View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Repository@USM

INVESTIGATION OF CHEMICAL COMPONENTS AND PURITIES OF EIGHT MALAYSIAN HONEYS AS COMPARED TO MANUKA HONEY

by

MOHAMMED MONIRUZZAMAN

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

June 2015

DEDICATION

To my wife and Baba

For their constant supports throughout my PhD

ACKNOWLEDGEMENTS

I would like to begin by expressing my sincere gratitude to my supervisor, Prof. Dr. Gan Siew Hua, for her valuable and appropriate guidance, endless enthusiasm, suggestions as well as advices during my study period. Special thanks to her valuable supports and inspirations during the time when I was much stressed with my research, she was the person who guided me all through the way and without her enormous assistance it was not possible for me to complete my research and to prepare this dissertation. Her friendly dealings and affectionate attitude highly inspired me to bring out the best from my side. The best thing I learnt from her is the writing skill which I believe something to be proud of as a researcher.

I would like to express my appreciation and respect to my co-supervisor, Prof. Dr. Siti Amrah Sulaiman, for her active encouragement, keen interest, valuable guidance and suggestions during the different stages of this research. I also convey my profound gratitude to Assoc. Prof. Dr. Isaac Rodriguez Pereiro, Department of Analytical Chemistry, University of Santiago de Compostela, Spain for supervising my GC-MS work, his appropriate guidance, good analytical techniques and inspiration, valuable suggestions and advice throughout my stay at that lab was praise worthy. In addition, I would like to thank Prof. Dr. Rafael Cela, head of the analytical lab for his precious support. I would also like to express my gratitude to USM fellowship programme and Erasmus Mundus scholarship programme for the financial support to conduct my study.

I am deeply indebted to my lovely beloved wife, Jannatul Nayma for her enormous support, caring and to tolerate all the pains. Her continuous support will be remembered with respect throughout my life. I am so grateful to my Baba, Muksudur

Rahman for his incessant supports, without those my way would be more difficult and special thanks to believe in me and making me feel special. I would also like to thank my beloved Amma (Samsun Nahar), Ma (Tuhin Akhter) for their love and care. Special thanks to my siblings Baro Apa and Dula Bhai, Nabila and Nobel. I would like to remember my respected father and a great man Late Abdul Awal Molla, whose lifestyle is a great inspiration for me always.

My sincere gratefulness is also for all lecturers and staff of my department, for their co-operation and assistance throughout my research work in the Pharmacology lab. Thanks to Dr. Md. Ibrahim Khalil and Dr. Alamgir Zaman Chowdhury from Bangladesh for their assistance. I also cordially thank to my colleagues and friends Mr. Chua Yung An, Mr. Jorge Casado, Mr. Tan Ka Liong, Mr. Munvar Mia Shaik, Ms. Tang Suk Peng, Ms. Wardah Yusof, Ms. Tamara Rodriguez Cabo for their invaluable assistance during different stages of my study.

This study was supported by Universiti Sains Malaysia Research University Grant (1001/PPSP/815058), Spanish Government and European Union FEDER funds (project CTQ2012-33080).

June, 2015 Mohammed Moniruzzaman

TABLE OF CONTENTS

CHAPTER 4

ASSESMENT OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION CONDITIONS FOR GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY IDENTIFICATION OF ORGANIC COMPOUNDS IN HONEY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AEAC	Antioxidant equivalent ascorbic acid content
AES	Atomic emission spectroscopy
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Ag	Silver
As	Arsenic
B	Boron
Ba	Barium
BD	Bangladesh
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
BSTFA	N,O-Bis(trimethylsilyl)-trifluoroacetamide
Ca	Calcium
CAS no	Chemical abstracts service number
CAT	Carnitine acylcarnitine translocase
CBA	Concentrated brown agouti
Cd	Cadmium
CEQ	Catechin equivalents
Cl	Chlorine
Co	Cobalt
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2

Cr	Chromium
Cu	Copper
DCPIP	2,6-dichlorophenolindophenol
DLLME	Dispersive liquid-liquid microextraction
DNSA	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	Electrical conductivity
EIC	Extracted ion current
EI-MS	Electron impact ionisation mass spectrometry
FAAS	Flame atomic absorption spectroscopy
FAES	Flame atomic emission spectroscopy
Fe	Iron
FRAP	Ferric ion reducing antioxidant power assay
GAL	Galicia
GAEs	Gallic acid equivalents
GC-MS	Gas chromatography mass spectrometry
GC-EI-MS	Gas chromatography electron impact ionisation mass spectrometry
GC-IT-MS	Gas chromatography ion trap mass spectrometry
GC-MS/MS	Gas chromatography tandem mass spectrometry
GC-QTOF-MS	Gas chromatography quadrupole time-of-flight mass spectrometry
GFAAS	Graphite furnace atomic absorption spectroscopy
GSH	Reduced glutathione
GI	Glycaemic index
GIT	Gastro intestinal tract
HCl	Hydrochloric acid

HMF	5-hydroxymethylfurfural
HNO_3	Nitric oxide
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
IHC	International honey commission
K	Potasium
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LC-QTOF-MS	Liquid chromatography quadrupole time-of-flight mass spectrometry
LLE	Liquid liquid extraction
LOD	Limit of detection
LogKow	The octanol/water partition coefficient
LOQ	Limit of quantification
MDA	Malondialdehyde
Mg	Magnesium
Mn	Manganese
MS	Mass spectrometer/Mass spectrometry
MW	Molecular weight
MY	Malaysia
Na	Sodium
$NaNO_2$	Sodium nitrite

NaOH	Sodium hydroxide
NF-κB	Necrosis factor-κB
Ni	Nickel
NSAIDS	Non-steroidal anti-inflammatory drugs
Na_2CO_3	Sodium carbonate
OPPs	Organophosphorus pesticides
OCPs	Organochlorine pesticides
ORAC	Oxygen radical absorbance capacity assay
P	Phosphorous
Pb	Lead
PCA	Principal component analysis
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
pKa	Acid-base dissociation constant
PTFE	Polytetrafluoroethylene
RDI	Recommended daily intake
ROS	Reactive oxygen substance
RSA	Radical-scavenging activity
RSD	Relative standard deviation
S	Sulphur
SD	Standard deviation
SDE	Simultaneous distillation extraction
SOD	Superoxide dismutase
Se	Selenium
Sn	Stannum

