Research Article

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Determination of honey adulteration with beet sugar and corn syrup using infrared spectroscopy and genetic-algorithm-based multivariate calibration

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Abstract

BACKGROUND: Fourier transform infrared spectroscopy (FTIR) equipped with attenuated total reflectance accessory was used to determine honey adulteration. Adulterated honey samples were prepared by adding corn syrup, beet sugar and water as adulterants to the pure honey samples in various amounts. The spectra of adulterated and pure honey samples ($n = 209$) were recorded between 4000 and 600 cm^{-1} wavenumber range.

RESULTS: Genetic-algorithm-based inverse least squares (GILS) and partial least squares (PLS) methods were used to determine honey content and amount of adulterants. Results indicated that the multivariate calibration generated with GILS could produce successful models with standard error of cross-validation in the range 0.97–2.52%, and standard error of prediction between 0.90 and 2.19% (% w/w) for all the components contained in the adulterated samples. Similar results were obtained with PLS, generating slightly larger standard error of cross-validation and standard error of prediction values.

CONCLUSION: The fact that the models were generated with several honey samples coming from various different botanical and geographical origins, quite successful results were obtained for the detection of adulterated honey samples with a simple Fourier transform infrared spectroscopy technique. Having a genetic algorithm for variable selection helped to build somewhat better models with GILS compared with PLS.

Keywords: multivariate calibration; genetic algorithms; FTIR spectroscopy; honey adulteration; corn syrup; beet sugar

INTRODUCTION

Honey is one of the most valuable natural food products, and there has been an increasing public awareness about consumption owing to its nutrient and therapeutic effects. The most common varieties of honey are the flower honeys that are produced by honey bees (*Apis mellifera* L.) from the nectars of various kinds of flowers depending on the geographical region and the season. The second type of well-known honey is honeydew honey, which is produced from secretions of living parts of pine trees, especially in late summer to early autumn. Depending on the type of honey and the geographical region where the honey is produced, the price of honey may vary significantly. As consumption demand for honey increases, adulteration of honey with low-cost sugar substitutes has become a serious public issue that should be addressed to protect consumers from possible health issues while promoting honest honey producers. It is also a very important subject for the sake of honest honey producers and local bee farmers, as they cannot compete with the price of adulterated honey products. Turkey is one of the leading countries in the production of honey. The Ministry of Health has tightened the control of honey producers and companies to prevent them from selling adulterated honey samples to consumers. Moreover, parameters concerning the quality and safe consumption of honey have become important for the protection of human health in recent years not only in Turkey, but also all over the world.

Consumption of adulterated honey samples causes human health problems due to the existence of 5-hydroxymethylfurfural (HMF) during the adulteration process. When adulterants are added into pure honey samples, the mixture is generally heated in order to obtain a homogeneous fake product that becomes very difficult to distinguish from unadulterated honey by ordinary consumers. As thermal treatment is applied under acidic conditions to sugar-rich food products, HMF can form as an intermediate in a Maillard reaction by direct dehydration of sugars.[1] HMF formation is not only a common problem at high temperatures but also at low temperatures, depending on the duration period. While it can also be seen in pure honey, adding inverted sugar and high-fructose corn syrup to adulterate honey might cause high amounts of HMF formation. All this information indicates

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Figure 1. Turkey map that shows the geographical regions (black areas) of the collected authentic honey samples which were used during the whole period of this study.

that adulteration of honey with cheap (i.e., corn syrup) or synthetic sugars for producing cheaper honeys poses a public health risk. Consequently, to be able to distinguish natural honey from adulterated fake products is very important.

There have been a large number of publications in the last couple of decades about determination of honey adulteration with cheaper sugar substitutes such as beet sugar, corn syrup, and so on. Scientists have aimed to detect honey adulteration by working on a variety of analytical methods. Among them, nuclear magnetic resonance spectroscopy[2–5] is one of the most widely used analytical techniques for detection of honey adulteration. Additionally, honey samples contain several types of sugars, such as glucose, fructose, sucrose and so on. Chromatographic methods have also been used to determine sugar composition, and these analytical methods have generally been coupled with mass spectrometry (MS) such as gas chromatography–MS and high-performance liquid chromatography–MS. Furthermore, gas chromatography[6,7] and high-performance liquid chromatography[8–13] are also used to detect adulterated honey samples. Moreover, carbon isotope ratio (C^{12}/C^{13}) analysis[14–20] (IR-MS) is used as a standard technique to detect the presence of artificial sweeteners in honey. Although there has been a great deal of study in the literature, IR-MS analysis requires more elaborate sample preparation techniques and therefore takes a lot of time. In addition, a recent report on the use of IR-MS for the quantitative determination of honey adulteration claims that it can be very difficult to assess adulteration based on the C^{12}/C^{13} ratio as the geographical and botanical origin of the honey might cause significant variations in these isotope ratios.[19] In recent years, researchers have focused on molecular spectroscopic methods, as they are cheaper, faster and simpler than chromatographic and hyphenated techniques. The most widely used molecular spectroscopic methods are fluorescence spectroscopy, Raman spectroscopy,[21] near-infrared spectroscopy[22–27] and Fourier transform infrared (FTIR) spectroscopy.[28–35]

