



**Development of a Rapid and Accurate System To
Differentiate Malaysian Honey Samples Using UV and
Colour Image**

by

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LIST OF ABBREVIATIONS

| | |
|-----------------|--------------------------------------------|
| SVM | Support Vector Machine |
| PCA | Principal Component Analysis |
| DPPH | 1,1-Diphenyl-2-picryl-hydrazyl |
| PFund | Colour Scale of honey |
| LDA | Linear Discriminant Analysis |
| E-NOSE | Electronic Nose |
| E-Tongue | Electronic Tongue |
| FIR | Fourier Infrared Spectroscopy |
| MIR | Mid Infrared Spectroscopy |
| NIR | Near Infrared Spectroscopy |
| HLF | High Level Fusion |
| LLF | Low Level Fusion |
| ILF | Intermediate Level Fusion |
| BPNN | Back Propagation Neural Network |
| PLS | Partial Least Squares |
| DPLS | Discriminant Partial Least Squares |
| ANN | Artificial Neural Network |
| SVR | Support Vector Regression |
| UV | Ultraviolet |
| SIMCA | Soft Independent Modeling of Class Analogy |

Pembangunan Kaedah yang Pantas dan Tepat Bagi Membezakan Sampel-Sampel Madu Malaysia Menggunakan UV dan Imej Warna

Abstrak

Malaysia adalah sebuah negara yang kaya dengan hasil hutan termasuk rempah-ratus, herba dan madu. Madu serta produk berasaskan madu semakin popular di Malaysia kerana berkait rapat dengan pemakanan sihat yang dipromosi oleh pelbagai agensi termasuklah kerajaan sebagai salah satu sumber pemakanan yang sihat. Ini seterusnya membuka peluang perniagaan untuk menyediakan produk madu serta produk sampingan yang berasaskan madu ke pasaran. Namun, fenomena ini juga menimbulkan permasalahan menentukan kualiti serta jenis madu yang ditawarkan di pasaran. Ini kerana jenis madu yang berlainan, sebagai contoh, mempunyai nilai khasiat yang berbeza, dan mungkin sesuai untuk sesuatu produk serta jenis pasaran. Pada waktu ini, kaedah menentukan jenis atau asal madu adalah melalui proses yang dilakukan di dalam makmal, yang kebanyakannya remeh, tidak mudah-alih, melibatkan penggunaan bahan kimia serta memerlukan kepakaran. Penyelidikan ini menampilkan kaedah yang lebih cekap menggunakan UV untuk pengkelasan madu. Penyelidikan ini juga menunjukkan bahawa penggunaan kamera digital RGB kos rendah juga boleh digunakan untuk tujuan yang sama. Selain dari itu, kerja yang dilaksanakan memperkenalkan fusion kedua-dua kaedah ini dan menunjukkan hasil pengkelasan yang lebih baik. Kerja ini mempamerkan pengkelasan madu berdasarkan dua ciri-ciri dari tiga (3) jenis madu tempatan yang berlainan, iaitu kandungan antioksidan serta warnanya. Kandungan antioksidan dibezakan dengan menggunakan gelombang spektroskopi UV pilihan, dan warna dibezakan menggunakan kamera RGB. Principal Component Analysis (PCA) digunakan di dalam kedua-dua kaedah untuk mengurangkan dimensi data yang terhasil. Support Vector Machine (SVM) pula digunakan bagi pengkelasan madu. Penilaian berasingan dilaksanakan bagi kedua-dua kaedah serta ke atas '*data fusion*', setelah terlaksana pengekstrakan ciri. Hasil pengkelasan menggunakan kaedah fusion meningkat sehingga 94% berbanding dengan dua kaedah tunggal yang dinyatakan. Ini menunjukkan kaedah yang disyorkan mampu menghasilkan pengkelasan madu yang tepat dan cepat mengikutinya. Kaedah yang disyorkan boleh diaplikasikan ke atas industri madu di Malaysia dan meningkatkan kualiti penilaian serta membolehkan penilai terhadap jenis-jenis madu.

