

Discrimination of various pure honey samples and its adulterants using FTIR spectroscopy coupled with chemometrics

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Abstract— Various honey samples and possible adulterants has been characterized using Fourier Transform Infrared (FT-IR) spectroscopy integrated with ATR sampling. Spectral data of twelve varieties of samples including mono-floral honey, multi-floral honey and different variety of adulterants has been collected in Mid-IR region (4000cm^{-1} - 400cm^{-1}). Spectral Mid-IR data has been corrected using baseline correction method and pre-processed using 2nd order derivative & Standard Normal Variate (SNV) methods for removal of any additive & multiplicative scattering effects. The principal component analysis (PCA) has been used for dimension reduction in the data set and exploratory data analysis of the honey and its adulterant samples. K-means, K-medians and Fuzzy C Means based classification model has been developed for the classification of pure honey samples and adulterants. Developed model has been cross-validated using external samples. The proposed method shows 97-100% discrimination ability for the given sample both in training and testing dataset.

Keywords—Honey, Adulteration, FT-IR Spectroscopy, Chemometrics.

I. INTRODUCTION

In India adulteration of the food is a common practice these days. There is a huge list of products both packaged and unpackaged available in the Indian market that are found adulterated and go into waste as they are unfit for consumption. This is such a violation of the rights of the customer who pay for these products, the farmers who work hard to grow the crops and the government who provide a multitude of resources for farming and food industry. Honey is one of the most ancient food product in the humankind. Honey possess many nutritionally, medicinally important substances which makes it very popular across the world. According to the norms of FSSAI no other additives or substances can be added to it apart from the other forms of pure honey. This is only the case with multi-floral honey but for mono-floral honey even other pure forms of honey cannot be added [1][2]. These mono-floral honey samples have several important properties in the medicine manufacturing industry [1][2]. India's export figures for pure natural honey stands at 61,333.88 MT worth of Rs. 732.16 Crore during the financial year of 2018-19. The market

is projected to reach Rs. 2805.7 Crore by 2024, growing at a CAGR of 10.2% during 2019-2024. All these financial figures show the importance of the honey industry for Indian economy & farmers. However, with the increasing environmental pollution in India, the honey bee population has decreased and it has hit the honey production units. On the other hand, demand from the market has been increasing rapidly and consequently rise in the honey adulteration. Several media reports can be found for the last few years indicating the problem of adulteration in honey expanding its roots in the country. Basically there are two ways by which honey can be adulterated; direct adulteration & indirect adulteration. Direct adulteration is performed by directly adding some foreign substance to the pure honey. High Fructose Corn Syrup (HFCS), glucose syrup, rice syrup, sugar syrups produced from beet, cane etc. and invert sugar syrups are some common adulterants which are added to the honey directly [3-4]. These adulterants can be tailored to mimic the natural constituents (e.g. sucrose, glucose, fructose etc.) profile of honey which makes these difficult to detect during analysis. High Fructose Corn Syrup (HFCS), is the most popular adulterant in the industry as it is much cheaper than the pure honey and its composition is much similar to the natural honey [5]. In India corn syrup, cane sugar and jaggery are most common adulterants for honey. In indirect adulteration, the adulterants are not directly added to the honey but are indirectly introduced by feeding these industrial sugar syrups to the honey bees at the stage when broods become available. Now the detection of these kinds of adulterated samples is a huge challenge as it is still unknown how the honey bees are converting the industrial sugars after being fed into the honey.

Now to detect adulteration in honey numerous analytical techniques can be applied such as Thin Layer Chromatography (TLC) [5], High Performance Thin Layer Chromatography (HPLC) [6] Gas Chromatography (GC) [7], Impedance Spectroscopy, Raman Spectroscopy, Stable Carbon Isotopic Ratio Analysis (SCIRA), Nuclear Magnetic Resonance (NMR), Mid Infrared Spectroscopy and NIR spectroscopy [8-10] etc. Mostly the chromatography based measurements involve the use of chemicals, time consuming and known to be the

destructive measurement techniques i.e. the sample under test cannot be reused. Therefore infrared spectroscopy coupled with chemometrics based multivariate data analysis (qualitative/quantitative) seems to be a prominent solution for detection of adulteration in honey. In fact from the last two decades, infrared (IR) spectroscopy has gained wide acceptance in the food industry over other analytical techniques due to many features e.g. ability to record spectra for solid and liquid without sample preparation, low cost, fast scanning, portable instrumentation etc.