In this study, FTIR spectroscopy coupled with a three-reflection diamond attenuated total reflectance (ATR) accessory was used to determine honey adulteration based on pure and adulterated honey samples synthetically prepared in the laboratory with three different adulterants (beet sugar, corn syrup and water). A genetic-algorithm-based inverse least squares (GILS) multivariate calibration method[36–38] was used to develop calibration models with pure and synthetically adulterated honey samples. In order to study the predictive performance of the GILS method, the partial least squares (PLS) method was also used to develop calibration models with the same data set, and these models were tested with 100 pure honey samples. Multivariate calibration models were generated for both the honey content of the samples and adulterants, and then predictions on the pure honey samples were conducted for all the components (honey and adulterants) and the results of both GILS and PLS were compared.

MATERIAL AND METHODS

Materials

A total of 115 pure honey samples were collected from various geographical and botanical origins around Turkey in the 2014–2016 harvest seasons. Among these 115 samples, 20 of them were commercial brands of honey collected from local markets, 23 of them were from the Ordu Apiculture Research Institute (Republic of Turkey Ministry of Food Agriculture and Livestock) and 72 of them were collected on site from various beekeepers. Botanical origins of the commercial honey samples were accepted as the labels on the products, whereas the samples coming from the Ordu Apiculture Research Institute were classified according to a pollen test that was done at the institute. On the other hand, the botanical origins of the samples collected directly from the beekeepers were accepted as statements of the owners as to the collection season and geographical region. The map in Fig. 1 indicates the geographical regions of the honey samples that were used in this study. All of these pure honey samples were stored at room temperature until analysis. Corn syrup and beet sugar were purchased from a local market to be used as adulterants for preparation of synthetic samples.

Sample preparation

Among the 72 original honey samples collected from beekeepers, six of them were selected as the stock pure honey samples for the

Table 1. Percentage composition (% w/w) of adulterated honey samples, prepared with pure honey, corn syrup, beet sugar and water

No.	Honey	Corn syrup	Beet sugar	Water	No.	Honey	Corn syrup	Beet sugar	Water
1	64.35	35.65	0.00	0.00	38	77.44	9.92	6.32	6.32
2	80.07	19.93	0.00	0.00	39	62.75	8.50	14.38	14.38
3	72.91	27.09	0.00	0.00	40	74.92	0.13	12.47	12.47
4	74.81	25.19	0.00	0.00	41	56.36	10.76	16.44	16.44
5	60.46	39.54	0.00	0.00	42	44.66	12.68	21.33	21.33
6	94.36	5.64	0.00	0.00	43	65.07	3.39	15.77	15.77
7	86.33	13.67	0.00	0.00	44	41.42	23.62	17.48	17.48
8	79.85	20.15	0.00	0.00	45	45.86	13.79	20.17	20.17
9	66.23	33.77	0.00	0.00	46	53.83	20.39	12.89	12.89
10	67.70	32.30	0.00	0.00	47	55.98	7.63	18.19	18.19
11	69.50	30.50	0.00	0.00	48	51.66	16.86	15.74	15.74
12	82.71	17.29	0.00	0.00	49	69.24	19.17	5.79	5.79
13	69.45	30.55	0.00	0.00	50	48.65	19.02	16.17	16.17
14	86.45	13.55	0.00	0.00	51	60.35	21.76	8.95	8.95
15	94.56	0.00	2.72	2.72	52	59.55	26.29	7.08	7.08
16	89.36	0.00	5.32	5.32	53	69.20	10.46	10.17	10.17
17	84.70	0.00	7.65	7.65	54	79.15	11.82	4.51	4.51
18	99.08	0.00	0.46	0.46	55	58.80	9.17	16.01	16.01
19	47.36	0.00	26.32	26.32	56	71.19	24.36	2.22	2.22
20	30.43	0.00	34.79	34.79	57	48.70	23.28	14.01	14.01
21	26.64	0.00	36.68	36.68	58	59.70	10.11	15.09	15.09
22	77.84	0.00	11.08	11.08	59	53.59	19.90	13.25	13.25
23	87.90	0.00	6.05	6.05	60	85.04	4.83	5.07	5.07
24	79.60	0.00	10.20	10.20	61	57.04	5.58	18.69	18.69
25	74.58	0.00	12.71	12.71	62	79.42	5.36	7.61	7.61
26	97.56	0.00	1.22	1.22	63	83.97	6.80	4.62	4.62
27	35.51	0.00	32.24	32.24	64	63.87	23.15	6.49	6.49
28	71.19	24.31	2.25	2.25	65	60.35	29.78	4.94	4.94
29	64.75	16.23	9.51	9.51	66	58.21	11.77	15.01	15.01
30	61.88	12.65	12.73	12.73	67	59.76	14.31	12.96	12.96
31	54.72	23.02	11.13	11.13	68	52.15	23.94	11.95	11.95
32	55.32	18.34	13.17	13.17	69	69.52	26.33	2.07	2.07
33	89.32	7.07	1.80	1.80	70	58.17	20.06	10.89	10.89
34	68.62	14.89	8.25	8.25	71	50.81	28.46	10.36	10.36
35	64.27	7.34	14.19	14.19	72	68.84	10.19	10.49	10.49
36	85.75	3.30	5.48	5.48	73	79.73	11.37	4.45	4.45
37	69.29	4.35	13.18	13.18	74	59.23	26.50	7.13	7.13

