Laser based imaging method to discriminate Riau Province pure honeys

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Abstract. Pure honey is defined as harvested honey without adulteration. This study proposes a laser induced fluorescence imaging (LIFI) method to discriminate 17 honey types which consisted of 9 pure forest honeys of Riau Province, Indonesia, and 8 national branded honey types. Measurement of sugar content and glucose were also performed. Three diode lasers of 405 nm, 532 nm, and 635 nm in wavelength were used to induce laser fluorescence on honey placed in a spectrometer cuvette. Fluorescence images of honey types were recorded using a colour CMOS camera and processed using ImageJ to obtain HSV values. Correlation and discrimination were performed using SPSS and K-Means Clustering. Fluorescence image colours range from bluish to orange hue for 405 nm laser related to honey light to dark colour. Excitation using 532 nm and 635 nm laser resulted in shifted colour to larger wavelength in slight difference in red colour respectively. Correlation of saturation-Brix for 635 nm resulted in higher R of 0.594, saturation-glucose for 405 nm of R of 0.495, and Hue-Brix for 532 nm of R of 0.494. K-Means clustering results also showed that excitation using green laser was able to discriminate 3 mixed honeys out of 17 honey types.

1. Introduction
Honey is one of Indonesian very important food substances which has health benefit and often used as sugar substitute such as for tea and baking. Recently, it becomes part of ingredient of food products to booze product sale profit using health benefit gimmick. Riau Province is one of Indonesian regions which are potential for forest honey production [1] due to still abundant forest vegetations. Most local honey products in Riau Province are forest honey based which come from small holder bee farmers surrounding forest. However, most of the honey products have not fulfilled the Indonesian National Standard for honeys which is due to traditional and simple way to produce honey and lack of access to measure their honey quality using qualitative and sophisticated devices.

Today, quality and purity of honey can be measured traditionally, manually, and sophisticatedly. Traditional methods used rely on human sense based on aroma, colour and taste. Most beekeeper use an analog refractometer to measure their honey quality which is limited to water or sugar contents measurement. The most qualitative and modern devices mostly used are HPLC, FT-IR spectrometer, and UV-Vis spectrometer. These devices are available commercially but expensive and needs a trained personnel to operate and often inaccessible by the small holders [2]. Efforts to find easier and faster
way to measure honey quality and purity are still ongoing such as FT-IR and chemometric [3], and FT-Raman spectroscopy combined with some simple and low cost chemometric devices [4]. Researches on applying artificial senses to imitate human senses for inspecting food products are also developed such as electronic nose and electronic tongue [5], electronic nose has been applied to assess honey quality [6].

Fluorescence spectroscopy has been used for food quality assessment. This method relies on the interaction of light with biology or organic material which composes of chemical molecules. Light induced Fluorescence method has been applied for food analysis such as for tea evaluation using Led [7] and classification, for quick distinguishing vegetables oils using diode laser and Led in the UV and Visible range [8], for olive oil using diode laser [9]. Fluorescence spectroscopy has been also applied to study honey and cane sugar syrup [10], to discriminate honey adulteration using laser diode [11] and to estimate honey quality [12].

Quality of honey is represented by its physical properties such as electric conductivity, acidity, viscosity, colour, and chemical properties such as sugar content (fructose, glucose, sucrose), water content, anti oxidant, and total phenol [13]. Honey types depend on many factors such as floral origin, region, and climate [14]. Colour is one of physical properties of honey quality. Traditional way to assess honey quality is by naked eyes to observe honey natural colours. Natural colours of honey can represent chemical contents such as dark colour honey due to less water contents and abundant phenolic content. However honey colours is also affected by floral origin and, long storage time, and process involved, Quality assessment of honey based on honey natural colour has been performed mostly using portable colorimeter [15, 16].

Assessment of honey quality using imaging technique has been developed recently since the availability of affordable CMOS/CCD cameras which is known as computer vision-CV [17]. Quality of honey has been evaluated using colour image of honey types using CCD camera to detect organic materials [18]. This method is very useful for quick measurement in field or remote area especially if the measurements integrated using a Smartphone [2]. This techniques has been applied for honey classification [19]. Imaging methods for quality assessment needs image processing technique. For measurement based on honey natural colours, Colour image processing algorithm are needed. CIE L*a*b* values are often used for honeys [17 ]. HSV values which composes of Hue, Saturation and Value parameter provides the perception human visual feature [20]. This technique can be also used to retrieve information from images. The image information is then correlated to measured physical and chemical properties of honey for further analysis such as honey classification or discrimination from adulterated honey.