In this work FT-IR spectroscopy with ATR sampling accessory coupled with chemometrics based qualitative/quantitative has been used for discrimination of pure honey samples and probable adulterants. K-means, K-medians and Fuzzy C means based classification model has been developed for discrimination of honey samples from various adulterant syrup samples.

II. MATERIALS AND METHODS

A. Preparation of samples

For this feasibility study total twelve different honey & adulterant samples were collected from different part of the country. The sample set comprises of two monofloral honey samples, two commercial honey samples, two pure multifloral honey samples collected directly from farmer and six different adulterant syrups procured from market. All these selected twelve varieties of the samples are as shown in the Table 1.

TABLE I. LIST OF VARIOUS HONEY AND ADULTERANTS UNDER TEST.

S.No.	Honey Variety	Category
1	Lemon Honey	Monofloral
2	Ginger Honey	Monofloral
3	Commercial Sample (2 no's)	Commercial multifloral
4	Pure honey collected from farmer (2 no's)	Pure multifloral
5	Cane Sugar Syrup	Adulterant
6	Corn Syrup	Adulterant
7	Jaggery Syrup	Adulterant
8	Maltose Syrup	Adulterant
9	Sucrose Syrup	Adulterant
10	Fructose Syrup	Adulterant

Each sample was analyzed using FTIR based instrument and the block diagram of the complete experimental work is shown in Fig.1.

B. Infrared Spectrophotometer and acquisition of spectra

An FTIR spectrophotometer with spectral range 4000cm^{-1} - 400cm^{-1} with resolution of 1.5cm^{-1} was used for acquisition of spectra from honey/adulterant samples. The equipment consists of Attenuated Total Reflectance (ATR) based sampling technique using diamond ATR crystal. The ATR based sampling offer several advantages such as very thin sampling path. Since the infrared rays has penetration depth typically in μm , therefore it requires very small sampling preparation.

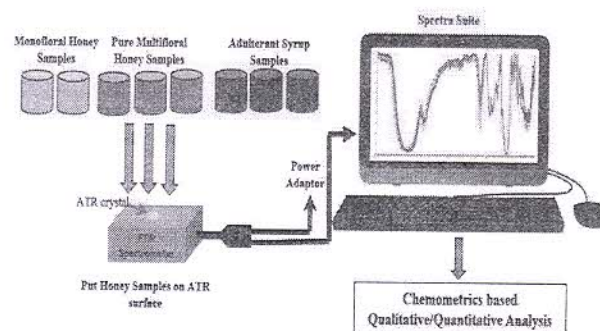


Fig.1. Block diagram of design of experiments for honey and its adulterant samples.

The samples were directly placed over the ATR crystal ensuring it covers it completely with no bubble formations. The spectrum was acquired in absorbance mode from the spectrometer utilizing the complete spectral range $4000\text{--}400\text{cm}^{-1}$ using the spectra suite software. The spectrum was acquired with the background subtraction and then corrected using preprocessing techniques such as baseline correction and normalization. The acquired spectra is as shown in the Fig.2.

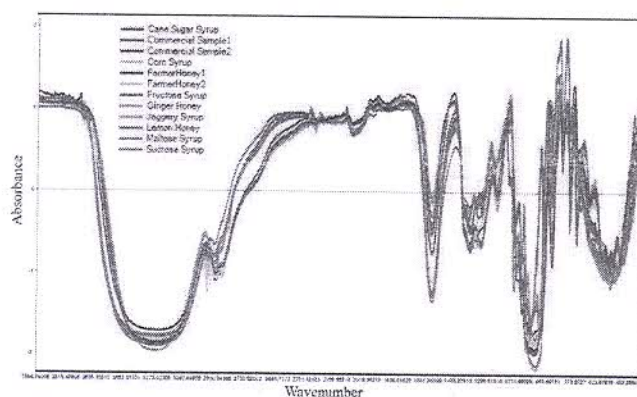


Fig. 2. Acquired spectrum of various honey and adulterant samples using FTIR.