preparation of the adulterated sample set. These six monofloral pure honey samples were also mixed in equal amounts in order to have a polyfloral honey sample, resulting in a total of seven stock samples (6 + 1 = 7). The selection of the six monofloral samples was made on the basis of their botanical and geographical origin in order to cover the maximum variability in adulterated samples. A total of 74 synthetically adulterated binary (honey and corn syrup), ternary (honey, beet sugar and water) and quaternary (honey, corn syrup, beet sugar and water) mixtures were prepared by mass percentage (Table 1). While preparing adulterated honey samples, corn syrup was used in its commercial form, which was a highly viscous water solution, and tap water was used to dissolve solid beet sugar as 50% (% w/w) solution. As can be seen from Table 1, the concentration ranges of each component were chosen to cover a wide range of possible real-life adulteration scenarios. Though all 74 samples given in Table 1 have some amount of honey, it is also important to introduce samples with no honey content for the models in order to detect samples with no honey content. For these, two additional sets of binary mixtures of corn syrup–water and beet sugar–water were prepared. and the percentage compositions of these samples are shown in Table 2. Among the 17 binary samples given in Table 2, nine of them were corn syrup–water and eight of them were beet sugar–water mixtures. While preparing the adulterated samples, two corn syrup stocks were used, and one of these undiluted samples was included in the calibration set and one of them reserved for future prediction. In addition, pure honey samples from various botanical and geographical origins were included in the calibration and validation sets in order to improve the predictive ability of the models. A total of 16 pure honey samples were reserved for the model construction. Honey samples that were included in the adulterated set were the ones specifically collected on site from the local producers.

To develop multivariate calibration models, a calibration set that contains 73 samples and an independent validation set with 30 samples were prepared. Among the 73 samples in the calibration set, 27 of them were quaternary mixtures of honey, corn syrup, beet sugar and water, 9 of them ternary mixtures of honey, beet sugar and water, 11 of them binary mixtures of honey and

Table 2. Percentage composition (% w/w) of corn syrup–water and beet sugar–water binary mixtures

No.	Honey	Corn syrup	Beet sugar	Water
1	0.00	9.97	0.00	90.03
2	0.00	20.02	0.00	79.98
3	0.00	30.07	0.00	69.93
4	0.00	40.22	0.00	59.78
5	0.00	50.01	0.00	49.99
6	0.00	60.15	0.00	39.85
7	0.00	69.96	0.00	30.04
8	0.00	79.82	0.00	20.18
9	0.00	90.29	0.00	9.71
10	0.00	0.00	10.20	89.80
11	0.00	0.00	19.97	80.03
12	0.00	0.00	30.05	69.95
13	0.00	0.00	39.99	60.01
14	0.00	0.00	49.87	50.13
15	0.00	0.00	60.06	39.94
16	0.00	0.00	70.08	29.92
17	0.00	0.00	77.75	22.25

corn syrup, 6 of them binary mixtures of corn syrup and water, 6 of them binary mixtures of beet sugar and water, 1 of them stock corn syrup and finally 13 of them pure honey samples ($27 + 9 + 11 + 6 + 6 + 1 + 13 = 73$). On the other hand, the independent validation set was constructed with 20 quaternary mixtures (honey, corn syrup, beet sugar and water), 4 ternary mixtures (honey, beet sugar and water), 3 binary mixtures (honey and corn syrup) and 3 pure honey samples ($20 + 4 + 3 + 3 = 30$). In addition to the calibration and independent validation sets, a secondary test set that contains 100 pure honey samples, 3 binary mixtures of corn syrup and water, 2 binary mixtures of beet sugar and water, and 1 undiluted corn syrup stock ($100 + 3 + 2 + 1 = 106$) were designed.