SPE	Solid phase extraction
SPME	Solid phase microextraction
Sr	Strontium
STZ	Streptozotocin
TBSA	Total body surface area
TDS	Total dissolved solids
TIC	Total ion current
TMS	Trimethylsilylation
TMSO	Trimethylsilyl oximes
TNF-α	Tumour necrosis factor-α
TPC	Total phenolic content
TPTZ	2,4,6-tris(1-pyridyl)-1,3,5-triazine
US FDA	United States Food and Drug Administration
V	Vanadium
VLDL	Very low density lipoprotein
VOCs	Volatile organic compounds
Zn	Zinc

SIASATAN KOMPOSISI KIMIA DAN KETULENAN LAPAN JENIS MADU MALAYSIA BERBANDING DENGAN MADU MANUKA

ABSTRAK

Pengenalan: Malaysia, sebuah negara tropika yang kaya dengan flora dan fauna mempunyai pelbagai jenis madu. Walaupun madu dihasilkan dan digunakan secara meluas di Malaysia, masih terdapat kekurangan maklumat berkenaan komposisi kimia madu-madu tersebut. Oleh itu, kajian ini bertujuan untuk menyiasat komposisi kimia (fizikal, kimia, parameter antioksida, mineral, unsur surih, bahan meruap dan kandungan gula) untuk lapan jenis madu Malaysia (akasia, nanas, gelam, longan, borneo, pokok getah, sourwood dan tualang) dengan membandingkan mereka dengan madu manuka.

Kaedah: Parameter-parameter fizikal dan antioksida yang berlainan diukur dengan menggunakan teknik spektrofotometri, sementara komposisi asid fenolik ditentukan dengan menggunakan teknik kromatografi cecair berprestasi tinggi. Kepekatan mineral-mineral dan unsur-unsur surih diukur dengan menggunakan spektrometri penyerapan atom. Kromatografi gas spektrometri jisim masa penerbangan caturkutub (GC-QTOF-MS) telah digunakan buat pertama kalinya untuk menganalisis bahan-bahan meruap madu dan seterusnya satu kaedah mikro-penyarian cecair-cecair serakan (DLLME) baharu telah dibangunkan untuk menganalisis bahan-bahan meruap. Analisis gula dijalankan dengan menggunakan GC-MS.

Keputusan dan perbincangan: Parameter fizikal madu-madu yang dikaji adalah dalam lingkungan had yang disarankan oleh International Honey Commission. Purata kepekatan bahan-bahan fenolik (325.59 ± 168.45 $mg_{asidgalik}$/kg) dan flavonoid

(62.52 ± 56.06 $mg_{katekin}$/kg), aktiviti skaveng radikal DPPH (43.02 ± 14.03%) dan kuasa penurunan ferik adalah 329.70 ± 209.16 μM Fe (II)/100 g. Asid benzoik merupakan bahan fenolik yang paling banyak (75%) diikuti oleh asid kafeik, katekin, mairisetina, asid galik dan naringenina. Kandungan mineral yang tinggi telah dikesan di dalam madu-madu yang dikaji di mana K, Na, Fe dan Ca merupakan unsur-unsur yang paling banyak (purata masing-masing, 1466.01, 230.15, 133.39 dan 144.48 mg/kg). Keseluruhannya, unsur-unsur surih berkenaan berada dalam lingkungan had yang disarankan dan tiada sisa pestisid dikesan dalam mana-mana sampel madu, menunjukkan madu-madu berkenaan berkualiti baik. Analisis seterusnya adalah dengan menggunakan mikroekstraksi fasa pepejal (SPME) ruang tutupan (HS). Keupayaan ketepatan jisim GC-QTOF-MS yang dinilai untuk pengesanan bahan-bahan menunjukkan jendela jisim yang sempit (0.005 Da) secara relatifnya. Akhirnya, satu kaedah DLLME yang baru telah dibangunkan dan dioptimumkan untuk menganalisis bahan-bahan meruap madu. Keseluruhan proses penyediaan sampel disempurnakan dalam masa lebih kurang 10 min. Penggunaan pelarut organik adalah sedikit (kurang daripada 4 mL) manakala sisihan piawai relatif kurang daripada 12% dan kira-kira 78 bahan organik dikenal pasti di dalam ekstraksi yang diperoleh. Selain itu, beberapa jenis gula juga dikenal pasti dan disukat di dalam madu-madu tersebut.

Kesimpulan: Madu sourwood, longan dan tualang mempunyai jumlah asid-asid fenolik dan flavonoid-flavonoid yang lebih tinggi di samping mempunyai potensi antipengoksidaan yang lebih baik berbanding dengan madu-madu Malaysia yang lain dan madu manuka. Secara keseluruhannya, keputusan kajian ini menunjukkan madu-madu Malaysia adalah berkualiti baik.

INVESTIGATION OF CHEMICAL COMPONENTS AND PURITIES OF EIGHT MALAYSIAN HONEYS AS COMPARED TO MANUKA HONEY

ABSTRACT

Introduction: Malaysia, a tropical country rich with flora and fauna has many different types of honeys. Although honey is produced and is greatly consumed in Malaysia, there is a lack of information on the chemical composition of these honeys. Thus, the present study was aimed to investigate the chemical composition (physical, chemical, antioxidant parameters, minerals, trace elements, volatile compounds and sugar content) of eight different Malaysian honeys (acacia, pineapple, gelam, longan, borneo, rubber tree, sourwood and tualang) compared to manuka honey.

Methods: Different physical and antioxidant parameters were measured using spectrophotometric techniques while phenolic acid composition was determined by high performance liquid chromatography. Minerals and trace elements were determined using atomic absorption spectrometry. Gas chromatography quadrupole time-of-flight mass spectrometry (GC-QTOF-MS) was used for the first time to analyse honey volatiles and subsequently a novel dispersive liquid-liquid microextraction (DLLME) method was developed to analyse volatiles. Sugar analysis was performed by GC-MS.

Results and discussions: The physical parameters of the investigated honeys were within the limit recommended by International Honey Commission. The mean concentration of phenolics (325.59 ± 168.45 $mg_{galicacid}$/kg) and flavonoids (62.52 ± 56.06 $mg_{catechin}$/kg), DPPH radical scavenging activity (43.02 ± 14.03%) and ferric

reducing power was 329.70 ± 209.16 μM Fe (II)/100 g. Benzoic acid was the most abundant phenolic compounds (75%) among the phenolic acids followed by caffeic acid, catechin, myricetin, gallic acid and naringenin. High mineral contents were observed in the investigated honeys with K, Na, Fe and Ca being the most abundant elements (mean 1466.01, 230.15, 133.39 and 144.48 mg/kg, respectively). Overall, the trace elements were within the recommended limits with no pesticide residues detected in any of the honey samples indicating their good qualities. Following analysis using headspace (HS) solid-phase microextraction (SPME), accurate mass capabilities of GC-QTOF-MS evaluated for compounds identification showed a relatively narrow mass window (0.005 Da). Finally, a novel DLLME method was developed and optimised to analyse honey volatiles. The whole sample preparation process was completed in only approximately 10 min, with a total consumption of organic solvents below 4 mL, relative standard deviations lower than 12% and approximately 78 organic compounds identified in the obtained extracts. Several sugars were identified and quantified in honeys.

