (mPa.s)		Electrical conductivity (mS/cm)		a_w	
Canonical 1	0.0000562		159.06603		−11.37593	
Canonical 2	0.0003293		−40.90448		62.376392	
Canonical 3	−1.43E−05		−1.316405		196.13578	
Chemical properties (I)						
	Lactone (meq/kg)		Diastase (DN)		Sucrose (%)	
Canonical 1	1.853		−0.139		−1.190	
Canonical 2	0.437		−0.892		2.371	
Canonical 3	0.343		0.933		2.075	
Chemical properties (II)						
	Free Acidity (meq/kg)		Ash (%)		HMF (mg/kg)	
Canonical 1	−0.044		−204.081		0.522	
Canonical 2	1.044		139.828		0.187	
Canonical 3	−0.379		326.743		0.357	

3. Results and discussion

The evaluation of physicochemical and rheological properties of honey was applied to verify authenticity and reveal the presence of adulterants.

3.1. Principal component analysis (PCA)

102 adulterated samples in six batches with 17 replications were considered. For each sample, the following parameters were measured: pH, free acidity (meq/kg), lactone (meq/kg), total acidity (meq/kg), moisture (%), ash (%), water insoluble solids (%), electrical conductivity (mS/cm), diastase activity (DN), HMF (mg/kg), reducing sugar (%), total sugar (%), sucrose (%), fructose/glucose ratio, viscosity (mPa.s), a_w, T_g (°C), L^*, a^*, b^*, chroma (C^*), hue angle (h^*), ΔE^*, glucose/moisture ratio, $t_{stringiness}$ (s), adhesiveness (N.s), F_{max} (N), surface stickiness (N), stringiness (mm), and $t_{start\text{-}stringiness}$ (s). PCA was used to extract the most important characteristics among variables with the most discriminating effect. As indicated by the Kaiser criterion, just the principal components with eigenvalue greater than one were regarded significant (Kaiser, 1960; Silvano, Varela, Palacio, Ruffinengo, & Yamul, 2014). In this work, 21 principal components (PCs) were selected in five sets from the original data. These five sets were found to be the most informative for classifying samples in a two-dimensional space. The discrimination rates, by means of cross-validation were used to optimize the number of PCs for consideration by LDA. The number of principal component factors is critical to the performance of the LDA discrimination model. Table 1 presents the results from PCA and Table 2 presents the mathematical models obtained for each PC to show the significance of each variable on the PCs.

3.2. Linear discriminate analysis (LDA)

LDA was performed on standardized data to calculate the class-scores for samples with respect to each batch. The statistical significance of each canonical was examined on the basis of a likelihood factor, which is a measurement of how well each canonical separate objects into batches. This factor is usually expressed as −2 log likelihood and its value (*i.e.* closest to zero) indicates the discriminatory power. Separation among batches was checked by plotting the first and second canonical, applying 95% confidence circle around the means (center of batches) of each batch. Table 3 shows the results obtained from LDA classification applied to the complete data matrix. All the samples were in the training set.

3.2.1. Color indices

The first and second canonicals described 73.83% and 19.99%, respectively, and 93.82% of the total variance. Fig. 1 shows a scatter plot for the first and second canonicals discriminatory values from six batches of adulterated honey. As can be seen from Table 3, for discrimination of samples by color indices, the −2 Log likelihood factor was 107.5 and, after cross validation, these indices correctly classified 64 samples (62.75% of samples).

Although, the samples were distinguished clearly with respect to the type of adulterant (IS and DS), batches of each adulterant

Table 3
Result of prediction ability for LDA model.

Counts: Actual rows by predicted columns							Correct classification (%)	−2 Log likelihood
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6		
Color indices							62.75	107.50
Batch 1	12	4	0	0	1	0		
Batch 2	2	15	0	0	0	0		
Batch 3	1	4	11	1	0	0		
Batch 4	1	0	0	11	4	1		
Batch 5	0	0	0	4	13	0		
Batch 6	0	1	0	1	3	12		
Rheological properties							67.65	69.88
Batch 1	7	6	4	0	0	0		
Batch 2	2	10	5	0	0	0		
Batch 3	5	3	6	1	2	0		
Batch 4	0	0	0	15	2	0		
Batch 5	0	2	0	1	14	0		
Batch 6	0	0	0	0	0	17		
Physical properties							97.06	10.48
Batch 1	17	0	0	0	0	0		
Batch 2	0	17	0	0	0	0		
Batch 3	0	0	17	0	0	0		
Batch 4	0	0	0	14	3	0		
Batch 5	0	0	0	0	17	0		
Batch 6	0	0	0	0	0	17		
Chemical properties (I)							95.10	17.06
Batch 1	16	1	0	0	0	0		
Batch 2	1	16	0	0	0	0		
Batch 3	0	0	17	0	0	0		
Batch 4	0	0	0	16	1	0		
Batch 5	0	0	0	1	16	0		
Batch 6	0	0	0	0	1	16		
Chemical properties (II)							100.00	0.00
Batch 1	17	0	0	0	0	0		
Batch 2	0	17	0	0	0	0		
Batch 3	0	0	17	0	0	0		
Batch 4	0	0	0	17	0	0		
Batch 5	0	0	0	0	17	0		
Batch 6	0	0	0	0	0	17		

