Identification of Pure and Adulterated Honey Using Two Spectroscopic Methods

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Abstract. Honey is a natural sweet substance which is often mixed with other liquids for health purposes or as a sugar substitute in variety of food. Due to high commercial profit, many fraudulent acts have been around to add other substances to pure honeys. This study used two spectroscopic methods which are the laser induced fluorescence (LIF) and Fourier Transform Infrared (FTIR) spectroscopy to differentiate pure and corn syrup adulterated honeys based on sugar content. LIF used a 405 nm diode laser as the excitation laser. Samples of 27 were prepared for this study. They composed of 15 pure honey and 2 non honeys, and 10 adulterated honeys which we coded from A to Q. Non honey samples were pure date syrup and corn syrup which coded as K and L. The sugar contents were measured manually using a brix refractometer which resulted the honey sugar contents range of 69.5 % to 78 %. The peak wavelengths observed range from 490.9 nm to 641.3 nm. LIF was able to differentiate between the pure honeys than the adulterated or mixed ingredient honeys except for sample C. The differences of FTIR spectrums were shown by honey samples which was not given corn syrup adulteration, where the difference begins to be seen clearly at the wave number range 1150 cm\(^{-1}\) to 650 cm\(^{-1}\).

1. Introduction
Honeys are food substances which have benefits for nutrition and medicine. Honeys are also export commodities for a country. Honeys are natural sweet liquids which are produced by honey bees from flower nectar or other parts of flower plants [1]. Honeys sold commercially are often adulterated by other substances for nutrition and medicine purposes but some are added to fake pure honeys. Many methods have been used and developed, traditional or modern ways, to discriminate, differentiate pure honeys from adulterated or non honeys. Fluorescence spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy have been proposed as potential methods to detect pure or adulterated honeys. Laser or LED induced fluorescence spectroscopy are also being proposed due to some advantages. Research on using fluorescence spectroscopy has bee carried out to differentiate pure than adulterated honey [2], Combinations of computer vision and laser induced fluorescence spectroscopy has also been carried out [3]. Light induced fluorescence Spectroscopy using LED has been developed and miniaturized using optical fiber [4]. Recently, the traditional method to test honey purity using human senses has been imitated using artificial senses. Honey qualities are represented by appearance such as color, aroma, and taste. These senses are being imitated by computer vision for appearance, electronic nose for aroma, and electronic tongue for taste [5]. Electronic nose has been used for many purposes
included honeys [6]. Hybrid methods which use electronic tongue and FTIR have been carried out to classify pure and adulterated honeys [7].

This study uses two spectroscopic methods, laser induced fluorescence spectroscopy and FTIR spectroscopy to differentiate pure honeys from non honeys, and honeys adulterated using corn syrup. Cheaper corn syrups have been around to substitute honey in food industry and are being a culprit for fake honey [8].

2. Methodology

2.1. Sample Preparation

Samples used in this study shown in Table 1, composed of 17 pure liquids and 10 adulterated honeys. The pure liquids consisted of 11 pure local forest honeys, 4 national brand pure honeys, and two non honeys. Non honey samples were pure date syrup and corn syrup which coded as K and L. Honey samples of A, B, D, E, G, H, J, M, O and P contain of 100% pure forest honeys (multiflora), while honey samples of C, N, and Q contain of mixed forest honeys with one or two monofloral honeys. Honey sample of I composes of mixed pure honeys, royal jelly, and date syrup while sample of F consists of royal jelly and bee pollen. There were 11 honey samples used for laser induced fluorescence (LIF) spectroscopy which have codes from A to K. Measurement using Fourier Transform Infrared spectroscopy used 10 pure honeys which have codes of C, F, G, H, I, M, N, O, P dan Q, 1 corn syrup sample labeled as L, and 10 mixed pure honeys with 30% corn syrup. All were coded with addition of letter L.

2.2. Sugar Measurement

Sugar content of each honey types and adulterated mixtures were measured using a portable and manual Brix refractometer with scale particularly assigned for honeys with scale of 58-90%. Sugar content is also defined as soluble solid content-SSC. Before measurement, the refractometer reading was first calibrated using distilled water. After calibration, a couple drops of a honey sample dripped on the blue surface of brix meter, smeared for even coating. Brix value can be found by looking into brix meter scale by viewfinder. Sugar content can be represented in Brix degree or simply % Brix. The measurements were performed in room temperature of (22–25°C). The measurements were done three times for each honey type. of the measurement are shown in Table 1.