Spectroscopic analysis

FTIR-ATR spectra of 209 authentic honey and adulterated samples (73 in the calibration set, 30 in the independent validation set and 106 in the secondary test set) were collected using with an FTIR spectrometer (Frontier FTIR/FTNIR, PerkinElmer Inc., MA, USA) equipped with a three-reflection diamond ATR crystal between 4000 and 600 cm^{-1} wavenumber ranges. Single-beam spectra of the samples were collected against the air background. The resolution of all these spectra was set to 4 cm^{-1}, and each spectrum was obtained using four replicate scans. The ATR crystal was cleaned with ethanol before each analysis and left to dry in order to collect a background spectrum before each sample.

Multivariate calibration

Multivariate calibration takes advantage of multiple variables for constructing models to predict the properties of interest of new samples – unlike univariate calibration, which relies on a single variable. For instance, a spectral peak that is assumed to be linearly proportional with the concentration of a compound might interfere with a peak of another compound. Also, in some cases the spectrum may be so complex that choosing a single wavenumber is not possible. Another point is the complementary information from the detector: readings in other wavenumbers can be taken into account by using multivariate calibration techniques in order to enhance the predictive power of the model.

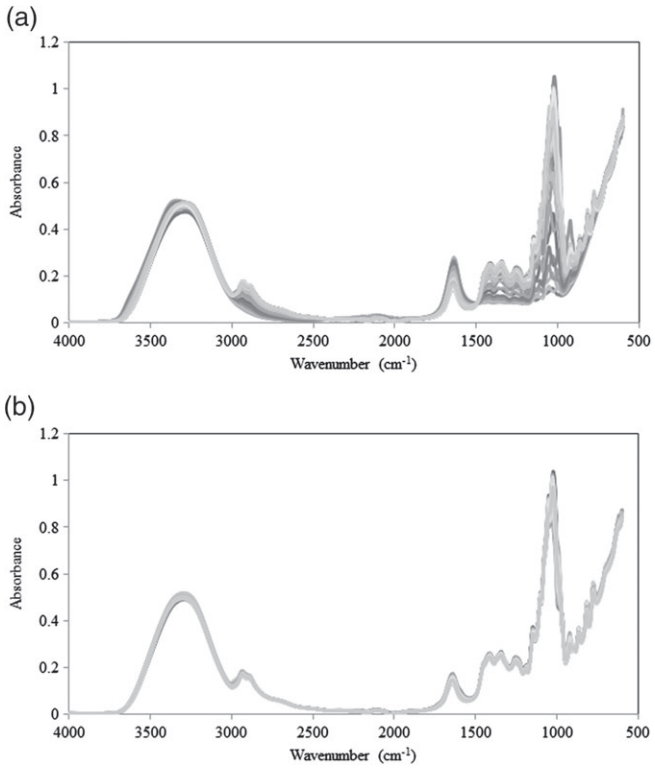

Figure 2. (a) FTIR-ATR spectra of 87 binary, ternary and quaternary adulterated honey samples prepared with pure honey, corn syrup, beet sugar and water together with 16 pure honey samples of which six of them were used to prepare adulterated samples. (b) FTIR-ATR spectra of 100 pure honey samples that were collected from different geographic areas and had different botanical origins.

GILS is multivariate calibration method in which a genetic algorithm (GA) is used as a variable selection and optimization method while constructing calibration models by using a standard inverse least squares method. Those variables that were selected by the GA were then put into an evolutionary process where the best subset of the variables was used in the models. The algorithmic details of the GILS algorithm were given in a number of previous studies[36,39,40] and will not be repeated here. PLS is a well-known factor-based multivariate calibration method originally proposed by Svante Wold[41] and has been used in many applications previously.[42,43]

Data analysis

Spectra of pure and adulterated honey samples were then transferred to another computer where data processing was carried out. The GILS and PLS methods were implemented in the MATLAB programming language using Matlab 2016a (MathWorks Inc, Natick, MA). PLS was used as a reference multivariate calibration method to compare the performance of GILS. The standard error of cross-validation (SECV) and the standard error of prediction (SEP) were calculated with the following equations for the assessment of the models:

$$\mathrm{SECV} = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m-2}} \qquad \mathrm{SEP} = \sqrt{\frac{\sum_{i=1}^{m}(c_i - \hat{c}_i)^2}{m}}$$

