An approach to assess the quality of honey using partial least square method

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Article Info	ABSTRACT
	The objective of the present study is to obtain the quantity of honey components such as moisture, glucose, fructose and sucrose in order to access the quality of honey. The tested honey samples are authenticated if the characteristics of a pure honey. The average ratio of 56% fructose to 44% glucose, but the ratios in the individual honeys ranged from a high of 64% fructose and 36% glucose to a low of 50% fructose and 50% glucose. The contents such as fructose and sucrose in honey is due to the presence of invertase enzymes. The organic acids present in the honey is responsible for the flavor and stability against the contamination of honey due to microorganisms. The natural food items are adulterated intentionally to increase the quantity and there by the quality gets affected. The main adulterants added in honey are sucrose, corn syrup, sugar syrup and jaggery syrup. The quantification deals in finding out the amount of basic constituents present in pure honey and adulterated honey using Fourier transform infrared (FTIR) spectrometer with the multivariate analysis and validating the same using chemical analysis method. The partial least square model is used in predicting the constituents of the samples.

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1. INTRODUCTION

Honey is a natural product obtained by honey bees from the nectar of flowers or excretions of plant sucking insects. Honey is a substance with the combinations of various substances such as carbohydrates, moisture, proteins, acids, hydroxymethylfurfural (HMF), and ashes [1]. Honey has a content 85% carbohydrates, 15 water, 0.3% proteins, 0.2% ashes, and less quantities of vitamins and amino-acids as well as other constituents in low levels of concentration [2], [3]. The composition and characteristics of honey varies due to botanical and geographical origin or the nectar used by the bees to due to which honey seems to be a complex mixture by nature. The floral origin determines the feature of the honey. A familiar problem in many parts of the world is adulteration of food stuff with different adulterants irrespective of the status of the country with respect to the economy. The adulteration is either intentional or unintentional rather to increase the quantity of the food substance. The addition of sucrose, beet syrup, corn syrup, molasses, banana, or any other harmless or harmful substances. This lead to the nutritional and health problems on consumers leading to the brutal influence on the national and international trade opportunities of the product [4]-[6]. Several honey purity tests conducted for the determination of sugar profile level by spectrophotometer, sugar profile

by gas chromatography, water content by hand held refractometer, determination of hydrogen peroxide and pH value by pH meter [7], [8].

Fourier transform infrared (FTIR) spectroscopy is a technique which is used to obtain infrared spectrum of absorption, emission, and photo conductivity of solid, liquid, and gas. It is used to detect different functional groups in PHB. FTIR spectrum is recorded between 4000 and 400 cm^{-1}. FTIR uses the mathematical process to translate the raw data into the actual spectrum. FTIR method is used to obtain the infrared spectrum of transmission or absorption of a sample. Depending on the infrared absorption frequency range 600–4000 cm^{-1}, the specific molecular groups prevailing in the sample will be determined through spectrum data in the automated software of spectroscopy [9]-[11]. The mathematical model and statistical procedures can be used in extracting information using Chemo metrics from the measurements obtained from a chemical system. There are two types of data, univariate which contains one variable to measure and the other for prediction. Multivariate data contains more variables to measure and predict. The chemo metric analysis starts with measuring and collection of data. The relevant information are extracted from the measured data (spectrometric data or chromatographic) using mathematical and statistical methods which facilitates in decision making. The next aim is to preprocess the obtained data to remove variation among the data that is not related to the analytical information. Typical preprocessing includes baseline correction, mean centering, normalization, orthogonal signal correction, multiplicative scatter correction, and savitsky-golay derivation. Many different methods for performing multivariate data analysis is available are principal component analysis (PCA), partial least squares (PLS), misclassification rate (MCR), and neural networks (NNs) [12], [13]. The qualitative analysis includes obtaining classification models followed by prediction and validation, whereas the quantitative analysis works with prediction models followed by validation. The quantification of common adulterants seen in milk samples using. The calibration is performed using least-squares support vector machine for the data obtained from Near-infrared spectroscopy using diffuse reflectance. The chemo metrics combined with Near-infrared spectroscopy is used to detect adulteration in honey using since there is a non-linear behaviour present when different adulterants are in the same data set, using the linear methods such as PLSR is difficult [14], [15].