Conclusion: Sourwood, longan and tualang honeys have higher number of phenolic acids, flavonoids with superior antioxidant potentials when compared to other Malaysian honeys and manuka honey. Overall, the results of this research indicate that Malaysian honeys are of good qualities.

CHAPTER 1

PHYSICOCHEMICAL, ANTIOXIDANT PROPERTIES, PHENOLIC ACID AND FLAVONOIDS CONTENT OF HONEYS FROM DIFFERENT REGIONS OF MALAYSIA COMPARED TO MANUKA HONEY

1. 1 INTRODUCTION

1.1.1 Background

Honey is a natural product produced by the honeybees and consists of a very concentrated solution of a complex mixture of sugars in which fructose and glucose are the main ingredients (Saxena *et al.*, 2010; Khalil *et al.*, 2011a; Cimpoiu *et al.*, 2013). In addition to carbohydrate content, it also contains minor but important constituents such as proteins, enzymes (invertase, glucose oxidase, catalase, and phosphatases), amino and organic acids (gluconic acid and acetic acid), lipids, vitamins (ascorbic acid, niacin and pyridoxine), volatile chemicals, phenolic acids, flavonoids and carotenoid-like substances as well as minerals (Blasa *et al.*, 2006; Saxena *et al.*, 2010; Khalil *et al.*, 2012). Although the composition of honey can be variable and is primarily dependent on its floral source, certain external factors such as seasonal and environmental factors and processing also play important roles (Bertoncelj *et al.*, 2007; Guler *et al.*, 2007; Alvarez-Suarez *et al.*, 2010). Honey is a functional food and has different biological properties such as antibacterial (bacteriostatic properties), anti-inflammatory, wound and sunburn healing, antioxidant, radical scavenging, antidiabetic and antimicrobial activities (Al-Mamary *et al.*, 2002; Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Blasa *et al.*, 2007; Ouchemoukh *et al.*, 2007; Gomes *et al.*, 2010; Erejuwa *et al.*, 2011; Sereia *et al.*, 2011; Mohamed *et al.*, 2012; Serem and Bester, 2012).

In recent years, there has been an increasing interest in determining the antioxidant potentials of honey (Bertoncelj *et al.*, 2007). It has been reported in many studies that the antioxidant activities of honey are widely variable, depending on the floral sources (Lachman *et al.*, 2010a). The botanical origin of honey has been reported to cover the greatest influence on its antioxidant activity, whereas processing, handling and storage can affect the antioxidant activity of honey only to a minor extent (Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005; Lachman *et al.*, 2010b). Moreover, it has been shown in several studies that the antioxidant potential of honey is strongly correlated with the concentration of total phenolics present (Al-Mamary *et al.*, 2002; Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Meda *et al.*, 2005; Blasa *et al.*, 2006; Bertoncelj *et al.*, 2007). Furthermore, the antioxidant activity has also been reported to be strongly correlated with honey colour, where dark coloured honey has been reported to have a higher total phenolic content and consequently higher antioxidant capacities (Frankel *et al.*, 1998; Beretta *et al.*, 2005; Bertoncelj *et al.*, 2007).

The antioxidant activity of honey has been attributed to both enzymatic proteins, including catalase (Schepartz, 1966), glucose oxidase and peroxidase (Ioyrish, 1974) and non-enzymatic substances such as ascorbic acid, α-tocopherol (Crane, 1975), carotenoids, amino acids, proteins, organic acids and Maillard reaction products (Al-Mamary *et al.*, 2002; Gheldof *et al.*, 2002; Schramm *et al.*, 2003; Aljadi and Kamaruddin, 2004; Baltrušaitytė *et al.*, 2007; Bertoncelj *et al.*, 2007; Ferreira *et al.*, 2009). There are more than 150 polyphenolic compounds that have previously been reported including phenolic acids, flavonoids, flavonols, catechins and cinnamic acid derivatives (Ferreira *et al.*, 2009). The composition and quantity of these components

vary widely according to the floral and geographic origins of the honey. Several studies on the identification and quantification of the antioxidant components of honeybee products originating from different countries have been reported (Ferreres *et al.*, 1994a; Ferreres *et al.*, 1994b; Ferreres *et al.*, 1994c; Gheldof *et al.*, 2002; Buratti *et al.*, 2007; Ferreira *et al.*, 2009). However, there is limited data available for Malaysian honey despite its high consumption rate by the general public.

Several types of honey are found in Malaysia. These are either directly or indirectly introduced into many types of foods in Malaysia and have also been used as a traditional medicine for the last few decades. Among the different types of honey available in the country, the antioxidant potentials of tualang and gelam honeys have been previously reported (Aljadi and Yusoff, 2003; Aljadi and Kamaruddin, 2004; Mohamed *et al.*, 2010; Hussein *et al.*, 2011; Khalil *et al.*, 2011b; Kishore *et al.*, 2011). However, there is lack of knowledge and scientific data on the other types of Malaysian honeys.

1.2.1 Literature Review

1.2.1.1 Physicochemical properties of honey

In general, most of the physical and chemical methods used in the analysis of honey are mainly intended for honey quality control and detection of possible adulteration present but some of them, particularly the determination of the electrical conductivity, the sugar composition, colour intensity or colour characteristics may help in the elucidation of the botanical origin. On the whole, physicochemical

properties of honey are very important features for honey quality and some of these properties are described in this section.

1.2.1.1.1 pH and moisture content

In general, honey is acidic in nature irrespective of its variable geographical origins due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage. Gluconic acid is the main acid present in honey found together with the respective glucono-lactone in a variable equilibrium (Mato *et al.*, 1997). Free acidity, total acidity and pH-value are important factors for the classification and/or discrimination of unifloral honeys, while lactones which are present in similar amounts in various unifloral honeys may be less useful for the determination of the botanical origin (Mato *et al.*, 1997; Persano-Oddo and Piro, 2004; Piazza and Persano Oddo, 2004). The pH values of Algerian, Brazilian, Spanish and Turkish honeys have been found to vary between 3.49 to 4.53, 3.10 to 4.05, 3.63 to 5.01 and 3.67 to 4.57, respectively (Azeredo *et al.*, 2003; Ouchemoukh *et al.*, 2007; Kayacier and Karaman, 2008).