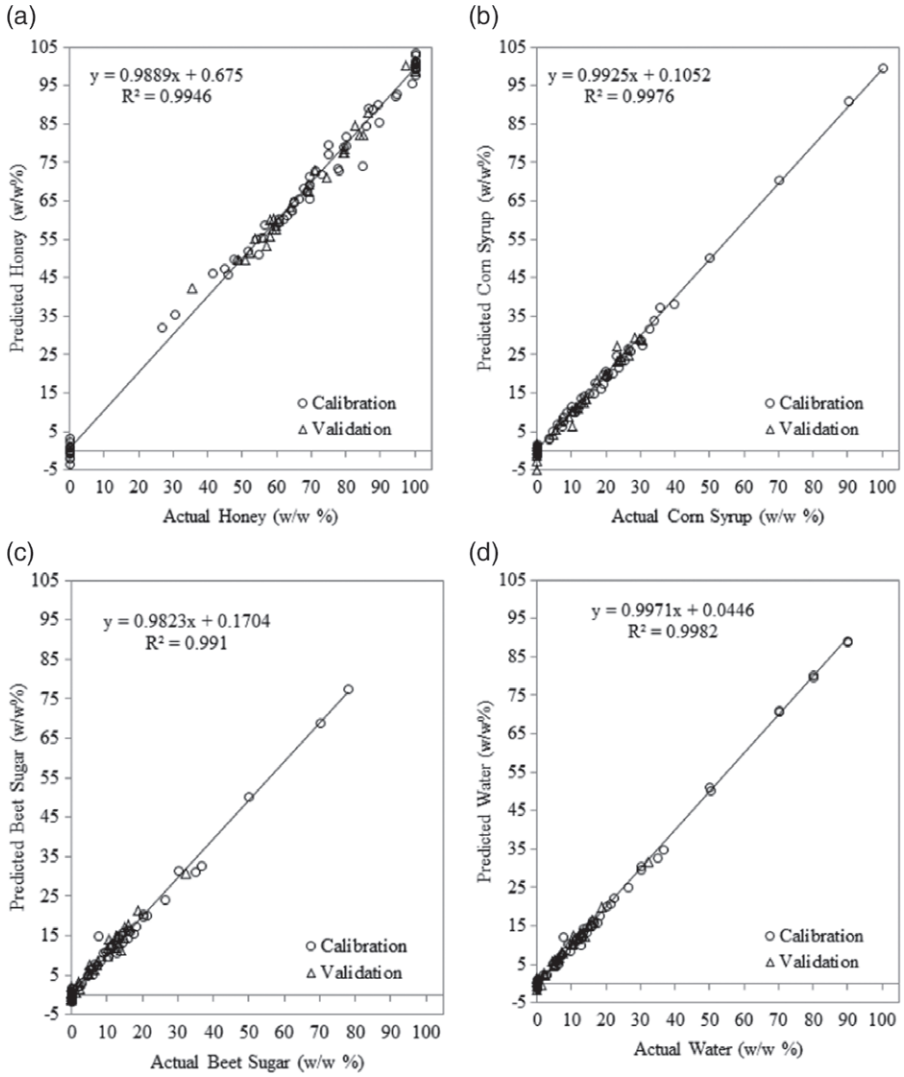

Figure 3. Actual *versus* predicted plots of GILS models for pure honey (a), corn syrup (b), beet sugar (c) and water (d).

where c_i is the reference and $\hat{c}_i$ is the predicted values of concentration of *i*th sample and *m* is the number of samples in the calibration and independent validation sets.

RESULTS AND DISCUSSION

The FTIR-ATR spectra of the samples from the calibration and independent validation sets (73 + 30 = 103 samples) are shown in Fig. 2(a), and Fig. 2(b) shows the FTIR-ATR spectra of the 100 authentic honey samples which were reserved for a secondary test set in order to test the performance of the models for the pure samples.

As can be seen from Fig. 2(a), there are significant spectral differences among the samples due to the concentration variations, especially in the fingerprint region (1500–500 cm^{-1}) of the FTIR spectra. It is expected that these differences will lead to successful calibration models throughout multivariate calibration for each component in the calibration set. When compared with the calibration and independent validation set spectra given in Fig. 2(a), the pure honey spectra shown in Fig. 2(b) demonstrates similar spectral features as expected, but there are some spectral intensity differences among those 100 pure honey samples possibly due to the geographical and botanical origins. These differences could also be partially attributed to the highly viscous nature of pure honey resulting in a slight scattering on the spectra. As a result, the predictive ability of the multivariate calibration with GILS and PLS could be affected not only for pure honey but also for adulterants. Nevertheless, multivariate calibration models generated with a large number of pure honey samples (16 pure honey samples in the calibration set) with various geographical and botanical origins are expected to account for these differences during the model building step.