Multivariate analysis (MVA): Multivariate data analysis determines the relationships existing between many variables. The multivariate analysis is rarely used to production processes but primarily applied in technical groups and laboratories. The MVA offers an insight in improving the performance of the process and product development. While analyzing the uni variate complex systems, the uni variate statistics leads to failure causing the process upset. The multivariate analysis tools helps engineers and production managers to understand the behaviour of the process and implement strategies in more robust control to control the quality, maximize the yield, run the processes closer to limits, to use components with lower cost, reduce energy, cycle time and minimize the waste [16]-[18].

Partial least squares (PLS): PLS is a widely used technique in chemo metrics, especially in the case where the number of independent variables is significantly larger than the number of data points [19], [20]. Partial least squares is a popular method for soft modelling in industrial applications. Partial least squares is a method for constructing predictive models when the factors are many and highly col-linear. A great advantage of PLS regression over classic regression are the available charts that describe the data structure. PLS regression is also used to build predictive models [21], [22]. The predicted values.

2. RESEARCH METHOD

The research work aims at finding the quality of honey samples using chemometric analysis. The contituents of honey and the adulteration in honey has been spotted using various chromatographic methods such as gas chromatography, high performance liquid chromatography and other physio chemical tests which are fast and accurate. The study from the previous research works reveals that application of multivariate data analysis such as canonical variate analysis, principal component analysis, partial least square regression, principal component regression. Linear discriminant analysis are promising methods of analysis for honey samples in identifying and developing a model to quantify the constituents of honey.

The different honey samples such as hill Honey; branded Honey were collected, 150 ml of honey is taken from a sample of 1-litre of hill honey. Sugar syrup is prepared using 150 ml of distilled water with 300 gm of sugar. 150 ml of honey is mixed with 15 ml of sugar syrup till the solution becomes homogeneous. The concentration of sugar syrup is increased as 20%, 30% and 40% with the hill honey. The spectral data is obtained for various honey samples, Sugar syrup, honey adulterated with sugar syrup with increased concentrations. The spectral data is acquired using FTIR spectrometer. The generalized block diagram is shown in Figure 1, which describes the process involved in the research work. The article illustrates only a part of the work rather than the work as whole.
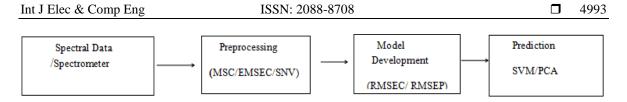
Figure 1. Generalized block diagram of the research work

The identification of the finger print regions of the key moisture, glucose, fructose and sucrose is followed by the preprocessing of the spectral data obtained for the samples of honey. The key constituents present in honey in larger composition are moisture, glucose, fructose and sucrose. The composition of moisture is measured to assess the shelf life of honey. The composition of glucose and fructose must be predicted to find the ratio between glucose and fructose whose allowable range is 1 for an authentic honey. The quantitative analysis of the components is performed in the research work in order to determine whether there is a change in the composition of the raw honey and the samples where the adulterant is added intentionally. Thus, a calibration model was developed using PLS to predict the constituents present in honey based on the spectral data obtained from the FTIR spectroscopy. The calibration curve is plotted between the reference values and predicted values for the acquired data from the samples to find out the RMSE and R^2 values. The detailed explanation is described in results and discussions section.