Moisture content is one of the most important physical characteristics of honey since it influences honey's storage and granulation (Bogdanov *et al.*, 2004). Its concentration is a function of the factors involved in honey's ripening, that includes weather conditions, original moisture of nectar, its rate of secretion and strength of the bee colony (as the bees use their wings to create a stream of dry air that constitutes the ventilation system of the hive) (Siddiqui, 1970). The moisture content of honeys originating from different geographical and botanical origins have

been reported to show a wide range (from 13% to 29%) (Kayacier and Karaman, 2008; Saxena *et al.*, 2010). The superior moisture content in honey could lead to undesirable fermentation of honey during storage caused by the action of osmotolerant yeasts and consequently ethyl alcohol and carbon dioxide are formed. Thereafter, the alcohol can be further oxidised to acetic acid and water resulting in a sour taste (Chirife *et al.*, 2006). Moreover, the moisture content of honey is also dependant on other factors including harvesting season, degree of maturity reached in the hive and climatic factors (Finola *et al.*, 2007; Saxena *et al.*, 2010).

1.2.1.1.2 Electrical conductivity

The measurement of electrical conductivity (EC) was established a long time ago (Vorwohl, 1964). Electrical conductivity is principally dependant on the mineral content of honey (Accorti *et al.*, 1983) as well as the ash and acid content in honey: the higher their content, the higher is the resulting conductivity (Bogdanov *et al.*, 2002). Therefore, this parameter was recently incorporated in the international standards replacing the determination of ash content (Alimentarius, 2001; Bogdanov *et al.*, 2004). Thus, it becomes one of the most essential quality parameters for the classification of unifloral honeys (Mateo and Bosch-Reig, 1998) which can be measured by relatively inexpensive instrumentation called conductometer (Bogdanov *et al.*, 2004) and was reported to be the most essential tool for the classification of unifloral honeys (Krauze and Zalewski, 1991; Mateo and Bosch-Reig, 1998; Piro *et al.*, 2002; Devillers *et al.*, 2004). The method for the determination of EC was described by Bogdanov *et al.,* (1997).

The range of EC in honey is usually between 0.06 and 2.17 $mScm^{-1}$. There are considerably higher amounts of minerals in honeydew honey compared to blossom honeys due to the fact that honeydew is directly sucked from the phloem by various insects and therefore, the minerals are mostly resorted before nectar secretion (Bogdanov *et al.*, 2004). Usually, honeydew honeys possess EC values higher than 0.8 $mScm^{-1}$, while the blends between blossom and honeydew honeys have EC values between 0.51 and 0.79 $mScm^{-1}$ and pure floral honeys exhibit EC values between 0.15 and 0.50 $mScm^{-1}$ (Bogdanov *et al.*, 2004). However, the exceptions should be applied for some blossom honeys including strawberry tree (*Arbutus unedo*), eucalyptus, lime (*Tilia sp.*), bell heather (*Erica*), ling heather (*Calluna vulgaris*), manuka or jelly bush (*Leptospermum*) and tea tree (*Melaleucasp.*) (Alimentarius, 2001; Kaškonienė *et al.*, 2010). Therefore, it is important to measure EC values since they can be unique to the investigated honey type.

1.2.1.1.3 Carbohydrates

More than 95% of the solids of honey are carbohydrate in nature (Kaškonienė *et al.*, 2010) while fructose and glucose are the major sugars. Fructose is the most abundant component in almost all honey types, with the exception of some honeys of dandelion (*Taraxacum officinale*), rape (*Brassica napus*) and blue curls (*Trichostema lanceolatumi*) origin, where glucose is present in higher amounts (Cavia et al., 2002). In addition, disaccharides, trisaccharides and other oligosaccharides are also present in honey in small concentrations (Sanz *et al.*, 2004a; Kaškonienė *et al.*, 2010). The concentration of fructose and glucose as well as their ratios are useful indicators for the classification of unifloral honeys (Oddo *et al.*, 1995; Persano-Oddo and Piro, 2004). Moreover, the monosaccharides fructose and glucose are the building blocks

for the more complex oligosaccharides and represent approximately 85-95% of the sugar content.

Many authors have proposed the use of sugar composition to establish honey authenticity (White and Doner, 1980; Goodall *et al.*, 1995; Low and South, 1995; Prodolliet and Hischenhuber, 1998; Sanz *et al.*, 2004b). On the other hand, the variation in the concentration of some carbohydrates, mainly monosaccharides (glucose and fructose) and trisaccharides (eg melezitose and raffinose) has been used to differentiate between nectar and honeydew honeys (Terrab *et al.*, 2002). However, several researchers conclude that sugar composition by itself is not enough to identify the botanical origin of nectar honeys (Földházi, 1994; Sanz *et al.*, 2004a; Sanz *et al.*, 2004b). Therefore, other parameters including honey colour, hydroxymethyl furfural (HMF) content, proteins, enzymes, vitamins, minerals and phenols should be measured.

1.2.1.1.4 Colour characteristics

Honey colour differs from water clear, through amber tones, until almost black, sometimes with typical bright yellow, greenish or reddish hues (Diez *et al.*, 2004). This is the first factor usually selected by consumers before purchasing honeys. In most countries, the pricing of honey depends to a great extent on colour. For example, light honeys like acacia (*Robinia pseudoacacia*) and orange (*Citrus spp*.) generally demands the highest prices due to their colour (Beretta *et al.*, 2005).

The most commonly used methods for measuring honey colour are dependent on simple optical comparison using the Pfund colour grader or the more sophisticated Lovibond instrument (Fell, 1978; Aubert and Gonnet, 1983) and the values of these instruments are the indicators of colour intensity. In general, the Lovibond instrument is easier to handle when compared to the Pfund graders therefore, honey is generally marketed according to the Pfund scale. The determination of colour is a useful classification criterion for unifloral honeys. Unfortunately, since honey colour darkens during storage this technique may therefore be only appropriate for the classification of fresh honeys. A strong interference of polyfloral honey with the unifloral honeys is also to be expected (Gonzales *et al.*, 1999; Terrab *et al.*, 2002). Moreover, it has been reported that variations in honeys geographical and botanical origins as well as composition are significantly reflected in their colour intensities (Terrab *et al.*, 2002).