2.3. Laser induced fluorescence Spectroscopy

Measurement of fluorescence wavelength using laser induced fluorescence spectroscopy was performed using an USB 2000+ Ocean Optics spectrometer which equipped with analyzing Spectrasuite software. The system consisted of a 405 nm diode laser with average laser power of 27.04 mW. Light collecting lens connected to a fiber optical cables, ND filters, mirrors, and the spectrometer. Each honey sample was placed in a visible polystyrene cuvette with capacity of 4.5 mL liquid and optical path length of 10 mm. The cuvette system and the lens were situated in a homemade black acrylic box with size of 5 cm x 5 cm x 10 cm. Recording of spectrum images for each honey type was done twice, the spectra were saved in bmp file for latter processing. Spectrasuite software was used to obtain the peak wavelength for each fluorescence spectrum.

2.4. Measurement Using FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectrometer was also used to differentiate the pure and adulterated honeys. FTIR spectrometer Shimadzu IR prestige 21 was used for this purpose. Honey samples which consisted of pure honeys, non honeys, adulterated honeys were prepared each in a test tube. Trained personnel performed the measurement of all prepared samples. A drop of honey sample was placed between two plates of KBr NaCl (Natrium Klorida) to create thin film then put in sample place in FTIR compartment. IRsolution software was used to acquire sample data and saved for latter analysis.
Measurement results can be graphed as absorbance or transmission plot which display Intensities (%) versus wavenumber (cm\(^{-1}\)).

3. Results and Discussion

3.1. Honey Sugar Contents

Table 1 shows sugar content for each honey types and adulterated honeys. The results show that all the honey samples have sugar contents comply to Indonesian National standard which are above 65% [9]. Sugar is the main component in honey substance beside water. High sugar content causes honeys appear viscous. Honeys should have high sugar content for better taste and prolonging honey storage time [10]. Sugar content is also responsible for osmotic effect of honeys hence able to prevent bacteria growth. Honey with less water content and higher sugar content appears darker because it contain higher phenolic compound than lighter color honey [11].

Table 1. Honey Sample Information

| No. | Sample Codes | Purity               | Sugar % | No. | Sample Codes | Purity               | Sugar % |
|-----|--------------|----------------------|---------|-----|--------------|----------------------|---------|
| 1.  | A            | Pure, Forest         | 69.0    | 15. | O            | Pure, Forest         | 78.0    |
| 2.  | B            | Pure, Forest         | 69.5    | 16. | P            | Pure, Forest         | 71.5    |
| 3.  | C            | Pure, Forest+2 flora | 75.0    | 17. | Q            | Pure, Forest+2 flora | 71.0    |
| 4.  | D            | Pure, Forest         | 69.0    | 18. | CL           | C + Corn syrup       | 73.5    |
| 5.  | E            | Pure, Forest         | 70.0    | 19. | FL           | F + Corn syrup       | 70.0    |
| 6.  | F            | Royal Jelly, Bee pollen | 72.0  | 20. | GL           | G + Corn syrup       | 67.5    |
| 7.  | G            | Pure, Forest         | 73.0    | 21. | HL           | H + Corn syrup       | 70.0    |
| 8.  | H            | Pure, Forest         | 75.0    | 22. | IL           | I + Corn syrup       | 73.5    |
| 9.  | I            | Mixed honey, date, royal Jelly | 76.0 | 23. | ML           | M + Corn syrup       | 67.5    |
| 10. | J            | Pure, Forest         | 77.0    | 24. | NL           | N + Corn syrup       | 72.0    |
| 11. | K            | Date Syrup 100 %     | 78.0    | 25. | OL           | O + Corn syrup       | 75.0    |
| 12. | L            | Corn Syrup 100 %     | 75.0    | 26. | PL           | P + Corn syrup       | 69.5    |
| 13. | M            | Pure, Forest         | 70.0    | 27. | QL           | Q + Corn syrup       | 69.5    |
| 14. | N            | Pure, Mono           | 73.5    |  |   |          |                     |         |