Development of a Rapid and Accurate System to Differentiate Malaysian Honey Samples Using UV and Colour Image

Abstract

Malaysia is a country rich with natural forest resources such as spices, herbs and honey. Honey and honey-based products in Malaysia is gaining popularity as a result to the healthy lifestyle promotions by various groups including the government. This leads to the business opportunities to provide honey and its derivatives into the market. However, this also creates another problem in determining the quality, and types of honey offered by the market. This is because different honey types, for example, have different properties, which may be desirable for different purposes, and market segments. At the moment, the methods in determining the botanical origins of honey are all laboratory-based, which may be tedious, not portable, involve chemical synthesis, time consuming and required expert personal. This research introduces a more efficient approach using UV for honey classification. This research work also shows that a low-cost approach using RGB digital camera, can also be used for the same purpose. In addition to that, the work introduces the idea of fusion using the two approaches and shows an improvement in classification. The work presented the classification of the honey based on two characteristics from three (3) types of local honey, namely the antioxidant contents and colour variations. The former uses the UV spectroscopy of selected wavelength range, and the latter using RGB digital camera. Principal Component Analysis (PCA) was used for both methods to reduce the dimension of extracted data. Support Vector Machine (SVM) was used for the classification of honey. The assessment was done separately for each of the methods, and also on the fusion of both data after features extraction. The overall classification of the fusion method improved significantly compared to single modality. Honey classification based on the fusion method was able to achieve 94% accuracy. Hence, the proposed methods have the ability to provide accurate and rapid classification of honey products in terms of its origin. The proposed system can be applied to the Malaysian honey industry and further improve the quality assessment and provide traceability.

CHAPTER 1

INTRODUCTION

1.1 Background and Research Motivation

Malaysia is a tropical country rich in natural forest resources such as herbs, medicinal plants, spices and honey. These traditional foods are one of the main sources of income for the Malaysian agricultural industry. This opens up more demand and hence creates the need for a better classification methods, which can provide an accurate dan dependable verification for these products. This will help the local industry such as honey which is gaining popularity day by day. At present, consumers usually classify honey in terms of its geographical distribution and different floral sources, smell, taste and their colour. As there are many different types of honey available in Malaysia, the identification of the botanical origin is important to classify it accurately.

There are few existing methods to classify type of honey. Sensory evaluation is one of the methods that currently being used, unfortunately it is not easy to set up and the subjective experiences among the panelist are often biased and inconsistent. Chemical test methods in laboratory (Serrano, Villarejo, Espejo, & Jodral, 2004), is more accurate than the sensory evolution method. However, it is quite time consuming and costly. In addition to that, this method does not guarantee the safety of consumers and hinders further development of apiculture.

This researchers have suggested to use sensory systems that make use of electronic nose and electronic tongue to discriminate bee honey (Zakaria et al., 2011), along with the application of physicochemical criteria such as: enzymatic activity, sugar content, pH, water content, electrical conductivity and so on to analyze different types

of Uni-floral honey (Conti, Stripeikis, Campanella, Cucina, & Tudino, 2007). Furthermore, identification of pollen counts for authentication has been used, although there are difficulties to provide guarantee a precise assignment of the origin (Corbella & Cozzolino, 2008; Ramsay, 2005).

There are also some researchers use Pfund colour scale (Bertoncelj, Doberšek, Jamnik, & Golob, 2007) to classify honey for commercial purposes. The Pfund colourimeter is a simple tool which is used to compare the colour of the honey sample with a standard coloured glass. The reference unit is on the Pfund scale (0 to 140 mm), starts with very light-coloured honey and rising up to the darkest honey. However, this method requires very large amounts of sample and depends on the person performing the analysis since different observers lead to different measurements. However, these drawbacks are still remained in these researches. Thus, it is essential to find out a rapid and accurate way of honey discrimination.

Recently, UV-Visible spectroscopy has received wide range of attention of the researchers as it is suitable for non-destructive analysis of biological and biomedical materials. For example, the UV-Vis spectroscopy can be used for discriminating tea beverages (Chen, Zhao, Liu, & Cai, 2008), milk (Balabin & Smirnov, 2011), coffee (Lv, 2011), and other materials (Sinelli, Cerretani, Di Egidio, Bendini, & Casiraghi, 2010; H Yang, Kuang, & Mouazen, 2012; Haiqing Yang, Kuang, & Mouazen, 2011). Some researchers used this technique such as (Gallardo-Velázquez, Osorio-Revilla, Zuñiga-de Loa, & Rivera-Espinoza, 2009; Zhu et al., 2010) to classify adulterants in some local origins of honey. Although these researches achieved high accuracy for honey discrimination, the calibration models were developed using full range of wavelengths, which brought about high complexity in computation and cause difficulty in practical applications.