Leave one out cross-validation was used with the calibration set defined in the 'Sample preparation' section for each method and the models generated were also tested with independent validation and test sets. Multivariate calibration models for each component (honey, beet sugar, corn syrup and water) were developed separately with the GILS and PLS methods.

GILS is a GA-based method for variable selection, and the algorithm was set to run with 30 genes where each gene represents a collection of randomly selected variables whose maximum size depends on the number of calibration samples. The variables are randomly selected from the whole spectral range, with an initial selection criterion of R^2 having a value of at least 0.50. The program

Table 3. Standard error of cross-validation (SECV), standard error of prediction (SEP) and regression coefficient (R^2) of honey, corn syrup, beet sugar and water contents obtained with GILS and PLS methods, along with number of principal components (PCs) and minimum and maximum concentrations of each component

	GILS results (% w/w)			PLS results				Range (% w/w)	
	SECV	SEP	R^2	No. PCs	SECV (% w/w)	SEP (% w/w)	R^2	Min.	Max.
Honey	2.52	2.19	0.9946	8	4.73	2.89	0.9807	0.00	100.00
Corn syrup	0.98	1.64	0.9976	10	2.43	2.55	0.9846	0.00	100.00
Beet sugar	1.43	1.54	0.9910	8	2.26	1.66	0.9772	0.00	77.75
Water	0.97	0.90	0.9982	7	1.47	1.18	0.9959	0.00	90.00

was set to run 100 times, in which the number of iterations was kept to 50 in each run. At the end, the best gene with the lowest SECV for the calibration set was selected to build the final model for each run, resulting in a total of 100 best models. These models were then used to predict the independent validation set, and the SEPs were determined. In addition, each model was also used to predict the honey content of the 100 pure honey samples and the six samples that contain only corn syrup–water or beet sugar–water binary mixtures in the secondary test set mentioned in the 'Sample preparation' section. In order to benefit from the averaging effect of the individual GILS models resulting from 100 best runs, the prediction values of the calibration set, independent validation set and the secondary test set were averaged. The results are plotted as actual *versus* predicted plots for the calibration and independent validation sets in Fig. 3.

As seen from Fig. 3, honey and corn syrup contents of the adulterated samples in the calibration and independent validation sets range from 0 to 100% (% w/w) and the average model generated from the results of the 100 best models produced R^2 values of 0.9946 and 0.9976 (Fig. 3(a,b)) for honey and corn syrup respectively. On the other hand, beet sugar content of the adulterated samples ranged from 0 to 80% (% w/w) and water content in the same samples ranged from 0 to 90% (% w/w). Owing to the fact that the corn syrup was purchased as a ready-to-use water solution and there was no indication of the actual water content, the water models were only constructed with the values given in Table 2. The R^2 values of the beet sugar and water models were also found to be over 0.99. Table 3 shows the SECV and SEP values for the GILS and PLS models, along with the number of principal components (PCs) for PLS.

Since the SECV and SEP values carry the same unit of the sample concentrations (% w/w), care must be taken while evaluating the magnitude of these values with respect to the dynamic range of the models as given in Table 3. The SECV and SEP values of honey content were found to be 2.52 (% w/w) and 2.19 (% w/w), respectively. For the corn syrup content of the samples, somewhat lower error values were calculated: 0.98 and 1.64 (% w/w). According to these results, the calibration model that was developed for corn syrup content can be evaluated as a slightly better model than the honey content model because both models have the same dynamic range; that is, between 0 and 100% (% w/w). Although the beet sugar model has a narrower operating range, both SECV and SEP values of this model are found to be slightly larger than those of the water model. The results of the PLS models are given Table 3 for the same data sets used in the GILS method.

The full spectral data were used in the case of PLS modelling, and no further data reduction was applied after mean centering. The optimum number of PCs for honey and beet sugar was 8, whereas for corn syrup it was 10 and for water it was 7. The R^2 values of mixture components honey, corn syrup, beet sugar and water were found as 0.9843, 0.9846, 0.9772 and 0.9959 respectively. When compared with the R^2 values obtained from GILS models, these regression coefficient values are relatively smaller. This is a reasonable result for an iterative method like GILS, and it is likely that the models may tend to be somewhat overfitted in favor

Figure 4. Predicted honey contents of pure honey samples with both GILS and PLS calibration methods.