3.2. Relation of peak wavelengths and honey sugar contents

Table 2 shows the colors of honey liquids before and after excited by 405 nm (violet) laser light. Laser or LED induced fluorescence spectroscopy is able to show different colors of different honey types, also to differentiate honey from non honeys. In fluorescence process, some light energy absorbed which cause light shifted to longer wavelength than the excitation wavelength. The peak wavelengths of the honey colors were measured by Ocean Optics Spectra suite program which comes with the spectrometer. The peak wavelengths ranges 490.9 nm to 641.3 nm for honey samples with A to K codes. The results also showed that fluorescence intensities decrease as sugar contents increase [2]. From Table 2, it is shown that as honey color darker, light intensities decreases. Honey colors are related to sugar contents. As mentioned before, sugar contents can darken the honey colors hence the higher the sugar contents or the less the water contents, honey colors become darker [11].
Table 2. Honey colors before and after excited by 405 nm laser light.

| Image of Honey Samples before excitation | A | B | C | D | E | F |
|-----------------------------------------|---|---|---|---|---|---|
|                                         | G | H | I | J | Non Madu K | GL |

| Image of Honey Samples After excitation | A | B | C | D | E | F |
|-----------------------------------------|---|---|---|---|---|---|
|                                         | G | H | I | J | Non Madu K | GL |

Relation between peak fluorescence wavelengths and sugar contents is described in Figure 1.. Figure 1 show that peak wavelengths linearly correlated to sugar content values. Higher sugar contents cause higher absorption of excitation light energy, less energy emitted. As consequences, fluorescence lights have longer wavelengths. It was shown that wavelength excitation of 405 nm is an effective wavelength to show fluorescence phenomena in honey samples [2].

Figure 1. Relation between peak wavelength to sugar contents of honey samples

The value of linear regression obtained ($R^2$) fro Figure 1 is 0.7972. Error (%) found from that is 1-$R^2$ which is 0.2028 or less than 20%. This result shows that there is another significant factor contributes to the relation. In another word, sugar contents have major factor to the shifting peak wavelength. Value of $R^2$ which close to one indicates strong correlation between Y to X variables.
3.3. FTIR Spectra of Pure and Adulterated Honeys.

Interaction of matter which contains complex compound molecules with energy from Infrared light will cause the molecules to vibrate. Energy of each molecule type is different which depends on its atom type and their bonding strength. This situation will result in different wavenumber (cm\(^{-1}\)) yang [12]. Characterization pure honeys and non honeys using FTIR spectroscopy can be done using resulted absorbance or transmission FTIR graphs which contain many peaks. Figure 2 shows the FTIR graph for all pure honeys and corn syrup (a), while b) shows FTIR graphs for each pure honey adulterated by 30 % of corn syrup.

![FTIR spectra all pure honeys](image1)

![FTIR spectra of adulterated honeys](image2)

**Figure 2.** FTIR spectrums of pure honeys and adulterated by corn syrup

![FTIR spectrum of pure honey C, mixed honey, corn syrup only](image3)

**Figure 3.** FTIR spectrum of pure honey C, mixed honey, corn syrup only

Figure 2 shows that there are difference absorbance intensities for pure and adulterated honeys. Figure 2a shows higher intensities than those in Figure 2b. Figure 2b shows a slightly difference peaks for each adulterated honeys. Figure 3 shows the difference between peaks of pure honey C, corn syrup, and honey C mixed with corn syrup. Honey C itself contains 3 honey types which are forest honey plus two monoflora honeys. The significant difference can be seen at wavenumber range of 3450 cm\(^{-1}\) to 800 cm\(^{-1}\). Other specific differences can be seen from 6 characteristic peaks (dash circles) which show some differences between pure honey C, non honey, pure honey mixed with corn syrup. .
4. Conclusion
Characteristics of pure and corn-syrup-adulterated honeys have been studied using two spectroscopic methods, laser induced fluorescence (LIF) and Fourier transform infrared (FTIR) spectroscopy. The sugar contents of honey samples used in this study range from 69 % - 78 %. LIF results show difference fluorescence color between pure honeys and non honeys, also shows different color due to royal jelly and honey mixed by corn syrup. There is a correlation of 0.80 between the peak wavelength and sugar content which also shows greater error for higher sugar contents. The results also show shifted peak wavelengths which are related to darker color of honey types and higher sugar content. Excitation using a 405 nm laser light resulted in shifted wavelength ranges of 490.9 nm to 641.3 nm. Results of FTIR spectroscopy measurements show a slight difference between characteristic curves of pure honey, corn-syrup, and honeys mixed by corn syrup. Both spectroscopic methods are potential to be used for discriminating pure than adulterated honeys, however the FTIR method is more expensive and unreachable by small holder honey farmers. The LIF method is more potential and can be developed and combined with computer vision method for low cost and portable system.

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