3. RESULTS AND DISCUSSION

The spectra of pure honey show the absorbance band 1062.45, 1646, 3372.31 nm corresponds to glucose, fructose and sucrose and the sample is pure hill honey containing 100% hill honey and 0% sugar syrup. The spectra of sugar syrup show the absorbance band at 1046.36, 1645.94, 3404.48 nm corresponds to glucose, fructose and sucrose and the sample is pure sugar syrup containing 0% hill honey and 100% sugar syrup. The spectra of hill honey with increasing concentration of sugar syrup show the absorbance and 1054.40, 1645.95, 2932.68, 3388.40 nm corresponds to glucose, fructose and sucrose and the sample contains increasing concentration of sugar syrup at 10%, 20%, 30%, 40% respectively. Partial least square analysis is applied in addition to the FTIR spectra of pure honey. The concentrations of the main constituents such as moisture and the main sugars such as glucose, fructose and sucrose are obtained using PLS technique. The chemical analysis is performed and used as a reference value to validate the FTIR predictions of the concentration of the main sugars. The results thus obtained has proved that the chemo metric analysis of linked with FTIR spectroscopy can be used to predict the contents of moisture, sugar [23], [24].

3.1. Prediction of moisture

The samples such as hill honey named as HH1, HH2, HH3, HH4, HH5, HH6 are analyzed using the FTIR spectrometer, out of which few samples are used for prediction and the remaining for validation. The graph is plotted for the predicted values vs reference values to obtain the concentration of the moisture content in the samples. From the Figure 2, it is observed that the RMSE=0.023 and R^2=0.9975 are the predicted values and RMSE=0.023 and R^2=0.9989 for the reference. The Table 1 shows the reference value, predicted values and the deviation from the reference values which is obtained from the chemical analysis in predicting the quantity of moisture. The score plot for the PLS calibration curve predicting the content of moisture in Honey is shown in Figure 3. The score plot is plotted for the samples of hill honey, branded honey, honey adulterated with sugar syrups in different concentrations as shown in Figure 4. The samples mentioned in the list are discriminated clearly in the score plot. The score plot is plotted for the factor-1 and factor-2.The score plot gives information about sample proximity and data set structure [25].

3.2. Prediction of glucose, fructose and sucrose

The samples such as hill honey named as HH1, HH2, HH3, HH4, HH5, HH6 along with the branded honey samples named as BH1, BH2, BH3, BH4, BH5, BH6 then the sugar syrups added to the hill honey with different concentrations labeled as HS1, HS2, HS3, HS4, HS5, HS6 are analyzed using the FTIR spectrometer, out of which few samples are used for prediction and the remaining for validation. The graph is plotted for the predicted values vs reference values to obtain the concentration of the glucose content in the samples. From the Figure 5, it is observed that the RMSE=0.013 and R^2=0.9973 are the predicted values and RMSE=0.0258 and R^2=0.9921 for the reference. The Table 1 shows the reference value, predicted values and the deviation from the reference values which is obtained from the chemical analysis in predicting the quantity of glucose [26], [27].
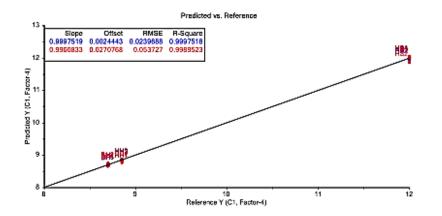
Figure 2. Partial least squares calibration curve predicting the content of moisture in honey

Table 1. The prediction vs reference values of moisture and glucose

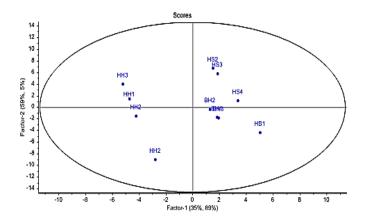| Sample	Reference value	Predicted value	Deviation	Sample	Reference value	Predicted value	Deviation
		Moisture				Glucose	
HH4	6.3500	6.3475	0.0099	HH4	8.8500	8.815	2.3918
HH5	6.3500	5.7937	0.6001	HH5	8.8500	8.815	2.3918
BH5	6.9000	6.8378	0.0154	BH5	8.7000	8.9197	0.0867
BH6	6.9000	6.8799	0.0104	BH6	8.7000	8.8321	0.0489
HS5	6.9000	6.8935	0.0065	HS5	12.000	12.0273	0.0287
HS6	6.9000	6.8812	0.0091	HS6	12.000	12.0432	0.0428