This thesis presents two methods for the classification of honey in terms of Botanical origin. Based on UV-Vis spectroscopy and Digital Camera, the difference in antioxidants and their hue colour were measured and later classified by the developed SVM classifier.

1.2 Problem Statements

Generally honey is classified in terms of taste, aroma, flavour and can also be distinguished by its colour (Lammertyn, Veraverbeke, & Irudayaraj, 2004). Due to these facts, human panelist has been the method of choice and often sought to provide quality assessment of honey. However, sensory evaluation is often inconsistent and varies from person to person (Massimiliano Magli, 2012). This is also due to personal experiences which may lead to bias and inaccurate assessment.

On the other hand, although artificial sensors, such as electronic nose and electronic tongue, are accurate (Guiyun Chen, 2013; Ulloa et al., 2013; Zakaria et al., 2011), they suffer some drawbacks as such tedious handling, requiring scheduled calibrations, involved with pattern recognition and may be sensitive to minute inconsistencies in the samples prepared.

Conventionally, chemical test is still acceptable but comes with the downside. Chemical test methods in laboratory, are accurate (Serrano et al., 2004). However, they are time consuming and costly. For example, UV-Vis spectroscopy is able to differentiate and classify honey with high accuracy (Chen et al., 2008). More than often, the computational analysis is getting complex as they use the full wavelength spectrum.

Hence, the improvement to the honey classification in terms of simpler procedures, more consistent results and portability is favorable. Therefore in this

research, two methods for honey classification were proposed and discussed separately. Also, the fusion of the two methods is presented.

1.3 Research Objectives

The main goal of this project is to provide an alternative method to allow the different types of honey to be classified. Also, it presents a fusion of the two methods implemented to improve the classification accuracy. The objectives of this project are as follow:

- To classify the origin of honey using antioxidant level by measuring the wavelengths from UV-Vis Spectroscopy.
- To classify the origin of honey based on the colour of honey images captured by Digital Camera.
- To implement data fusion technique by combining both UV and Digital Image for honey classification.
- To evaluate the performance of the fusion and compared with single method

1.4 Scope of This Research

The major contributions of this research are as follows:

- Develop procedure to prepare honey samples for the UV-Vis and Colour Image, also to cover the different age of the samples
- Perform data conditioning to allow the classification using SVM, k-NN and LDA to be performed.
- Implement classifications of honey samples using single methods, and implement fusion approach and the results were compared.

1.5 Motivation of The Work

The work highlights a new way of classification of honey product. The classification of honey products are no longer based only on the sensory evaluation and subjective human experience such as: taste, aroma and colour. As the discriminating factors. Instead, highly accurate techniques to classify honey products in terms of its botanical origin and level of colour have been utilised and demonstrated in this work.

In this research, the discrimination of honey in terms of different floral and level of colour has been performed. The success of using high accurate techniques allow further classification made not only based on the brands, but allowing the user to group the sample based on the combination of colour and antioxidants.

The scope of the work does not include:

- To find or to propose new algorithm or compare the performance of pattern recognition.
- Work on the comparison or suggestion to persuade or to determine the accuracies of the proposed approach compared to the existing approaches. If we are trying to introduce more accurate classification system, the direct comparison will be performed.
- Comparison with human expert panels. At the moment in these research areas, there are no trained personnel that are able to provide classification for honey samples.

1.6 Thesis Outline

This thesis is organized in five chapters.

Chapter 1 discusses the background, problem statements, objectives, scope and motivation for this research.

Chapter 2 presents an overview of previous studies and researches on classification of honey. Main components of honey and the proportions of these components have been presented in this chapter. Previous methods used to classify the honey in terms of Botanical Origin and colour has been described including the methods used in this research to classify honey.

Chapter 3 presents the methods used for classifying honey, terms of antioxidant using UV-Vis spectroscopy and various of colour using digital camera, and also present the overview of the research methodology.

Chapter 4 presents the data dimension reduction using PCA and classification using SVM on single methods as well as fusion of multiple classifiers.

Chapter 5 concludes the thesis by summarizing the research findings. It ends by proposing future work.