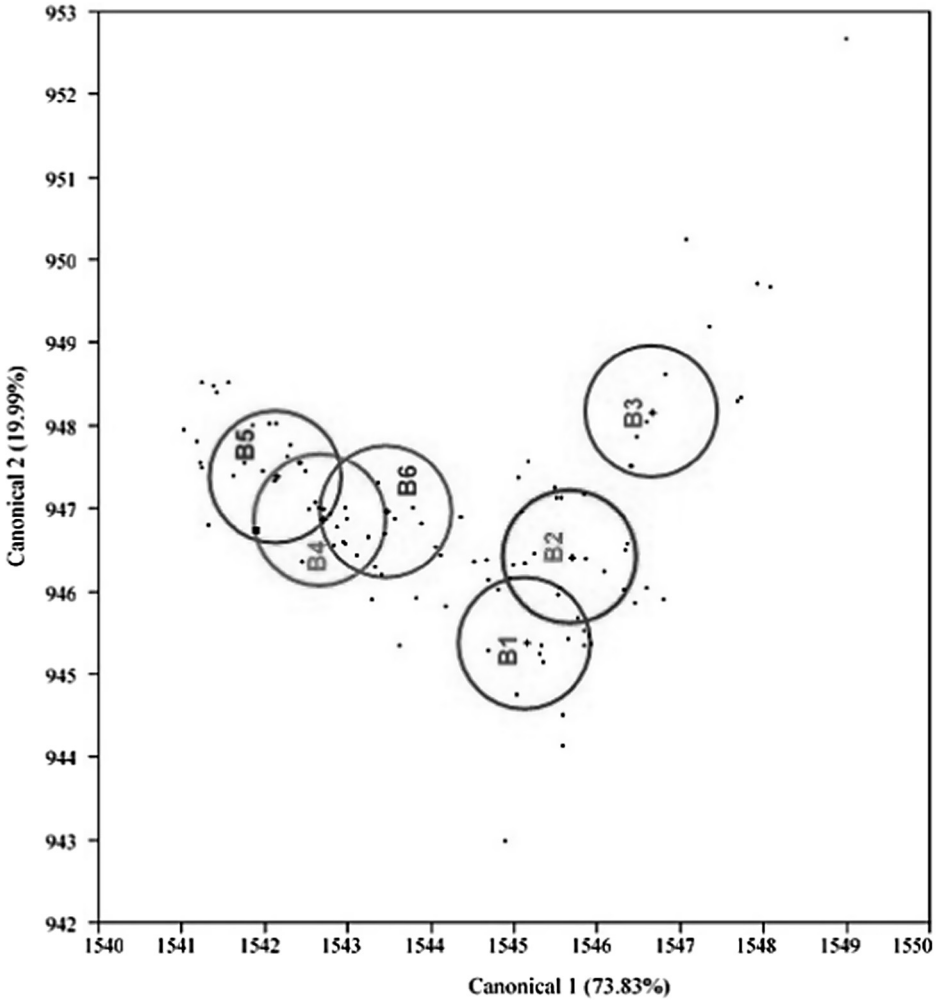

Fig. 1. Adulterated honey batches based LDA on color indices. (+) Group centroid.

overlapped at the concentrations used, especially for DS. The 7% and 15% IS concentrations also overlapped, and the predictive model misclassified six samples at 30%. Color indices, as principal components, could be used to separate the type of adulterant (*i.e.* IS and DS), but not different concentrations, especially for DS. This is in agreement with Corbella and Cozzolino (2006).