Laser induced fluorescence spectroscopy method has been used to assess honey quality using laser or LED light. Color feature of honey has also been explored using digital camera or colorimeter.. However, the combination of laser induced fluorescence method and imaging methods using CMOS/CCD camera has not been explored much. The availability of low cost colour cameras and diode lasers make a laser based imaging method is possible to discriminate pure from adulterated honeys, and further studies could integrate the methods with Smartphone to help customers, distributors or small holder bee keeper to be able to scan honey characteristics fairly faster and accurate.

This study was aimed to use a laser induced fluorescence imaging technique to discriminate 17 honey types which consisted of 9 local uncertified and certified honeys from seven regions of Riau Province, Indonesia, 7 national branded honeys and 1 date syrup. Physical and chemical characteristics of the honey samples were measured, correlated and discriminated. The color characteristic of 17 honey types were measured using laser induced fluorescence imaging. Fluorescence images of honey types were processed to obtain HSV values which represent the color characteristics of honeys. Brix % and glucose contents were also measured which represent the chemical properties of the honeys. Color characteristics were correlated to the chemical properties of 17 honey types. HSV values were then used to discriminate honey types using K-means clustering method [21].
2. Materials and Methods

2.1. Materials
Samples of this study were 17 honey types consisting of 9 local honeys, 7 National honeys, and 1 non honey. The local honeys represent local honeys from seven regions of Riau Province, Indonesia, non honey, and National branded sold in supermarkets. There were 9 local honeys found which comprised of 5 unbranded and 4 branded honeys. Branded is defined as it has label which mention ingredients and has certification. Certification can be from local (regency or province) Department of Health. Most National branded honeys have certification from Indonesia National Agency of Drug and Food Control, some even complemented by kosher certification from Indonesian Council of Islamic Clergy. All the honey samples were coded randomly from A to Q. Information and honey codes are shown in Table 1. Honey compositions are found from label and from honey distributor and farmer.

Table 1. Honey information and Brix %, Glucose content measured

| Honey Code | Colour Observed   | Composition                           | Brix (%) | Glucose (g/mL) |
|------------|-------------------|---------------------------------------|----------|----------------|
| A          | Dark Brown        | Forest honey                          | 72       | 61.61          |
| B          | Light Brown       | Forest honey                          | 69       | 44.37          |
| C          | Brown             | Forest honey, royal jelly, bee pollen | 72.5     | 48.27          |
| D          | Light Brown       | Randu honey, forest honey, longan     | 77       | 43.44          |
| E          | Black             | Honey, date syrup, royal jelly        | 77       | 42.90          |
| F          | Brown             | Forest honey                          | 68.5     | 37.69          |
| G          | Yellow            | Forest honey                          | 69       | 42.16          |
| H          | Black             | Forest honey                          | 71       | 42.63          |
| I          | Light Brown       | Forest honey                          | 70       | 39.51          |
| J          | Dark Brown        | Pure forest honey                     | 78       | 50.17          |
| K          | Black             | Date syrup                            | 74       | 17.13          |
| L          | Brown             | Pure honey                            | 76.5     | 37.95          |
| M          | Light Brown       | Pure honey                            | 77.5     | 30.80          |
| N          | Yellow            | Pure wasp honey                       | 71.5     | 37.30          |
| O          | Brown             | Honey, royal jelly, bee pollen        | 78.5     | 36.83          |
| P          | Light Brown       | Forest honey                          | 69.5     | 45.74          |
| Q          | Brown             | Randu honey, forest honey, longan     | 78       | 38.89          |

2.2. Sugar and glucose measurement
A portable analog Brix Refraktrometer or Brixmeter was used to measure the sugar content of each honey sample. Sugar content is also defined as soluble solid content-SSC. This apparatus is also used to measure water content and Baume number. The Brixmeter used for this experiment is designated for sugar content of honey (RHB-90ATC) which has 58-90 % Brix scale and 12-27 % scale for water content. Brix % of each honey sample was measured three times. All honeys were stored in air conditioned room. Results of the measurement are shown in Table 1.