CHAPTER 2

LITERATURE REVIEW

This chapter presents an overview of previous studies and researches on classification of honey. The main differences in composite and physicochemical parameters of honey types are presented in this chapter. Previous methods used to classify the honey in terms of Botanical Origin and colours have been described including the methods used in this research to classify honey.

2.1. Overview of Honey

Honey is a natural food produced by bees from the nectar of blossom (nectar honey) or from the secretions of living plants or excretions of plant sucking insects of the living part of plant (honeydew honey) that transform and combine with specific substances of their own, and keep this mixture in the honey comb to ripen and mature (Sanz, Gonzalez, De Lorenzo, Sanz, & Martinez-Castro, 2005). Honey is composed primarily by a mixture of sugars (85–95%) and water (16–18%) approximately, and minority components such as proteins, free amino acids, organics acids, phenolic compounds, vitamins and minerals (Castro-Vázquez, Díaz-Maroto, De Torres, & Pérez-Coello, 2010; Kahraman, Buyukunal, Vural, & Altunatmaz, 2010; Pires, Estevinho, Feás, Cantalapiedra, & Iglesias, 2009; Sanz et al., 2005). Bee foragers collect nectar and honeydew from plants and carry it by means of their honey sac and bring it to their colony. When a worker bee drinks the nectar from flowers, a maximum of about 25 mg of nectar is stored at the bottom of the esophagus in a widened region called the honey

stomach or honey sac. On their way, they already add enzymes from their two glands, salivary and the hypopharyngeal, and transfer the nectar to colony bees. These nurse bees pass it over to each other and finally fill the honey into the combs. During this process the bees fan with their wings, thus lowering honey's humidity, and when the water contents reaches 30–40% the honey is filled into the combs. However, the bees add additional enzymes to the honey. These enzymes include diastase and invertase, both of which break down to larger saccharides, especially invertase, into monosaccharide (fructose and glucose). While glucose oxidase oxidizes the glucose to gluconic acid and hydrogen peroxide; the latter acting as an agent against bacterial spoilage. Thus, chemical transformation of the nectar into honey begins almost immediately. The warm colony temperature (35 °C), and further fanning lowers further the honey humidity. Bees also suck out the honey and deposit it back into the combs, and by this process further lower the water content of the honey. This transformation process takes place in 1 to 3 days. Generally, when the ripe honey has humidity lower than 20% the bees cap the combs, thus preventing absorption of moisture by the ripe honey. Only rarely, under very humid or tropical conditions can honey with more than 20% be capped by bees. The aim of the beekeeper is to harvest honey with less than 18% humidity (Ball, 2007).

2.2. Types of Honey

Honeys can be classified into two types: nectar honey and honeydew. Nectar is a sugar solution of varying concentrations: from 5–80%. About 95% of the dry substances are sugars, the rest are amino acids (0.05%), minerals (0.02–0.45%), small amounts of organic acids, vitamins and aroma compounds (Al et al., 2009; Manzanares, García, Galdón, Rodríguez, & Romero, 2011). The sugar value ranges widely, from 0.0005 to 8

mg. The sugar composition is also typical for each plant species, the main component of sugar are sucrose, glucose and fructose. Most plants have nectars consisting predominantly of fructose and glucose (60–85%); but in some plants the nectar is mainly sucrose (De La Fuente, Ruiz-Matute, Valencia-Barrera, Sanz, & Castro, 2011; Lazaridou, Biliaderis, Bacandritsos, & Sabatini, 2004). The sugar concentration depends on different climatic factor such as temperature, soil humidity and weather season. When humidity is higher, the nectar quantity is greater, but the sugar concentration is smaller. Temperature also plays a very important role in the process of honey production. Optimum temperatures are 25 to 35 °C. Strong winds reduce nectar secretion. The nectar secretion depends also on the day time. Therefore it is not possible to foresee nectar production. Maximum secretion is at early afternoon. Bees prefer nectar with higher sugar contents, e.g. around 50% and will not forage if it is below 5%, bees gather nectar for their energy needs. The higher the sugar value of a plant, the more it is visited by bees for foraging (Manzanares et al., 2011; Weryszko-Chmielewska & Chwil, 2007).