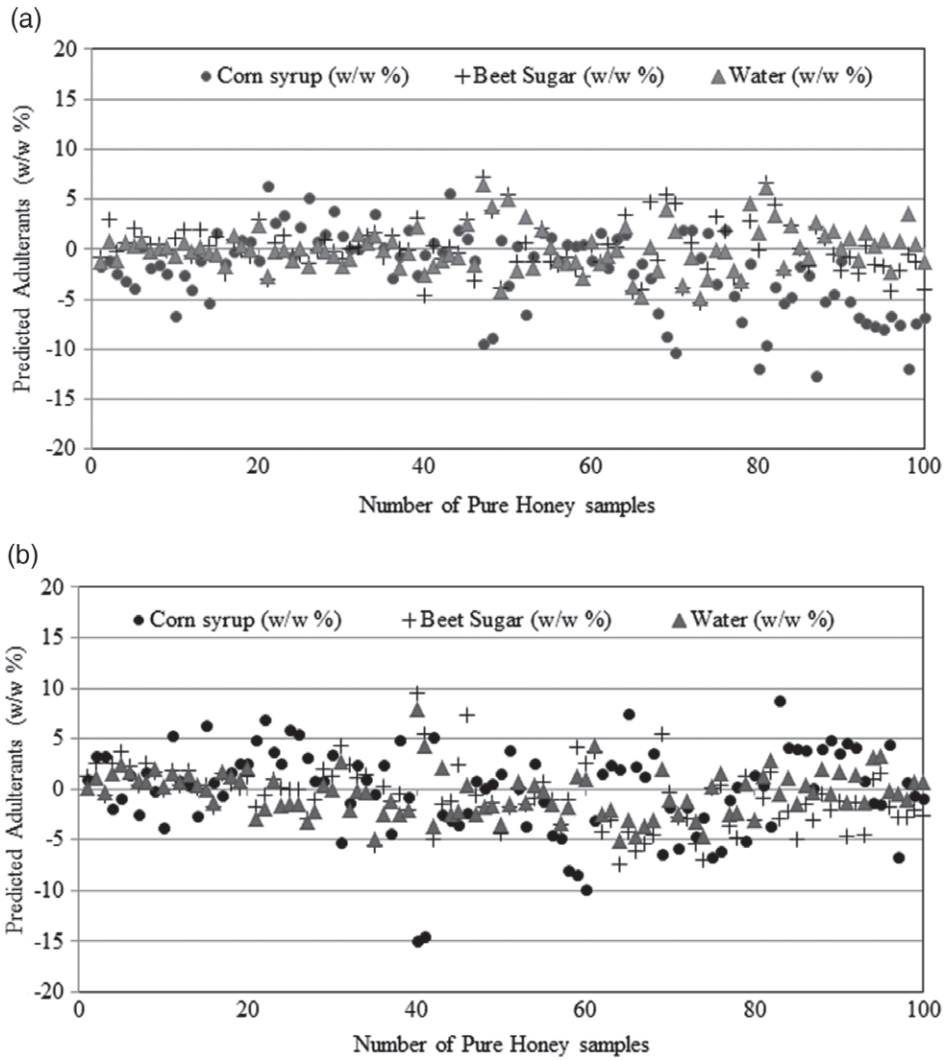

Figure 5. Predicted corn syrup, beet sugar and water contents of pure honey samples with both GILS (a) and PLS (b) calibration methods.

of calibration samples even though cross-validation is applied. However, the SEP results of GILS models for honey and water content do not seem to indicate any overfitting, as their values are even lower than the SECV values of the models. On the other hand, SEP values of corn syrup and beet sugar models are somewhat larger than SECV values, as can also be seen from Table 3.

On the other hand, the SECV and SEP values of honey, corn syrup, beet sugar and water obtained with PLS were higher than those obtained from GILS. In particular, the honey and corn syrup results are almost double the SECV and SEP values of the corresponding GILS models, whereas the differences for the beet sugar and water models in terms of SEP values were less significant. In addition, except for the corn syrup model, the SEP models for the other three components resulted in lower values when compared with the SECV values of the PLS models. These results indicate that while there is a slight overfitting problem in GILS, no such problem is observed in PLS. After completing the modelling studies with the GILS and PLS methods, the successful models were also evaluated with the secondary test set, which contains 100 pure honey samples. Figure 4 shows the honey content predictions of these samples with the GILS and PLS methods.

As can be seen from the comparison of the two methods, the predicted honey content of the samples was found to range between 90 and 110% (% w/w), resulting in a ±10% variability at the extreme limits. However, when the graph is examined in detail it is seen that the majority of samples (about 80) were predicted within ±5% (% w/w) error rate within both the GILS and PLS models. Considering the SECV and SEP values of the honey models generated with both GILS and PLS, the 5% variability in the honey content predictions of these pure honey samples is a reasonable error for 80% of the test set samples. On the other hand, both methods showed similar prediction ability for the overall comparison of all the secondary test samples in honey content. The prediction results of corn syrup, beet sugar and water content of the samples are given in two separate graphs in Fig. 5 for GILS (a) and PLS (b).