UV-VIS spectrometer [19] was used to measure the glucose content. Due to viscous honey samples, Dilution 100 times was done. Each honey sample has concentration 0.05 gr/5 ml (100 ppm). The measurement results are shown in Table 1.

2.3. Laser Induced Fluorescence Imaging and Image Acquisition
The laser induced fluorescence imaging system used consisted of three diode lasers, honey samples in cuvettes, an USB CMOS colour camera. The diode lasers used were 20 mW 405 nm (violet), 5 mW 532 nm (green), and 1.2 mW 635 nm diode laser. The USB camera is an microscope digital camera which has 3.2, spectral range of 380 nm-650 nm MP resolution, USB 2.0 connection, with sensor size
of 1/2 " . The cuvettes used were 4.5 mL visible polystyrene cuvettes for spectrophotometer which has about 1 % absorption.

The experiment procedure was started by setting the optical stage for a honey filled cuvette in front of one of the diode laser. The camera was set perpendicular of the laser beam path aiming to the cuvette surface. Each honey type was filled in a cuvette and labeled according to the designated code. Driver software of the camera was used to record and saved the images of each honey type before and after excited by each laser. Recording of the images was performed for each laser excitation, twice for each honey type which resulted of 102 fluorescence images and 34 images with no laser excitation. Such measurement was repeated twice at different days. Image data were saved in JPEG and Bitmap for further data analysis.

2.4. Data Analysis

All the image data of honey types were processed using ImageJ software to obtain HSV (Hue, Saturation Value) values. The ImageJ Plugin called colour transformer by M Barilla, University of Birmingham was used to convert RGB image to HSV. Region of Interest (ROI) applied for each image is varied particularly for fluorescence images which depends on the fluorescence characteristics (penetration depth and colour distribution), for most images, 46 ×22 pixels was used as ROI. HSV total for each honey type was the average HSV from two images taken.

Correlation analysis was performed for the honey image data. Physical and chemical characteristics involved were Brix %, glucose content, Hue, Saturation, Value component from 17 honey types. Linear correlation analysis of the parameters was carried out using SPSS 16.

K-means clustering was used to analyze the possible clustering between all honey types based on their HSV values. K-means algorithm consists of two phases. The first phase is to choose a K value randomly and set as a center value. Next phases are to take every object data to nearest centre [21].

Here, the data used were obtained by HSV colour feature segmentation of HSV. The segmentation results were stored on n × 3 matrices where n is the number of honey types involved. The number of cluster sets was 2 which represented pure honey and adulterated or mixed honey. Euclidean distance used as follow

\[ d(x, y) = \|x - y\|^2 = \sum_{i=1}^{n} (x_i - y_i)^2 \]

Here \(d_{xy}\) is the image distance between x and y, \(x_i\) is the feature cluster centre at \(i^{th}\) and \(y_i\) is feature value at \(i^{th}\) data respectively.

3. Results and Discussion

3.1. Brix values and UV-Vis spectrometer results of absorbance and glucose measurement

Table 1 shows the results of Brix refractometer and UV-Vis spectrometer measurements. The Brix values ranges from 68.5 – 78.5 % which shows the variations in sugar contents which is also defined as solid soluble content (SSC). These values are inversely proportional to water contents which are also related to honey natural colour. Darker honey colour has lower water content and higher sugar content which has negative correlation to sugar content of -0.707 [15]. Table 1 shows that some black and brown honeys have higher sugar content which means lower water content. The good quality of honey according to Indonesian National Standard (SNI) should have minimal 22 % of water content and 65 % of sugar content. Table 1 also shows that mixed honey (nectar types), adulterated with date syrup, and not honey (date syrup) have the highest Brix values. It also shows that most local, unbranded honeys from Riau Province, Indonesia have higher water content which can be due to traditionally process of honeys by small holder bee keepers.
3.2. Laser induced fluorescence images

Table 2 shows the relationship between honey natural colours, luminescence colour after excitation by different laser wavelengths, and the status of honey. The status of honey (purity and brand) was determined by labels on their container after purchased and by interviewing some local bee keepers. Excitation using 405 nm is able to show the relationship between the natural colour of honey and the luminescence colours. Light colour has bluish hue, darker honey has orange colour except for some honey such as M, C, and J honey codes which can be investigated further. The fluorescence colours and the natural honey colours were determined by observation from images of honey in a cuvette recorded by the CMOS camera. The 405 nm excitation can discriminate the honey types effectively, the pure honeys have fluorescence peaks at 500 nm [11] using 405 nm laser and measured using an Ocean Optics spectrometer, or specifically ranges 495-510 nm using 405 nm LED [12].