1.2.1.1.5 HMF content

Usually fresh honey does not contain HMF (Bogdanov *et al.*, 2004) and thus, determination of this compound is useful for evaluation of the quality of honey (Zappala *et al.*, 2005). Overheating of honey samples during processing or storage for very long periods could lead to the conversion of sugars to HMF and it was reported that heating of unifloral honey leads to different HMF levels in honey (Fallico *et al.*, 2004) while in a previous study of our research group, it was found that some Malaysian honeys stored for more than one year contained HMF in very high concentrations (Khalil *et al.*, 2010). Usually, HMF is formed during acid catalysed dehydration of hexoses (Zappala *et al.*, 2005) and is connected to the chemical properties of honey like pH, total acidity, mineral content (Hase *et al.*,

1973; Anam and Dart, 1995; Singh and Bath, 1997; Singh and Bath, 1998; Bath and Singh, 1999). Therefore, a low level of HMF is an indicator of the freshness of honey. In another study, the HMF content of Indian honey samples was found to be higher than the internationally recommended limit of 80 mg/kg (Saxena *et al.*, 2010).

1.2.1.1.6 Proteins, enzymes and amino acids

Honey contains approximately 0.5% proteins which is mainly enzymes and free amino acids (Bogdanov *et al.*, 2008). The contribution of that fraction of protein present in honey to human protein intake is marginal and the three main enzymes present in honey are diastase (amylase), which degrades starch or glycogen into smaller sugar units, invertase (sucrase, glucosidase), that decompose sucrose into fructose and glucose, as well as glucose oxidase, that produce hydrogen peroxide and gluconic acid from glucose (Bogdanov *et al.*, 2008). Proline, the main amino acid in honey, originates predominantly from the bee and its concentration is used as a sign of honey ripeness as well as for the detection of adulteration (von der Ohe *et al.*, 1991).

Proline content in honey shows characteristic values in different unifloral honeys (Bogdanov *et al.*, 2004; Persano-Oddo and Piro, 2004) and is broadly correlated with the enzyme activity (Bogdanov *et al.*, 2004). However, the difference of this parameter in different unifloral honeys is relatively high and therefore, it is not possible to classify unifloral honey on the basis of proline content only (Persano-Oddo and Piro, 2004; Sanz *et al.*, 2004a). In addition to the proline content, for the purpose of determining the geographical origin of honey, free amino acid profiles

have primarily been proposed (Gilbert *et al.*, 1981; Davies and Harris, 1982). In a previous study on lavender (*Lavandula spp.*) and eucalyptus (*Eucalyptus spp*.) honeys, high concentrations of phenylalanine (906 - 1830 mg/kg) and tyrosine (229 - 382 mg/kg) were detected in lavender honeys which could be characteristic for lavender honeys and allowed a differentiation from eucalyptus honeys (Bouseta *et al.*, 1996). Tryptophan and glutamic acid were used to distinguish honeydew from blossom honeys (Iglesias *et al.*, 2004).

1.2.1.1.7 Vitamins, minerals and trace compounds

The quantity of vitamins and minerals is little and the contribution of honey to the recommended daily intake (RDI) of the different trace substances is minor (Bogdanov *et al.*, 2004). It is known that different unifloral honeys contain varying amounts of minerals and trace elements (Bogdanov *et al.*, 2004). The mineral content of honey is mainly dependent on the plant's absorption of the minerals from the soil and from the environment (Gonzalez-Miret *et al.*, 2005). It has been reported that the mineral honey content is 0.04 - 0.20%, depending on whether it is the light or dark honey type (Vanhanen *et al.*, 2011) and this type of honey tends to contain higher levels of minerals. To date, twenty-seven different mineral elements have been identified and measured in honey from nine different countries (Vanhanen *et al.*, 2011). However, no honey has been shown to contain all 27 elements so far. In most studies, particular groups of minerals have been found in honey from different floral and geographical origins (Al-Mamary *et al.*, 2002; Fernandez-Torres *et al.*, 2005; Golob *et al.*, 2005; Lachman *et al.*, 2007; Madejczyk and Baralkiewicz, 2008) indicating that mineral content is unique to each honey type.

1.2.1.1.8 Aroma compounds and polyphenols

There is a wide range of varieties of honeys having different tastes and colours, depending on their botanical origins (Crane *et al.*, 1984). As mentioned previously, sugars are the main taste building compounds with a high fructose content followed by glucose concentration. Honey aroma depends also on the quantity and type of acids and amino acids present in it. In the past decades, extensive research on aromatic compounds has been conducted with more than 500 different volatile compounds identified in different types of honey. Thus, it can be assumed that most aroma building compounds differ in various types of honey depending on its botanical origin (Bogdanov *et al.*, 2004). Most importantly, honey flavour is an important quality for its applications or uses in food industry and is also a selection criterion for consumer in choosing honey type (Bogdanov *et al.*, 2008).

Moreover, volatile organic compounds (VOCs) in honey are obtained from different biosynthetic pathways and are extracted by using a variety of methods associated with varying degrees of selectivity and effectiveness (Manyi-Loh *et al.*, 2011). However, the composition of VOCs in honey is subjective to both nectar composition and floral origin, which could also be attributed to the honey's geographical origin (Cuevas-Glory *et al.*, 2008). Additionally, differences occur in the level of volatile components found in honey during storage as a result of the temperature at which it is exposed and also the duration of exposure (Manyi-Loh *et al.*, 2011). It was assumed that these changes in heated or stored honey have been attributed to two main causes: compounds that are heat labile and may be easily destroyed and volatile compounds produced by non-enzymatic browning (Maillard reaction) (Manyi-Loh *et al.*, 2011). Moreover, in a previous study conducted by

Castro-Várquez *et al*., (2008), there was a reduction in the concentrations of terpene derivatives and methyl anthranilate in contrast to an increase in concentration of linalool, linalool oxides and dien-diols in stored citrus honeys indicating that the effects are variable.

In addition to the aforementioned compounds reported to be present in honeys, polyphenols are another important group of compounds with respect to the appearance and the functional properties of honey (Bogdanov *et al.*, 2008). It was reported that total polyphenols present in different honey types at 56 to 500 mg/kg (Al-Mamary *et al.*, 2002; Gheldof *et al.*, 2002). Polyphenols that are present in honey are mainly flavonoids (e.g. quercetin, kaempferol, chrysin, luteolin, apigenin, galangin), phenolic acids and their derivatives (Tomas-Barberan *et al.*, 2001). These are compounds known to have antioxidant properties. Phenolic acids and polyphenols are plant-derived secondary metabolites (Bogdanov *et al.*, 2008). These compounds have been used as chemotaxonomic indicators in plant systematic and have been suggested as possible markers for the determination of botanical origin of honey. Considerable differences in composition and content of phenolic compounds between different unifloral honeys have been reported (Ferreres *et al.*, 1993; Tomas-Barberan *et al.*, 2001). Dark-coloured honeys are reported to contain more phenolic acid derivatives but lower amounts of flavonoid when compared to the light coloured ones (Ampuero *et al.*, 2004).