III. DATA PRE-TREATMENT AND CHEMOMETRICS

In IR spectroscopy, the acquired spectra will contain some redundant information due to scattering effect. There can be several factors which contribute towards these scattering such as uncontrolled environmental conditions, any small malfunctioning of the electronic component, changes in the surface roughness of the sample etc. All these parameters collectively may contribute towards additive, multiplicative and wavelength specific scattering effects. As a result of the scattering, significant differences in terms of baseline shift, variation in slope or unwanted peaks may be noted in the acquired spectra [11]. It becomes even more serious problem when one has to distinguish between the spectral data with very minute variations. Therefore application of pre-processing techniques becomes very important step before going for any qualitative or quantitative analysis. The various steps involved in chemometrics based qualitative analysis is as shown in Fig. 3.

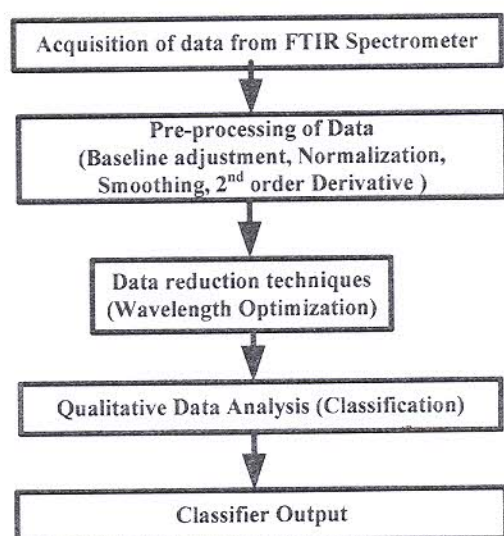


Fig.3 Flow diagram for data preprocessing and qualitative analysis

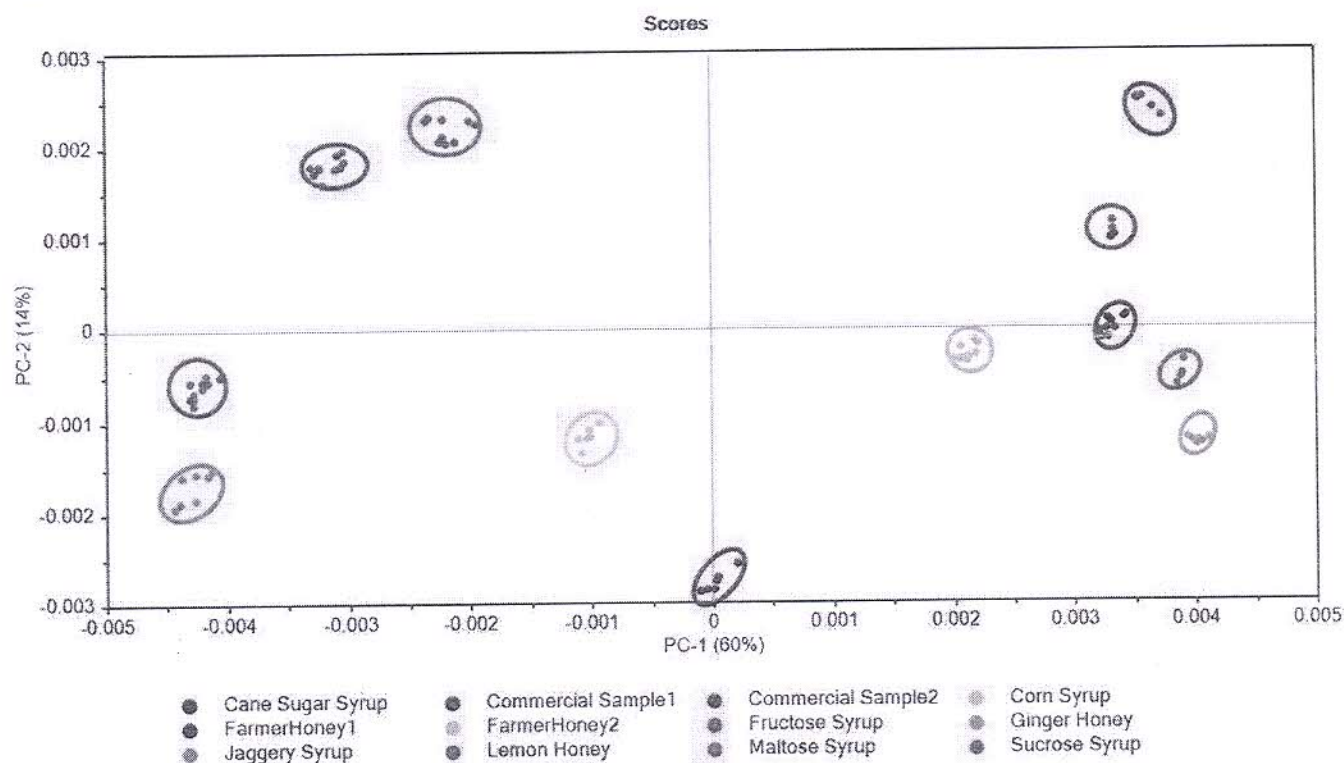


Fig.4. Score plot of PC1 vs PC2.

Principal Component Analysis (PCA) algorithm basically transforms the original data coordinates with the principal components (PCs). This transformation of the coordinates is done in such a way that the 1st PC will attain the maximum variance than other preceding components [13]. Therefore only few PCs will require to represent the most of the variation or information in the original data set, hence the data reduction is achieved. In this case 99% of the variance in the original data was represented through seven principal components only and

A. Data Pre-treatment of acquired spectra

Data pre-treatment of the acquired spectra plays very important role in any chemometrics based data analysis. In this work complete data analysis performed using Unscrambler X, from M/S CAMO, a standard tool for multivariate data. Firstly the baseline of the acquired spectra was corrected followed by the normalization of the data. Standard Normal Variate (SNV) and derivative based preprocessing techniques [11] have been used to correct additive & multiplicative scattering effects.

In general multivariate spectral data consists of higher dimensions and it is very difficult to process or visualize the data. In this case total twelve samples have been scanned ten times over 2515 spectral points in the range $4000-400\text{ cm}^{-1}$ i.e. the data matrix size in this case study becomes $[120 \times 2515]$ for calibration and another data set of size $[120 \times 2515]$ for validation. Here Principal Component Analysis (PCA) [12] algorithm is utilized to reduce the dimensionality of the data by keeping the maximum information in terms of variance.