Honeydew is the secretion product of plant-sucking insects (Hemiptera, mostly aphids). These insects pierce the foliage or other covering parts of the plant and feed on the sap. The ingested sap is passed through the insect's gut, and the surplus is excreted as droplets of honeydew, which are gathered by the bees. There are different sorts of honeydew producing insects. Most plants are trees; the coniferous trees yield worldwide the highest amounts of honeydew. However, other plants, e.g. cotton, lucerne and sunflower can also provide honeydew (Manzanares et al., 2011; Sanz et al., 2005). And in Malaysian have a few types of honeydew plants as Star fruit and honey melon. Honeydew is a solution with varying sugar concentration (5–60%), containing mainly fructose, besides higher sugars (oligosaccharides). There are also smaller amounts of

amino acids, proteins, minerals, acids and vitamins. In addition, honeydew contains cells of algae and fungi, however there are not specific for the honeydew origin. Some insects produce high amounts of the trisaccharide melezitose, which is only very slightly soluble in water, thus yielding honey, which can crystallize in the combs (Manzanares et al., 2011). Honeydew production is even less predictable than the nectar flow, as it depends on the buildup of plant sucking insects. By evaluating the populations of the plant-sucking insects, the potential for a possible honey flow can be estimated. However, the honeydew flow depends also on favorable weather conditions during the honey flow period (De La Fuente et al., 2011; Manzanares et al., 2011). Table 2.1 shows the differences in composition between nectar and honeydew honey for some physicochemical parameters these two kinds of honeys.

Table 2.1: Differences in composition and physicochemical parameters of nectar honey and honeydew honey; values (g/100 g) (Al et al., 2009; Manzanares et al., 2011).

| Component | Nectar Honey | Honeydew Honey |
|----------------------------------|---------------------|-----------------------|
| Water | 15–20 | 15–20 |
| Carbohydrates | 72–85 | 73–83 |
| Fructose | 30–45 | 28–40 |
| Glucose | 20–40 | 22.9–40.7 |
| Sucrose | 0.1–4.8 | 0.2–7.6 |
| Melezitose | <0.1 | 0.3–22.0 |
| Erlose | 0.56 | 0.16–1 |
| Threhalose | 1.2–2.4 | 1.2–2.9 |
| Turanose | 1.0–2.1 | 0.7–2.2 |
| Other Disaccharides (maltose) | 28 | 16 |
| Other Oligosaccharides | 0.5–1.0 | 0.1–6.0 |
| Total sugars | 79.7 | 80.5 |
| Acids | 0.2–0.8 | 0.8–1.5 |
| Water activity | 0.56–0.61 | 0.57–0.61 |
| Minerals | 0.1–0.5 | 0.6–2 |
| Amino acids, Proteins | 0.2–0.4 | 0.4–0.7 |
| pH | 3.5–4.5 | 4.5–6.5 |

This study focused on the nectar honey whereby three types of Malaysia's honey are selected based on the most popular and highly demanded. These honey types were also used in the local community for the treatment of various diseases, as it contains a large amount of antioxidants, vitamins and essential ingredients such as minerals.

2.2.1 Tualang Honey

Tualang honey is a Malaysian multifloral jungle honey. The honey is produced by the rock bee (*Apis dorsata*), which builds hives on branches of tall Tualang trees located mainly in the north-western region of Peninsular Malaysia (Ahmed & Othman, 2013). In general, Tualang honey has been used in the local community for the treatment of various diseases, as it contains a large amount of antioxidant. The internal composition of the different types of honey vary with different floral sources as well as climatic and environmental conditions (Aljadi & Kamaruddin, 2004; Gheldof, Wang, & Engeseth, 2002; Küçük et al., 2007). Studies on the antioxidant activity of different types of honey from different countries and different botanical origins have been carried out (Al-Mamary, Al-Meerri, & Al-Habori, 2002; Aljadi & Kamaruddin, 2004; Beretta, Granata, Ferrero, Orioli, & Facino, 2005; Estevinho, Pereira, Moreira, Dias, & Pereira, 2008; Socha, Juszczak, Pietrzyk, & Fortuna, 2009). However, the antioxidant properties of the local Tualang honey have not been well documented in terms of botanical origins.

2.2.2 Acacia Honey

Acacia honey is quite a unique honey in Malaysia produced by *Apis mellifera* bees. It is one of the brightest honey type. When it is in a liquid form, acacia honey is almost transparent but once it crystallizes, it becomes white or very pale yellow in