
UNIVERSITI PUTRA MALAYSIA

# HPLC SUGAR PROFILES OF MALAYSIAN HONEYS

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# HPLC SUGAR PROFILES OF MALAYSIAN HONEYS

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| | |
|---|---|
| HPLC | High Performance Liquid Chromatoragphy |
| RID | Refractive Index Detector |
| GC | Gas Chromatography |
| MS | Mass Spectroscopy |
| HPAEC | High-Performance Anion-Exchange Chromatography |
| PAD | Pulsed Amperometric Detection |
| NMR | Nuclear Magnetic Resonance |
| UV | Ultraviolet |
| RI | Refractive Index |
| ELSD | Evaporating Light Scattering Detector |
| CAD | Charge Aerosol Detector |
| HCA | Hierachical Clustering Analysis |
| PCA | Principal Components Analysis |
| NIR | Near-Infrared Spectroscopy |
| PC | Principal Components |
| F/G | Fructose to Glucose Ratio |
| C8 | Carbon-8 |
| C18 | Carbon-18 |
| $NH_2$ | Amino group |
| HMF | Hydroxymethylfurfural |
| % | Percentage |
| mg | Milligram |
| ml | Millilitre |

| | |
|---|---|
| nm | Nanometer |
| °C | Degree Celcius |
| Cal | Calorie Unit |
| ms | Millisecond |
| cm | Centimeter |
| g | Gram |
| v/v | Volume per volume |
| mm | Millimeter |
| µl | Microliter |
| mAU.s | Milliabsorbance unit per second |
| µm | Micrometer |
| *et al.,* | and friends |

## ABSTRACT

Sugar profiles of 34 honey samples from different regions of Malaysia which coming from Perlis, Kedah, Kelantan, Perak, Terengganu, Kuala Lumpur, Selangor, Pahang, Melaka, Negeri Sembilan, Johor, Sabah and Sarawak are analysed by HPLC with UV detector at 195 nm. These samples consist of 10 Kelulut honeys, 2 Acacia honeys, 3 Hutan honeys, 9 Tualang honeys and 10 unknown honeys (unknown types). Five sugars are identified which are three monosaccharides and two dissacharides. Fructose (32.66±1.19%) is the major sugar in all samples followed by glucose (31.57±1.20%), galactose (2.62±1.94%), maltose (2.06±0.35%) and sucrose (2.95±0.39%). Fructose and glucose are the main monosaccharides in all samples. Galactose, sucrose and maltose are only present in certain honey samples. Hierarchical clustering analysis (HCA) and principal component analysis (PCA) used to classify honeys correctly based on sugar composition. Euclidean distance shows the concentration of each sugars presence in the samples which differentiate low and high concentration of total sugar. Cosine distance study recognized the pattern of sugar in honey samples from different region of Malaysia. Based on principal component analysis (PCA), honey samples are scattered according to their PC1 and PC2, thus allows the authenticity prediction.

## ABSTRAK

Profil gula 34 sampel madu dari kawasan-kawasan yang berlainan di Malaysia yang terdiri dari Perlis, Kedah, Kelantan, Perak, Terengganu, Kuala Lumpur, Selangor, Pahang, Melaka, Negeri Sembilan, Johor, Sabah dan Sarawak dianalisis oleh HPLC dengan menggunakan pengesan UV pada 195 nm. Sampel ini terdiri daripada 10 madu Kelulut, 2 madu Acacia , 3 madu Hutan, 9 madu Tualang dan 10 madu Unknown (jenis yang tidak diketahui). Lima gula dikenal pasti iaitu tiga monosakarida dan dua disakarida. Fruktosa (32.66 ± 1.19%) adalah gula utama dalam semua sampel diikuti dengan glukosa (31.57 ± 1.20%), galaktosa (2.62 ± 1.94%), maltosa (2.06 ± 0.35%) dan sukrosa (2.95 ± 0.39%). Fruktosa dan glukosa adalah monosakarida utama dalam semua sampel. Galaktosa, sukrosa dan maltosa hanya hadir dalam sampel madu tertentu. Analisis hierarki kelompok (HCA) dan analisis komponen utama (PCA) yang digunakan untuk mengkelaskan madu dengan betul berdasarkan komposisi gula. Jarak Euclidean menunjukkan kepekatan setiap kehadiran gula dalam sampel yang membezakan kepekatan rendah dan tinggi daripada jumlah kesuluruhan gula. Kajian jarak Cosine mengenalpasti corak gula dalam sampel madu dari kawasan yang berlainan di Malaysia. Berdasarkan analisis komponen utama (PCA), sampel madu bertaburan mengikut data PC1 dan PC2, sekali gus membolehkan ramalan keaslian.