hence rest of the data was discarded. Score plot of PC1 vs PC2 is as shown in Fig. 4. From the score plot it can be observed that pure honey and adulterants are separated along PC1.

B. Classification of the samples under test

Classification is a process of assigning a pre-defined class to the given object. In this case there are twelve different classes as twelve different varieties were chosen for this study. Therefore, each sample recorded from the spectrometer has to

be assigned to one of the classes. Based on best fit to the build classification model for each sample there can be three different outcomes [11,13]

- a) A test sample belongs to a single class.
- b) A test sample belongs to many classes.
- c) A test sample belongs to none of the classes.

Three different classification models were used to classify the data. K-means, K-medians and Fuzzy C Means were used as classification models. First using training data set [120x2515] all these models were built and then tested using test data set [120x2515]. Before building these models, the raw data acquired from the spectrophotometer was first pre-processed with some basic spectral correction techniques (Baseline correction, smoothing, normalization, differentiation, component analysis). The comparison of the performance of these classifiers with complete set of variables as well as optimized variables is shown in the Table 2.

TABLE II. VARIOUS CLASSIFIERS WITH PREDICTION ACCURACY

Model	Dataset	Total sample	True Positive	True Negative	Accuracy
K-means	Training	120	117	0	97.5%
K-medians	Test	120	120	0	100%
Fuzzy C Means	Test	120	120	0	100%

IV. CONCLUSION

In this work, application of FT-IR spectroscopy with ATR sampling accessory for discrimination of honey samples and its probable adulterants has been demonstrated. Total 12 varieties of sample including two samples of mono-floral honey, two samples of multi-floral honey directly collected from farmers, two samples for pure commercial honey and six different varieties of adulterant samples has been analysed. FT-IR spectral data of all the samples has been pre-treated with various pre-processing techniques. For discrimination of honey samples and adulterants three chemometrics models namely K-means, K-medians and Fuzzy C means has been developed. All the models was able to correctly predict the pure samples and adulterants in both the training and testing data set. Hence, FT-IR spectroscopy coupled with chemometrics strategy can be used for detecting various adulterants in honey samples.