3.2.2. Rheological properties

The first two canonicals explained 96.61% of total variance (75.58% for canonical 1 and 21.03% for canonical 2). The scatter plot of the first and second canonical discriminations is shown in Fig. 2 (a). In addition, Table 3 illustrates that the −2 Log likelihood factor for the discrimination model of six batches, using rheological properties, was 69.88% and the correct classification was 67.65%. Rheological properties, as PCs, could separate IS and DS (type of adulterant) and concentration of DS but not different concentrations of IS (Fig. 2) a. As shown in Table 3, the sixth batch (containing 30% DS) with 17 correct classification was the best prediction model whilst concentrations of 7% and 30% IS produced seven and six correct classification, respectively.

3.2.3. Physical properties

As shown in Fig. 2 (b), canonical 1 vs. canonical 2, described 97.77% of the variance (65.58% for canonical 1 and 32.19% for canonical 2). This scatter plot depicts a 2D plot constructed with canonical 1 and canonical 2, and six batches of adulterated honey. Using this set, the −2 Log likelihood factor of discrimination was 10.48, and generated 97.06% correct classifications, which implies a powerful model capable of distinguishing the samples. As can be seen in Table 3, there was good separation for IS adulteration as well as 15% and 30% DS, and only three samples in batch 4 (7% DS) were classified incorrectly. Thus, separation based on viscosity, electrical conductivity and a_w as PCs had good discriminatory power. Previous studies showed that electrical conductivity is one of the best factors to detect adulteration of honey (Corbella & Cozzolino, 2006). Our results can be compared with those of Paradkar and Irudayaraj (2002) where 96% honey of adulterated with beet and cane invert sugar was classified correctly using canonical variate analysis and FT-Raman.

3.2.4. Chemical properties (I)

All adulterated honey samples discriminated along two chemical property canonical axes, confirming the presence of six batches. Canonical 1 explained 66.42% of variance and canonical 2 explained 33.30% of variance. The total accumulative contribution rate of variance from canonical 1 and 2 was 99.77%. The scatter plot for canonical 1 vs. canonical 2 (Fig. 3a) clearly shows the separation of all six batches of adulterated honey, regardless of type or concentration. In Table 3, it can be seen that all replications in batch 3 (containing 30% IS) were predicted correctly; other batches presented at least one misclassified sample. The PCs of this set were useful parameters for discrimination of type and concentra-