In a previous study, the flavonoid profile of nine European unifloral honeys was analysed by high performance liquid chromatography (HPLC) (Tomas-Barberan *et*

al., 2001). Hesperetin was confirmed as a marker of citrus honey with no specific compounds detected in robinia and lavandula honeys. Abscisic acid, formerly reported as a characteristic compound of calluna honey (Ferreres *et al.*, 1994a) was also detected in brassica, tilia and robinia honeys while gallic and dimerellagic acids were reported to be useful marker of calluna honey (Ferreres *et al.*, 1994a). These findings are in agreement with that for heather honeys from erica and calluna species (Andrade *et al.*, 1997). Thus, the determination of the flavonoid patterns is useful for the classification of most unifloral honeys.

1.2.2 Quality of honey

The quality of honey is determined by its sensorial, chemical, physical and microbiological characteristics (Alvarez-Suarez *et al.*, 2010). The criteria that define the physicochemical quality of honey are specified by the European Commission Directive 2001/110 (Council Directive of the European Union:, 2002) and codex Alimentarius (Alimentarius, 2001). The major criteria of interest are moisture content, EC, ash content, reducing and non-reducing sugars, free acidity, diastase activity and HMF contents (Blasa *et al.*, 2006; Alvarez- Suarez *et al.*, 2010; Alvarez-Suarez *et al.*, 2010). According to the international regulations for honey (Alimentarius, 2001; Council Directive of the European Union:, 2002), the maximum prescribed limit is (≤20%) for honey's moisture content. As mentioned previously, HMF content of honey is an important factor for determining its quality. Thus, the Codex Alimentarius (Alimentarius, 2001) established that the HMF content of honey after processing and/or blending should not be higher than 80 mg/kg.

The European Union (Council Directive of the European Union:, 2002) recommended the ideal HMF limit in honey as 40 mg/kg with the following exceptions: 80 mg/kg for honey originating from countries or regions with tropical temperatures or 15 mg/kg for honey with low enzymatic level (8-3 Schade Units). Since Malaysia is a tropical country, the HMF content of the honeys produced in this country should be less than 80 mg/kg to meet the international standard. Moreover, the protein content of honey is normally less than 5 mg/g (Anklam, 1998; Bogdanov *et al.*, 2004) while according to the European Community Directive, the total sugar content of honey sample is recommended to be more than 60% (Council Directive of the European Union:, 2002) and the maximum prescribed limit of sucrose content for honey is 5% as recommended by the Codex standard (Alimentarius, 2001). Overall, it can be concluded that honey samples meeting the above criteria is considered as good quality honey.

1.2.3 Biological Importance of honey

Honey has been traditionally used from ancient times in the treatment of different types of diseases. The medicinal or biological importance of honey has been known from the ancient times. Honey possesses several medicinal and/or biological properties which is beneficial for human health. Due to its beneficial properties, in recent years, an alternative medicine branch named apitherapy, has been developed, offering treatments depending on honey type and other types of bee products against many diseases (Bogdanov *et al.*, 2008). Some of the important biological features of honey are described in the following sections.

1.2.3.1 Antibacterial activity

Antimicrobial agents are basically vital in reducing the global burden of infectious diseases. However, as resistant pathogens develop and multiply, the efficiency of the antibiotics is reduced. This type of bacterial resistance to the antimicrobial agents causes a very serious threat to public health and for all kinds of antibiotics, including the major last-resort drugs with the frequencies of resistance increasing worldwide (Levy and Marshall, 2004; Mandal *et al.*, 2009; Mandal *et al.*, 2010a). As a result, alternative antimicrobial strategies are urgently needed leading to a re-evaluation of the therapeutic application and use of ancient remedies such as plants and plant-based products, including honey (Basualdo *et al.*, 2007; Mandal *et al.*, 2010b; Mandal *et al.*, 2010a; Mandal and Mandal, 2011; Vallianou *et al.*, 2014).

Honey has a number of properties which make it appropriate as an antibacterial agent. Molan and Cooper (2000) reported that the variation in antimicrobial potency among the different honeys can be more than hundred fold, depending on their botanical, geographical, seasonal, source, processing, harvesting and storage conditions. The antimicrobial properties of honey is mainly attributed to the osmotic effect of the substance's sugars, its pH, and particularly its peroxidase activity (Alnaqdy *et al.*, 2005; Ghazali, 2009; Tan *et al.*, 2009; Nasir *et al.*, 2010; Vallianou *et al.*, 2014). The antimicrobial effects are also because of the presence of non peroxidase substances such as phenolic acids, flavonoids and lysozymes (Alnaqdy *et al.*, 2005; Tan *et al.*, 2009; Nasir *et al.*, 2010; Khalil *et al.*, 2014). The antibacterial properties of honey differ according to its source and reported to be high in New Zealand's manuka honey derived from the *Leptospernum* species (Lusby *et al.*, 2005).

The *in vitro* antibacterial properties of different types of honey have previously been reported (Willix *et al.*, 1992; Cooper and Molan, 1999; Cooper *et al.*, 1999; Cooper *et al.*, 2002; Wilkinson and Cavanagh, 2005). The antibacterial property is thought to be due to the presence of hydrogen peroxide which is released by the action of peroxidase, an enzyme added by the bees to the collected nectar (Molan, 1992b). Other than the major-wound infecting bacteria, honey has also been shown to have significant antibacterial activity against resistant gram-positive cocci such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Cooper *et al.*, 2002). In addition, honey forms a physical barrier on the wound surface due to its high viscosity which prevents bacterial penetration and colonisation attributed by the low honey (pH 3.6) (Efem, 1988). It also provides a non-adherent interface between the dressing and the wound bed which generates a moist healing environment, thus preventing newly-formed tissue from tearing when the dressing is removed (Wijesinghe *et al.*, 2009). In addition, honey has been reported to have deodorising properties (Dunford *et al.*, 2000) and have been reported to reduce the malodour from wounds infected with anaerobes.

A group of researcher in Malaysia performed an *in vitro* experiment on antibacterial activities of five different types of Malaysian honey (*Tualang, Hutan, Gelang, Pucuk Daun* and *Ee Feng Gu)* and found significant variation in the composition of the honeys. For example, *Tualang, Pucuk Daun* and *Ee Feng Gu* honey showed significant antibacterial activities against *S. typhi, S. aureus, S. Sonnie* and *E. coli in vitro* (Tumin *et al.*, 2005). Tualang honey was reported to be more effective than manuka honey against some gram-negative bacterial strains in burn wounds management (Norizah *et al.*, 2004) which may be attributed to the higher content of

phenolics, flavanoids and HMF. Moreover, the bactericidal effect of the acidic fraction of tualang honey is greater against some bacterial strains than the non-extracted or non-fractionated fraction (KirnpalKaur *et al.*, 2011). In addition, tualang honey reduces the growth of wounds infected with *Pseudomonas aeruginosa*, *Acinetobacter baumanii* or *Klebsiella pneumonia* (Nasir *et al.*, 2010) which are common causes of hospital infections.