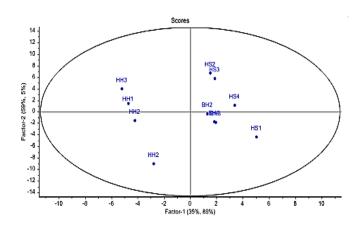Figure 3. Partial least squares calibration curve predicting the content of moisture in honey

Figure 4. Score plot for the FTIR spectral data of hill honey, branded honey, adulterated honey
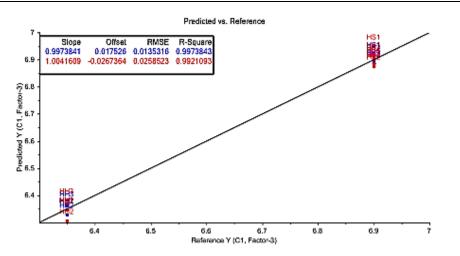
Figure 5. Partial least squares calibration curve predicting the content of glucose in honey

The graph is plotted between the predicted values and reference values to obtain the concentration of the fructose content in the samples. From the Figure 6, it is observed that the RMSE=0.627 and R^2=0.9983 are the predicted values and RMSE=1.274 and R^2=0.9979 for the reference. The Table 2 shows the reference value, predicted values and the deviation from the reference values which is obtained from the chemical analysis in predicting the quantity of fructose [28].
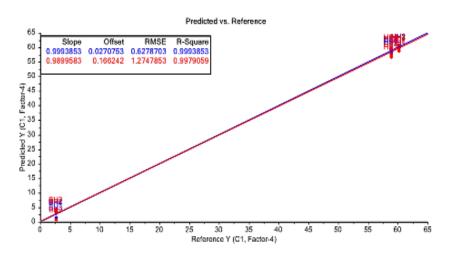

Figure 6. Partial least squares calibration curve predicting the content of fructose in honey

Table 2. The prediction vs reference values of fructose and sucrose

Sample	Reference value	Predicted value	Deviation	Sample	Reference value	Predicted value	Deviation
		Fructose				Sucrose	
HH4	60.2000	59.6728	1.0006	HH4	2.4000	1.9562	0.5367
HH5	60.2000	135.8681	53.4479	HH5	2.4000	0.9773	22.9930
BH5	2.7000	8.5649	1.8865	BH5	2.5000	4.1670	0.8689
BH6	2.7000	6.0807	1.1251	BH6	2.5000	3.4932	0.5565
HS5	58.9000	59.8127	0.6687	HS5	30.2000	30.4123	0.3775
HS6	58.9000	60.9223	0.9544	HS6	30.2000	30.4823	0.4885

Figure 6 shows the plot between the predicted value and reference value using PLS model in predicting the sucrose content in the samples of hill honey, branded honey and hill honey adulterated with sugar syrups in different concentrations. From the Figure 7, it is observed that the RMSE=0.160 and R^2=0.999 are the predicted values and RMSE=0.810 and R^2=0.9972 for the reference. The Table 2 shows the

reference value, predicted values and the deviation from the reference values which is obtained from the chemical analysis in predicting the quantity of fructose.
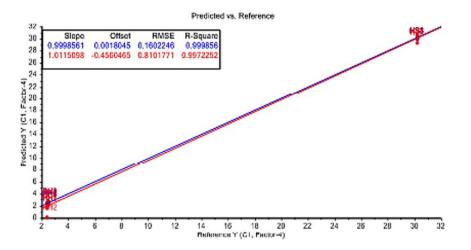

Figure 7. Partial least squares calibration curve predicting the content of sucrose in honey