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REFERENCES

- [1] Blanka Záborská, Lenka Vorlová, "Adulteration of honey and available methods for detection – a review", *Acta Veterinaria Brno*, 2014, pp.85-104.
- [2] Liming Wu, Bing Du, Yvan Vander Heyden, Lanzhen Chen, Liuwei Zhao, Miao Wang, Xiaofeng Xue, "Recent advancements in detecting sugar-based adulterants in honey - A challenge", *Trends in Analytical Chemistry*, 2017, pp. 25-38.
- [3] D. Cozzolino, E. Corbella & H. E. Smyth, "Quality Control of Honey Using Infrared Spectroscopy: A Review", *Applied Spectroscopy Reviews*, 2011, pp. 523-538.
- [4] Consuelo Pita-Calvo, María Esther Guerra-Rodríguez, and Manuel Vazquez, "A Review of the Analytical Methods used in the Quality Control of Honey", *Journal of Agriculture and Food Chemistry*, 2017.
- [5] Irene Kushnir, "Sensitive Thin Layer Chromatographic Detection of High Fructose Corn Sirup and Other Adulterants in Honey", *Journal of Association of Official Analytical Chemists*, 1979, pp. 917-920.
- [6] Anitta Puscas, Anamaria Hosu, Claudia Cimpoiu, "Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration" *Journal of Chromatography A*, 2013, pp.132-135.
- [7] A I Ruiz-Matute, A C Soria, I Martínez-Castro, M L Sanz, "A New Methodology Based on GC-MS to Detect Honey Adulteration With Commercial Syrups", *J Agric Food Chem*, 2007.
- [8] J. F. Daniel Kelly, Gerard Downey And Vanessa Fouratier, "Initial Study of Honey Adulteration by Sugar Solutions Using Midinfrared (MIR) Spectroscopy and Chemometrics", *J. Agric. Food Chem.*, Vol. 52, No. 1, 2004.
- [9] Xiangrong Zhu, Shuifang Li, Yang Shan, Zhuoyong Zhang, Gaoyang Li, Donglin Su, Feng Liu, "Detection of adulterants such as sweeteners materials in honey using near-infrared spectroscopy and chemometrics", *Journal of Food Engineering*, 2010, pp. 92–97.
- [10] Gerard Downey, Vanessa Fouratier and Daniel Kelly, "Detection of honey adulteration by addition of fructose and glucose using near infra transreflectance spectroscopy", *Journal of Food Engineering*, 2010, pp. 92–97.
- [11] Navjot Kumar, PC Panchariya, Surendra Singh Patel, AH Kiranmayee, Rishi Ranjan, "Application of various Pre-processing techniques on Infrared (IR) Spectroscopy data for classification of different ghee samples", *International Conference on Computing, Communication, Control and Automation (ICCUBEA 2018)*.
- [12] Magnus Borga Tomas Landelius Hans Knutsson, "A Unified Approach to PCA, PLS, MLR and CCA", *Digitala Vetenskapliga Arkivet (DIVA)*, 1997.
- [13] Navjot Kumar, PC Panchariya, A H Kiranmayee, Punit Khatri, K Srinath "Rapid classification of different types of ghee using mid infrared spectroscopy" *Trends in Industrial Measurement and Automation (TIMA)*, 2017.
- [14] Davide Ballanjo, Viviana Consonni, "Classification tools in chemistry Part I: Linear Models. PLS-DA", *Analytical Methods*, Vol. 5, 2013, 37-90-3798.
- [15] Ranjan R., Kumar N., Kiranmayee A.H., Panchariya P.C. "Characterization of Edible Oils Using NIR Spectroscopy and Chemometric Methods", *Intelligent Systems Design and Applications. ISDA 2018. Advances in Intelligent Systems and Computing*, vol 941. Springer.