Table 2 also shows that excitation using green laser shifts the luminescence colours to higher wavelength. However some of honey types do not follow the trend such as G and H honey codes. Honey fluorescence is dominated mostly by flavonoids which is responsible for characteristics of honey colour [11] and others such as carotenoids and antioxidant [1, 15]. Green luminescence for olive oil due to excitation using green light is due to the presence of chlorophyll while the yellow colour is due to Lutein existence which represents the maturity of olive fruit before harvested [9].

Red laser excitation does not show much colour change. However illuminated images shows that there is a difference in beam pattern when red light pass the cuvette. All honey types can be penetrated by red light due to its longer wavelength except Honey K which pure honey but black colour. Some honeys show straight beam pattern transmitted while others scatter and diverse the beam. Date syrup (not honey) has diverse beam pattern unlike other honey types. This could be due to larger particle size contained and thicker syrup. Excitation using red laser is potential to be used for viscosity measurement using optical methods.

| Colour Observed | Honey Code | Laser Wavelength | Purity | Brand |
|-----------------|------------|------------------|--------|-------|
| Yellow G        | Blue       | Half             | Blue-white | Through | White | Through | Pure | local |
| Yellow N        | Blue       | Through          | White-Green | Through | Light red | Through | Not bee | National |
| Light Brown B   | Blue       | Through          | White    | Through | Red     | Through | Pure | local |
| Light Brown D   | Blue       | Through          | White-yellow | Through | Scatter red | Through | Mixed Honey | National |
| Light Brown I   | Blue       | Through          | White    | Through | Red     | Through | Pure | Local |
| Light Brown M   | White-purple | Through       | White-blue | Through | Red     | Through | Pure | National |
| Light Brown P   | Blue       | Through          | White-blue | Through | Red     | Through | Pure | Local |
| Brown C         | Green      | Half             | Yellow   | Through | Red     | Through | Mixed Honey | Local |
| Brown F         | Blue       | Half             | White    | Through | Red     | Through | Pure | Local |
| Brown L         | Blue-green | Half             | White-yellow | Through | Light red | Through | Pure | National |
| Brown O         | Blue-green | Half             | White-Yellow | Through | Light red | Through | Mixed Honey | National |
| Brown Q         | Blue-green | Half             | White-Yellow | Through | Light red | Through | Mixed Honey | National |
| Dark Brown A    | Yellow     | Low              | Yellow   | Half     | White-red | Through | Pure | Local |
| Dark Brown J    | Green      | Low              | Yellow-orange | Half     | Scatter red | Through | Pure | Local |
| Black H         | Orange     | Very Low         | Orange   | Very Low | Red     | Half     | Pure | Local |
| Black E         | Orange     | Low              | Red-orange | Half     | White-red | Through | Mixed Honey and date syrup | National |
| Black K         | Orange     | Very Low         | Yellow-orange | Low     | Diverse | White red | Through | Date syrup | National |

\[a\] \text{FC} = \text{Fluorescence colour} \\
\[b\] \text{P} = \text{Light penetration depth}
Using laser induced fluorescence imaging is more potential which is opposite for just using a spectrometer where excitation by 405 nm is more efficient [11]. LIFI gives more information on honey characteristics to be collected in data base for further application such as Smartphone based lab [2] or a portable system using Photosensitive detectors. Physical and chemical properties of honey types can be related to LIFI images information for sorting and grading honey using machine vision.

3.3. Correlation of HSV values and honey chemical contents

In this study, sugar and glucose contents were measured using a manual Refractometer and an UV-Vis spectrometer respectively. HSV (Hue, saturation and value) of colour images can be used to represent the colour characteristics of materials [20]. Table 3 shows correlation level between HSV components and measured chemical contents. For 405 nm wavelength, correlation of saturation–glucose is the highest, this could be related to colour change, penetration depth in Table 2 and variation of glucose content in Table 1. In order to correlate imaging. Excitation using 532 nm light resulted in higher correlation between Hue of the image to Brix contents or water contents. Red light excitations shows higher correlation for saturation values to Brix contents. It could be the cause of thicker or not thicker beam transmitted through the cuvette.