4. CONCLUSION

The regression models were developed using PLS method to predict the quantity of constituents such as moisture, glucose, fructose and sucrose. The RMSE value of 0.023 and R^2 of 0.9975 and the predicted values and RMSE=0.023 and R^2=0.9989 and the reference for the moisture content. The RMSE=0.627 and R^2=0.9983 are the predicted values and RMSE=1.274 and R^2=0.9979 and r the reference for glucose content. The RMSE=0.627 and R^2=0.9983 are the predicted values and RMSE=1.274 and R^2=0.9979 for the reference for fructose content. The RMSE=0.160 and R^2=0.999 are the predicted values and RMSE=0.810 and R^2=0.9972 for sucrose. The composition of reducing sugars in pure samples of honey must be less than 60% as per European Standards. Due to the high humidity the % the reducing sugars is lower than 60% in a country like Malaysia which falls in a tropical region. But still the ratio between fructose/glucose observed for pure honey vary from 0.9-1.35. The total percentage of reducing sugars in pure and adulterated honey might have the indistinguishable characteristics. One of the studies reveals that the level of sucrose exceeding 5% but not more than 5% has been confirmed by the European standard for pure honey. The allowable moisture content in pure honey is up to 20%. The permissible value of glucose ranges between 22.89 and 40.75%, the acceptable range of fructose is from 30.91 to 44.26%, and tolerable value of sucrose ranges from 0.25 to 7.59%. The spectra obtained from the FTIR has shown a promising result in finding out the amount of constituents that contributes much in samples of honey and adulterated honey with the corn syrup. The obtained result shows that there is no much deviation in the quantity of moisture, glucose fructose and sucrose from the allowable range to the obtained results using multivariate model using FTIR spectroscopy technique.

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G. Rajalakshmi has completed the under gradutate program from Sethu Institute of Technology, Madurai Kamaraj University in the discipline of Instrumentation and Control Engineering in the year 1999. The master's degree was attained in Electronics & Control Engineering in the year 2007, form Sathyabama University.She started her carrier as a lecturer which is her passion in an Engineering College in the department of Electronics and Instrimentation Engineering. Now she is working as an Assistant Professor in the Department of Electronics and Instrumentation Engineering, Sathyabama Institute of Science & Technology, Chennai, pursuing the Ph.D programme in Signal processing. The author is much interested in the real time applications based research work, which could serve the society also. The adulteration has been a massive problem in the food industries which paved the way for her to undergo the research work in quality analysis of food substances. The title of her Ph.D work is "Quality Evaluation of honey using spectroscopic techniques". As a result of her reserach work she has Published the papers in National & International Journals and also in International conferences.

A. Gopal obtained his degree and, post graduation from Madras Christian College, Chennai in the field of Physics with Electronics as specialization. He received his Ph.D from University of Madras during the year 1995 in the filed of electronic-instrumentation and underwent Post Doctoral Fellowship for a period of one year at Oakland University, Detroit, Michigan, USA in 1998. He started his career at CEERI Centre, Chennai as Junior Research Fellow (JRF) and presently working as Chief Scientist & Scientist-in-charge, CEERI Chennai and also Professor of AcSIR, CSIR. So far he has involved in the design & developed of twenty industrial application prototype instruments, which has got application towards Paper Industry, Fruit exporting Industries, Baking industries herbal industries. As on date he has published around 90 papers in reputed journals/Conference Proceedings. He has got 12 awards (including Two from IEEE) and also got Prof. LIPTAK award from ISA, USA for his contribution to instrumentation & automation. He has edited around 23 conference/international conference proceeding materials. He has involved in organizing 15 international conferences and conducted several workshops. Also, he guided several Undergraduate and Post Graduate students of engineering institutions. Presently guiding 8 Ph.D scholars and as a D.C member given technical guidance to around 50 research scholars. Under his guidance two scholars has got Ph.D degree.

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