The GILS prediction results of corn syrup, which should be zero in the ideal case, demonstrated much larger variations compared with beet sugar and water in the pure honey samples. In fact, deviations from 0% are the smallest for water predictions, especially in first 60 samples. Overall, the majority of the samples yielded ±3% variability for sugar beet and water content, whereas for the corn syrup content there were 20 samples whose predictions were below the −5% boundary line and five samples above the +5% boundary line. On the other hand, there were only five samples whose beet sugar predictions were above the +5% boundary line,

Table 4. GILS predicted results of binary mixtures of corn syrup with water and beet sugar with water

	Corn syrup (% w/w)		Beet sugar (% w/w)		Water (% w/w)		Honey (% w/w)	
No.	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	40.22	38.96	0.00	−0.13	59.78	59.82	0.00	0.92
2	60.15	60.96	0.00	0.70	39.85	40.90	0.00	−2.38
3	79.82	79.62	0.00	−1.19	20.18	21.06	0.00	−0.63
4	100.00	97.84	0.00	−0.64	0.00	−1.24	0.00	4.96
5	0.00	−0.79	39.99	42.13	60.01	61.10	0.00	−2.24
6	0.00	−2.64	60.06	59.22	39.94	36.12	0.00	9.04

Table 5. PLS predicted results of binary mixtures of corn syrup with water and beet sugar with water

	Corn syrup (% w/w)		Beet sugar (% w/w)		Water (% w/w)		Honey (% w/w)	
No.	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	40.22	35.19	0.00	0.53	59.78	61.05	0.00	7.03
2	60.15	61.64	0.00	0.88	39.85	40.35	0.00	0.54
3	79.82	82.10	0.00	0.91	20.18	20.72	0.00	−1.85
4	100.00	91.27	0.00	−3.00	0.00	−2.09	0.00	12.53
5	0.00	0.92	39.99	45.32	60.01	61.18	0.00	−11.01
6	0.00	−2.18	60.06	60.65	39.94	36.43	0.00	4.63

and the rest of the 100 samples were all in between ±5% intervals. In terms of water content predictions, there was no sample outside the ±5% boundary lines. The results of the PLS predictions shown in Fig. 5(b) display more uniform variability, which ranges mostly in the ±5% interval for all three adulterants in the secondary test set. When compared with GILS predictions, it is seen that GILS predictions are less scattered than PLS predictions in the first 40 samples.

In order to clarify the figures, the GILS and PLS predicted results of the samples with no honey content (sample numbers 101–106 in the secondary test set) are given in Tables 4 and 5 respectively.

As shown in Tables 4 and 5, the first four samples are the corn syrup–water mixtures, and their actual corn syrup contents are 40.22%, 60.15%, 79.82% and 100% (% w/w). On the other hand, the subsequent two samples had been prepared as binary mixtures of beet sugar and water, with 39.99% and 60.06% (% w/w) beet sugar content. The corn syrup, beet sugar, water and honey contents were predicted with GILS and PLS models in order to evaluate their prediction ability, and the actual and predicted results are given in four distinct columns for each model. When Table 4 is examined, it is clear that corn syrup determinations of the six samples are quite accurate, with less than ±3% (% w/w) deviation from the actual values. However, the PLS predictions of corn syrup for samples 1 and 4 deviated around 5–9% (% w/w) in the negative direction. The results of the remaining samples predicted by both methods were in good agreement with the actual values. When the predicted beet sugar concentrations were assessed, GILS results were found to be more reliable than PLS predicted results for the fourth and fifth samples. Additionally, results of water content predictions demonstrated that sample 6 was predicted with a relatively larger deviation (approximately 4%) by both GILS and PLS models but the other five sample prediction results were in good agreement with the actual values. The models developed for honey with both GILS and PLS were also used to assess the prediction ability, although these binary mixtures had no honey content. Among the PLS predicted results, honey contents for the first, fourth and fifth samples were predicted above the ±5% (% w/w) interval. When the GILS predicted results were examined for the same content, only the last sample was predicted over the ±5% (% w/w) range.

CONCLUSION

As a general concluding remark it can be said that GILS offers some advantages over the conventional PLS method due to the variable selection with a GA. In terms of honey content determination of pure honey samples, both methods gave quite successful results regardless of the botanical and geographical origin of the samples. The results revealed that both GILS and PLS can be used for initial detection of honey adulteration coupled with FTIR-ATR spectroscopy, and even onsite when the models are transferred to a portable FTIR system. FTIR spectroscopy is a simpler, faster and cheaper method for field application in comparison with the other analytical techniques, which require tedious sample preparation.

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