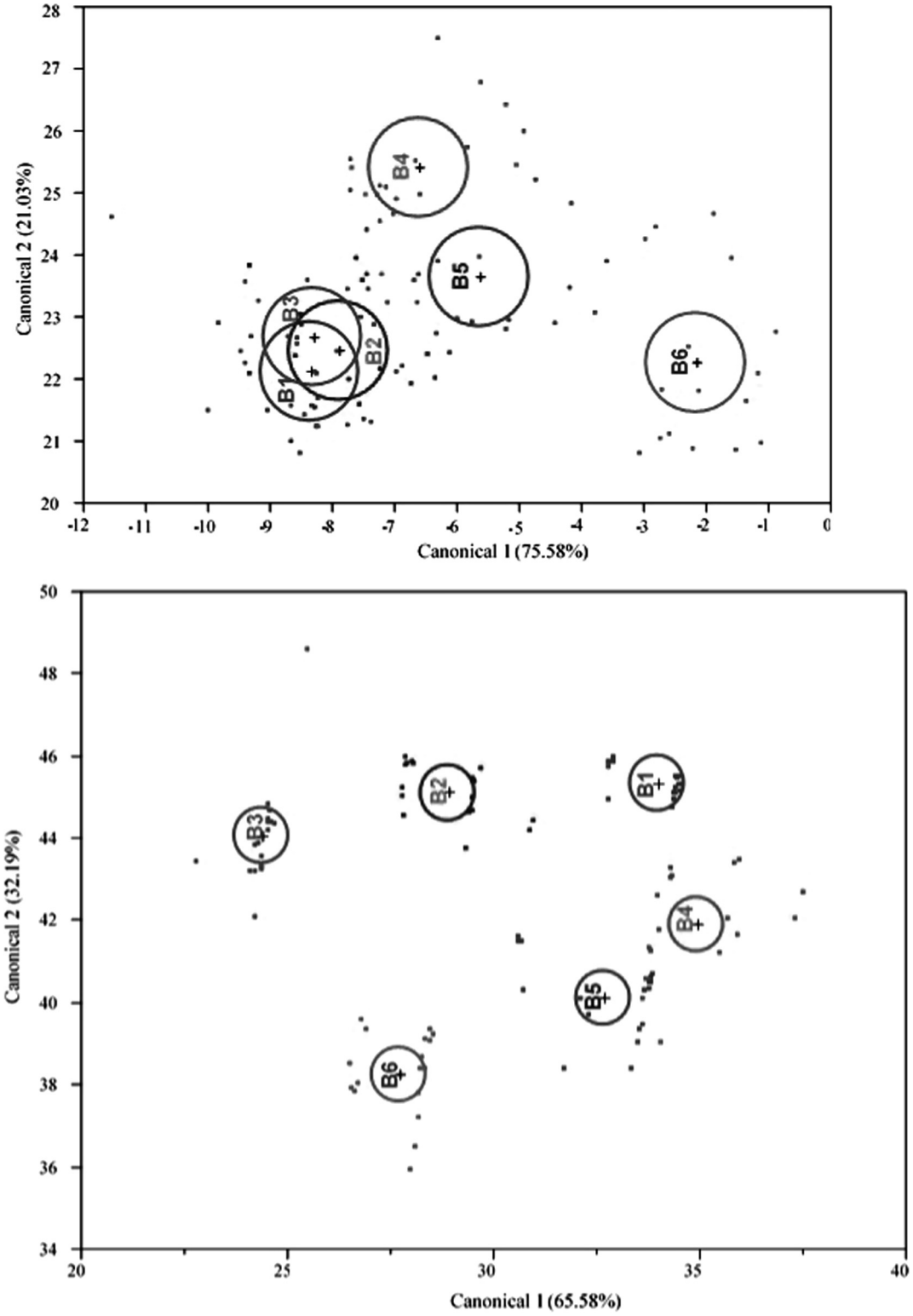

Fig. 2. Adulterated honey batches based LDA on rheological parameters (a) and physical parameters (b). (+) Group centroid.

tion of adulterants with 95.10% correct classification and a −2 Log likelihood factor of 17.06. This study confirmed that sucrose could be used as a PCs to detect honey adulteration, as reported by Cotte, Casabianca, Chardon, Lheritier, and Grenier-Loustalot (2003). Also, precision of the classification in this set are comparable with those of Paradkar and Irudayaraj (2002).

3.2.5. Chemical properties (II)

In this case, the first two canonicals explained 97.31% of the total variance (variance corresponding to canonical 1 was 77.43% and the variance corresponding to canonical 2 was 19.88%). Fig. 3 (b) shows a scatter plot for the first two canonicals obtained using LDA for 7% (batch 1), 15% (batch 2), and 30% (batch 3) IS