**Table 3.** The highest correlation between HSV components and Brix %, sugar content for each excitation laser wavelength

| Laser Wavelength | Parameter       | R    | R²   | Errors |
|------------------|----------------|------|------|--------|
| 405 nm           | Saturation –glucose | 0.495| 0.245| 0.137  |
| 532 nm           | Hue –Brix       | 0.494| 0.224| 0.032  |
| 635 nm           | Saturation –Brix | 0.594| 0.352| 0.282  |

3.4. Honey types discrimination using K-means clustering

Figure 1 a) and b) show relationship between H, S and V values. Figure 1 a) represents the clustering result of HSV values for natural honey images where the laser lights have not been applied. For this figure, X axis represents H values and Y axis represents S values. The clustering resulted in 3 honey types discriminated as mixed or impure honeys. Figure 1 b) shows the clustering result using 532 nm or green laser where X axis represents V values and Y axis represent S values. The clustering results for the 532 nm laser which uses S and V component values are shown in Table 4. Symbol (O) in Figure 1 a) and b) represents honey types included in cluster 1 (pure) while symbol (*) represents honeys in cluster 2 (impure or not honey). Symbol x corresponds to the cluster centre.

![Figure 1](image1.png)

**Figure 1.** K-Means Clustering results based on HSV Values (a) without laser and (b) 532 nm laser
Table 4. HSV values for each honey type and K-means clustering results

| Honey Code | H     | S     | V     | K-means |
|------------|-------|-------|-------|---------|
| A          | 0.999 | 0.93925 | 0.26375 | 1       |
| B          | 1     | 0.9785 | 0.33475 | 1       |
| C          | 0.976083 | 0.99825 | 0.171 | 1       |
| D          | 0.99925 | 0.81725 | 0.312 | 1       |
| E          | 1     | 0.86175 | 0.036 | 2       |
| F          | 0.99525 | 0.98325 | 0.33625 | 1       |
| G          | 0.9965 | 0.99675 | 0.33625 | 1       |
| H          | 1     | 0.80025 | 0.004 | 2       |
| I          | 1     | 0.98425 | 0.33375 | 1       |
| J          | 0.99625 | 0.983 | 0.15075 | 1       |
| K          | 0.9635 | 0.99325 | 0.1445 | 1       |
| L          | 0.917 | 0.99025 | 0.19775 | 1       |
| M          | 1     | 0.99425 | 0.3335 | 1       |
| N          | 0.99175 | 0.95075 | 0.335 | 1       |
| O          | 0.8735 | 0.9925 | 0.1765 | 1       |
| P          | 1     | 0.99275 | 0.33325 | 1       |
| Q          | 0.95225 | 0.67575 | 0.18225 | 2       |

4. Conclusion
A laser induced fluorescence imaging (LIFI) method was used to discriminate 17 honey types which consisted of 9 pure forest honeys of Riau Province, Indonesia, and 8 branded honey types sold nationally. Three diode lasers of 405 nm, 532 nm, and 635 nm in wavelength were used to induce laser fluorescence on honey placed in a spectrometer cuvette. Fluorescence images of honey types were processed, analyzed, and classified using ImageJ, SPSS, and K-means clustering respectively. Fluorescence image colour ranges from bluish, greenish, yellowish, and orange hue for 405 nm laser and shifts to yellowish, greenish, and orange colour for 532 nm laser. Based on the image colours, excitation using the 532 laser was able to discriminate mixed, not and pure honey. Excitation using 650 nm results in a slight difference in red colour. Saturation-Brix correlation resulted in higher R = 0.594 for 650 nm laser, followed by saturation-glucose for 405 nm of R = 0.495, and Hue-Brix for 532 nm of R = 0.494 laser. K-Means clustering results also showed that excitation using green laser was able to discriminate 3 mixed honey and not honey out of 17 honey types. LIFI is potential imaging method to discriminate pure honey and mixed honey which can be applied in developing a low cost, portable Smartphone based system for small holder beekeepers. Advanced study using LIFI can be done to include intentionally adulterated honeys using beet or corn syrups with varied concentrations.

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