1.2.3.2 Wound healing properties

Honey dressings are a traditional therapy for burns and wounds and has a number of characteristics that could potentially relieve healing in the treatment of burns (Suguna *et al.*, 1993; Subrahmanyam, 1998; Dunford *et al.*, 2000; Wijesinghe *et al.*, 2009; Hadagali and Chua, 2014; Vallianou *et al.*, 2014). It is the oldest medication for treating wounds, dating back to the sixth century AD (Golder, 2003; Khoo *et al.*, 2010). The ancient Egyptians applied honey in a grease-honey-lint dressing to act on infected wounds. However, the traditional cure was stopped in the 1940s, before bacteria were discovered to be the reason of infection followed by the discovery of antibiotics. It has recently been rediscovered by the medical profession, particularly where conventional modern therapeutic agents fail and with the trend increasing prevalence of antibiotic-resistant wounds (Khoo *et al.*, 2010). In the midst of the increasing number of antibiotic resistant bacteria, honey is gaining a new attention as an alternative treatment. Unprocessed, undiluted honey has been revealed to speed healing for first and second degree burns (Subrahmanyam, 1991; Wijesinghe *et al.*, 2009). Available evidence confers a greater benefit of honey when compared with alternative dressing treatments for superficial or partial thickness burns (Molan, 2002; Wijesinghe *et al.*, 2009) (Table 1.1).

Usually, honey helps in wound healing by narrowing the oedema, inflammation or irritation and exudation that commonly occur in all types of wounds. Honey stimulates the growth of epithelial cells and fibroblasts (Tonks *et al.*, 2003; Visavadia *et al.*, 2008). It was reported in previous studies that manuka honey can cure humid burns and several other types of wounds (Molan *et al.*, 1988; Visavadia *et al.*, 2008) (Table 1.2). Malaysian tualang honey was reported to show wound healing properties in several studies (Nur Azida *et al.*, 2008; Khoo *et al.*, 2010; Nasir *et al.*, 2010).

Studies indicated that in full-thickness burn wounds treated with tualang honey and conventional hydrofibre silver-treated wounds, the wounds treated with tualang honey yielded a reduction (by 32.26%) in wound size (Khoo *et al.*, 2010). Moreover, it was also noticed that patients prefer tualang honey hydrogel dressings than conventional dressings because they claimed the treatment to be soothing and gave minimal pain while providing a pleasant odour (Imran *et al.*, 2011). In another research, both tualang and manuka honeys have been reported to be effective in the treatment of diabetic foot (Imran *et al.*, 2011).

Table 1.1: Antibacterial activity of honey against life-threatening infectious bacteria to human

Bacterial strain	Clinical importance	References
S. aureus	Nosocomial and community acquired infections	(Taormina *et al.*, 2001; Chauhan *et al.*, 2010; Sherlock *et al.*, 2010)
E. coli	Diarrhoea, urinary tract infection, wound infections and septicaemia	(Sherlock *et al.*, 2010)
V. cholerae	Cholera	(Molan, 1992b)
S. marcescens	Wound infections and septicaemia	(Molan, 1992b)
S. maltophilia	Blood stream infection, pneumonia, urinary tract infection and nosocomial infection	(Tan *et al.*, 2009)
A. baumannii	Opportunistic pathogen infects immune-compromised individuals through open wounds, catheters and breathing tubes	(Tan *et al.*, 2009)
P. aeruginosa	Diabetic foot ulcer, wound infection and urinary infections	(Mullai and Menon, 2007; Chauhan *et al.*, 2010; Sherlock *et al.*, 2010)
H. pylori	Peptic ulcer, chronic gastritis and gastric malignancies	(Ndip *et al.*, 2008)
A. schubertii *H. paraphrohaemlyticus* *M. luteus* *C. cellulans* *L. anguillarum* *A. baumannii*	Burn-wound infection	(Hassanein *et al.*, 2010)
M. tuberculosis	Tuberculosis	(Asadi-Pooya *et al.*, 2002)
S. enteric (serovar Typhi)	Enteric fever	(Molan, 1992b; Chauhan *et al.*, 2010)

Table 1.2: Studies reported on wound healing properties of honeys

Burn Type	Honey	Control	References
Superficial and deep partial thickness burns	Undiluted, applied once daily and covered with sterile gauze	Silver sulphadiazine (1%) applied once daily	(Mashhood *et al.*, 2006)
Superficial burns total body surface area (TBSA) (<40%)	Unprocessed, undiluted apply (16-30 mL) on alternate days. Cover in sterile gauze.	Silver sulphadiazine impregnated gauze to be replaced daily	(Subrahmanyam, 1998)
Partial thickness burns TBSA (<40%)	Unprocessed, undiluted apply (15-30 mL) on alternate days. Cover in sterile gauze.	Autoclaved potato peel bandages to be replaced every 2 days	(Subrahmanyam, 1996)
Partial thickness burns TBSA (<40%)	Honey impregnated gauze, covered with absorbent dressing to be inspected every 2 days	Amniotic membrane obtained in a fresh condition. To change on day 8 then every two days	(Subrahmanyam, 1994)
Partial thickness burns TBSA (<40%)	Honey impregnated gauze to be changed every 2 days	Bio-occlusive, moisture-permeable, polyurethane dressing (OpSite). To be inspected on day 8	(Subrahmanyam, 1993)
Superficial burns TBSA (<40%)	Unprocessed, undiluted apply (15–30 mL) daily. Cover in sterile gauze.	Silver sulphadiazine impregnated gauze to be replaced daily	(Subrahmanyam, 1991)
Superficial burns TBSA (<15%) (children)	Details not given	Silver sulphadiazine (details not given)	(Bangroo *et al.*, 2005)
TBSA (<40%)	Unprocessed, undiluted apply in quantities of 15–30 mL	Silver Sulphadiazine impregnated gauze to be replaced alternate days	(Subrahmanyam *et al.*, 2001)

TBSA: total body surface area. Source: Wijesinghe *et al.,* (2009).