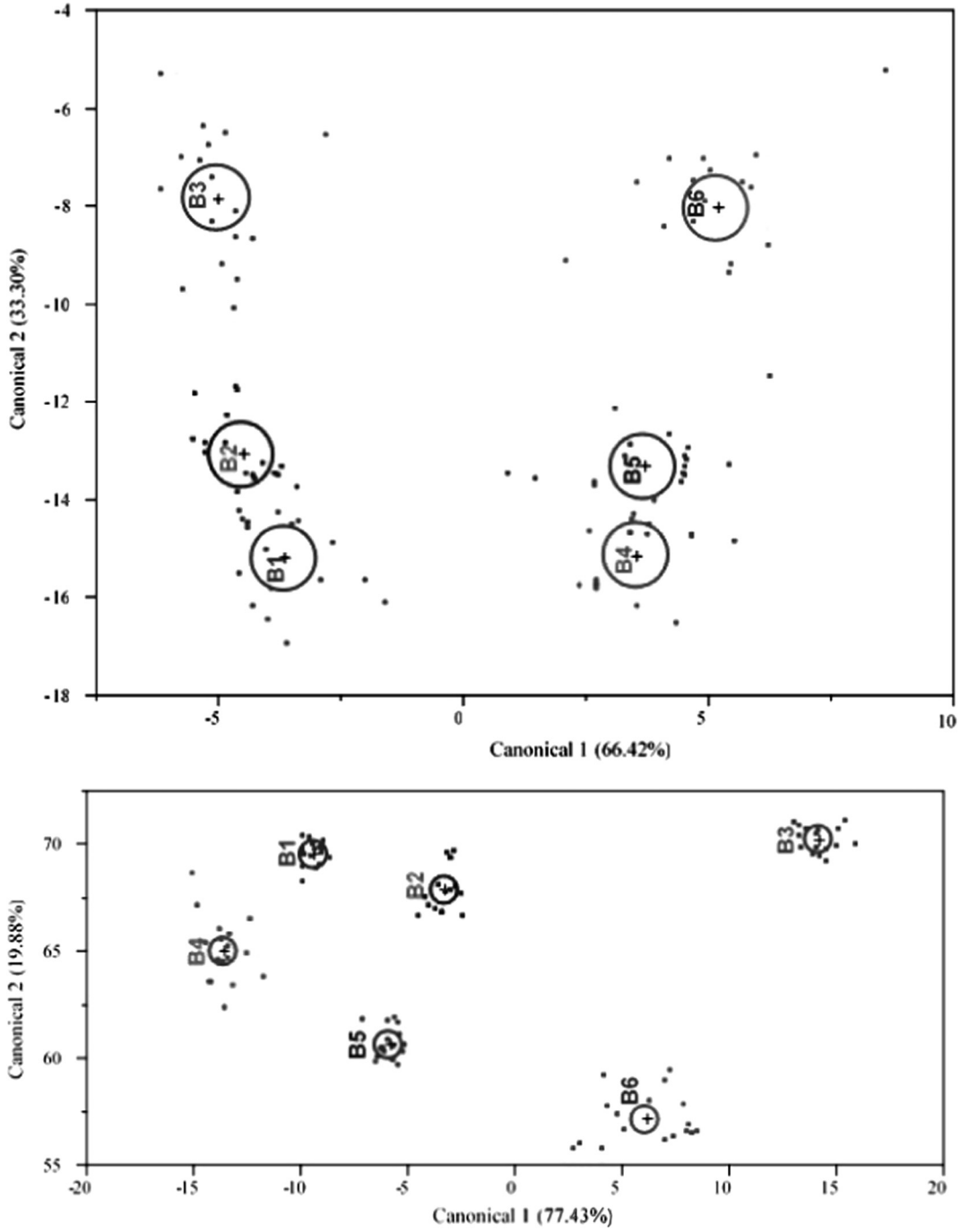

Fig. 3. Adulterated honey batches based LDA on chemical parameters I (a) and chemical parameters II (b). (+) Group centroid.

adulterated honey as well as 7% (batch 4), 15% (batch 5), and 30% (batch 6) DS adulterated honey. Free acidity, HMF and ash, as PCs in LDA, with a zero −2 Log likelihood factor and 100% correct classification, showed the best discrimination for type and concentration of the adulterants. According to previous studies, HMF is a useful property to detect honey adulteration (Corbella & Cozzolino, 2006). Our results can be compared with those of Sivakesava and Irudayaraj (2002) where 100% correct classification of adulterated honey was achieved using LDA and mid-infrared analysis. In this study, beet- and cane invert sugar-adulterated honey (7% and 25%) were separated successfully. Results from the LDA prediction our dataset are shown in Table 3. The scatter plot in Fig. 3(b) shows clear separation of six batches in the 2D space represented by canonical 1 and 2. The chemical background of honey and adulterants might explain such good separation in this 2D space using PCA-LDA methods. This result indicates the potential of chemical properties to investigate honey adulteration, and the method could be helpful in distinguishing pure and adulterated honey.

4. Conclusions

This study focused on multivariate statistical analysis to investigate the capacity of rheological and physicochemical properties of honey as PCs to detect and classify adulteration with complex sugars. The results indicated the feasibility of PCA to create new variables, in the form of PCs based on the rheological and physicochemical properties of pure honey. Thus, for discrimination and

classification processes, these PCs were use as reliable predictors. Routine quality control methods for honey (physicochemical properties), in combination with LDA, were found to be able to classify the honey, taking into account the type and concentration of the adulterants. These results highlight the use of physicochemical properties to detect honey adulteration. Color indices and rheological properties were much less accurate than chemical and other physical properties. These results support the idea that addition of any amount of every substance(s) to a food modifies the certain component ratios or creates an irregularity in its composition. This study also demonstrated the acceptability of PCA and LDA in discriminating complex sugar adulteration of honey, even at low concentrations following routine analyses. Furthermore, rheological properties could not only detect adulteration with complex sugars, like IS and DS, but also the concentration of adulteration. These results show that HMF, ash, free acidity, diastase activity, lactones, sucrose, viscosity, electrical conductivity, and a_w have an important role in detecting and classifying types and concentrations of potential adulterants in honey.

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