# CHAPTER 1

## INTRODUCTION

According to Codex Standard for honey, honey is defined as the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature (Codex Standard., 2011).

Honey mainly composes of 80-85% of carbohydrates and other minor substances such as protein and amino acids, minerals, vitamins, enzymes and organic acids (Arvanitoyannis *et al*., 2005). Sugars in honey are obtained from several reactions of enzymes on the nectar sources. The enzymes involved are: invertase, diastase and glucose oxidase (Crane, 1980). The main enzyme responsible to sugar content in honey is invertase which converts sucrose to subunits fructose and glucose by breaking their glycosidic bonds.

Malaysia is a country that rich in biodiversity. There are several type of honey found in Malaysia which are Tualang, Kelulut, Hutan, Acacia and so on which highly influenced by type of honey bees. Honey bees come from *Apidae* with single genus, *Apis* comprises four species: *Apis dorsata* (Tualang), *Apis florea*, *Apis mellifera* (Acacia) and *Apis cerana* (Yong and Othman, 2007). One major problem faces by the Malaysian public is the question of honey purity and quality. The adulteration of honey slightly increased in honey products by adding the natural carbohydrates content of honey with cheaper or artificial sweeteners to gain more

profit. Many adulterants such as starch and dextrin (Wang *et al*., 2015), isoglucose syrups and acid-inverted syrups (Crane, 1980), corn syrups (Ribeiro *et al*., 2014), and beet sugars (Canabero *et al*., 2006) are reported in past research.

Therefore, the methods of quality control to identify the authenticity of honey are required. Several methods have been developed to detect adulteration of honey. Many analytical methods for sugar analysis have been reported: high-performance liquid chromatography coupled with refractive index detector (HPLC-RID) (Can *et al*., 2015) or gas chromatography-mass spectroscopy (GC-MS) (Pasini *et al*., 2013), high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Ouchemoukh *et al*., 2010) and nuclear magnetic resonance (NMR) (Consonni *et al*., 2013).

Sugar profiling of different types of honey has been reported by many scientists to detect adulteration of honey (Arvanitoyannis *et al*., 2005). The profiling showed that total sugar content in honey samples generally composed of monosaccharides, disaccharides and oligosaccharides (Fuente *et al*., 2011). As stated in Codex Standard, the sum of both fructose and glucose not less than 60/100 g and sucrose not more than 5/100 g has been used as a standard to measure sugar composition. The sugar profiles were used determined in multifloral and unifloral honeys from Algeria (Ouchemoukh *et al*., 2010).

In this study, 34 honey samples were analysed by using high-performance liquid chromatography with ultraviolet detector (HPLC-UV) at 195 nm. Sugars profiling are expected to be capable to detect the adulterated honey samples, detect sugar composition and identify pattern of various type of honeys. Multivariate statistical analysis is carried out to obtain graphic representation which shows best

summary of the information. Hierarchical clustering analysis (Euclidean distance and Cosine distance) and principal component analysis are used to characterize the information of honey.

Thus, in this study, there are three major objectives that will be accomplished which are:

1. To identify different types of sugars in honey samples.
2. To analyse sugar pattern of Malaysia honey based on sugar composition.
3. To predict authenticity of honeys by using statistical methods and based on Codex Standard.

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