1.2.3.3 Antioxidant activity

The phrase “oxidative stress” defines the lack of balance between the production of free radicals and the antioxidant protective activity. It is believed that the protection against oxidation can help prevent some chronic diseases (Ames *et al.*, 1993). The oxidative alteration of the lipoproteins is considered to be a vital factor for the pathogenesis of arteriosclerosis (Parthasarathy *et al.*, 1992). Chronic or degenerative diseases are more vulnerable to oxidative stress and honey has been reported to have antioxidant activities which may suppress oxidative stress (Mohamed *et al.*, 2010). Honey contains several compounds including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins which are responsible for its significant antioxidant activities (Frankel *et al.*, 1998; Nagai *et al.*, 2001; Al-Mamary *et al.*, 2002; Fahey and Stephenson, 2002; Gheldof and Engeseth, 2002; Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Inoue *et al.*, 2005; Blasa *et al.*, 2006; Pérez *et al.*, 2007).

It is noteworthy that the botanical origin of honey has the utmost influence on its antioxidant activity, while processing, handling and storage affects its antioxidant potential only to a minor extent (Chowdhury, 1999; Beretta *et al.*, 2005; Arráez-Román *et al.*, 2006; Castro-Vázquez *et al.*, 2009; Sereia *et al.*, 2011; Vallianou *et al.*, 2014). In addition, it has been revealed in several studies that the antioxidant activity of honey strongly correlates with the content of the total phenolics (Frankel *et al.*, 1998; Lee *et al.*, 1998; Rashed and Soltan, 2004; Blasa *et al.*, 2006; Lachman *et al.*, 2007; Khalil *et al.*, 2011a; Khalil *et al.*, 2011b; Moniruzzaman *et al.*, 2014) making the measurement of the total phenolic content (TPC) an important step in this study.

In addition to the phenolic compounds, a strong correlation has been observed between antioxidant activity and the colour of honey. Many researchers have reported that dark honey has higher total phenolic content and consequently higher antioxidant capacities (Tewari and Irudayaraj, 2004; Islam *et al.*, 2012; Khalil *et al.*, 2012). It was also reported that the antioxidant activity is found in both the ether and the water fractions of honey, indicating that the phenolics and flavonoids contents in honey may vary depending on the extraction solvent used which can be made available to various compartments of the human body where they play different physiological effects (Blasa *et al.*, 2006; Yaghoobi *et al.*, 2008).

(a) Antioxidant properties of honey from in vitro studies

The *in vitro* antioxidant properties of natural or synthetic substances including honey are usually measured in the form of antiradical activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, oxygen radical absorbance capacity assay (ORAC) and ferric reducing antioxidant power assay (FRAP) (Gheldof and Engeseth, 2002; Hussein *et al.*, 2011). The antioxidant properties of different varieties of honeys originating from various countries and geographical regions in various reports were measured by applying these tests. It was reported that some Bangladeshi honeys showed higher DPPH scavenging property with greatest FRAP values (Islam *et al.*, 2012; Moniruzzaman *et al.*, 2014) while Turkish red pine honey produced by *Marchalina hellenica* showed higher DPPH scavenging activity which is indicative of its antiradical activities (Akbulut *et al.*, 2009). Some Algerian and Saudi Arabian honey samples were also confirmed to exhibit antioxidant activities (Al-Hindi *et al.*, 2011; Khalil *et al.*, 2012). Similar antioxidant properties were also reported for Australian honey produced by *Trigona carbonariawas* (Oddo *et al.*,

2008) and Peruvian honey (Rodriguez-Malaver *et al.*, 2009) and also reported to have high antioxidant properties.

Malaysian tualang honey produced by *Apis dorsata* has been shown to exhibit good antioxidant and antiradical activities (Mohamed *et al.*, 2010; Khalil *et al.*, 2011b; Kishore *et al.*, 2011). In several studies, antioxidant activities have also been shown by honeys originating from different countries including Indian (Saxena *et al.*, 2010), Portuguese (Estevinho *et al.*, 2008), Romanian (Cimpoiu *et al.*, 2013), Venezuelan (Vit *et al.*, 2009), American buckwheat (Van den Berg *et al.*, 2008), Cuban (Alvarez-Suarez *et al.*, 2010), Croatian oak honeydew (Jerković and Marijanović, 2010), Ecuadorian (Guerrini *et al.*, 2009) and Spanish honeys (Pérez *et al.*, 2007). Moreover, several studies conducted recently suggested that gamma irradiation may increase the antioxidant capacities and total phenolic contents in honey (Hussein *et al.*, 2011; Khalil *et al.*, 2012). On the whole, the data on the *in vitro* antioxidant activities of honey indicate that honey is not only an antioxidant itself but is also a rich source of different antioxidants compounds which may act synergistically together.

(b) Antioxidant properties of honey from *in vivo* studies

It was revealed that honey, like other antioxidant agents, protects against damage or injury. This protective effect of honey is partially intervened through improvement of oxidative stress in tissues such as gastro intestinal tract (GIT), liver, kidney, pancreas, eye, plasma, red blood cells and reproductive organs (Gharzouli *et al.*, 2002; Al-Waili *et al.*, 2006; Kassim *et al.*, 2010; Zaid *et al.*, 2010; Mohamed *et al.*,

2012). For that reason, the *in vivo* antioxidant effect of honey will be described with regard to its ability to improve oxidative stress in various cells, tissues, organs or body fluids.

The finding from several studies suggests that honey exerts gastroprotective effect in rodents administered with indomethacin, ethanol, aspirin or ammonia (Ali *et al.*, 1990; Gharzouli *et al.*, 2001; Gharzouli *et al.*, 2002; Ali, 2003). Although the sugars present in honey mainly fructose, glucose, sucrose and maltose may have a role in its gastroprotective effect (Gharzouli *et al.*, 2001; Gharzouli *et al.*, 2002), there is a chance that the antioxidant effect of honey may also contribute to its gastroprotective effect. This statement is based on the finding which indicates that increased mucosal lipid peroxide and reduced glutathione (GSH) levels may intensify gastric haemorrhagic ulcer in diabetic rats (Hung, 2005). Further evidence to support the role of antioxidant present in honey as gastroprotective is provided by Kim, (2009). Several different studies suggested that the liver is susceptible to oxidative stress and damage with the beneficial effects of antioxidants on hepatic oxidative stress reported (Dias *et al.*, 2005; Gumieniczek, 2005). In rats with obstructive jaundice which is generally associated with increased hepatic reactive oxygen substance (ROS) formation, oxidative stress and inflammation (Liu *et al.*, 2001; Celebi *et al.*, 2004), it was reported that honey supplementation significantly decreased the levels of malondialdehyde (MDA) and increased GSH content in the liver (Kılıcoglu *et al.*, 2008).