UNIVERSITI PUTRA MALAYSIA

IMPACT OF SUGAR AND ACID ADULTERATION ON SAFETY OF HONEY PRODUCED BY Apis mellifera LINNAEUS AND Heterotrigona itama COCKERELL BEES

RAFIEH FAKHLAEI

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By

RAFIEH FAKHLAEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Doctor of Philosophy

June 2021

DEDICATION

Specially dedicated to,

My parents: the reason of what I become today,
my sister for her moral support and encouragement,
and my life partner for his understanding, tolerance and moral support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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June 2021

Chairperson: Professor Jinap Selamat, PhD
Institute: Tropical Agriculture and Food Security

Honey is the natural sweet substance produced by honeybees from the nectar of plants or excretions of plant-sucking insects on the living parts of plants. Honey, has been used for anti-ageing, enhancing libido and immune system, treatment of bronchial phlegm, and relieving sore throat, cough, and cold. Honey possesses various pharmacological properties and health benefits such as anti-inflammatory, antioxidant, anti-cancer activities. Thus, honey is prone to be adulterated through inappropriate labelling and fake mixing with cheap and low-quality honey, sugars, and other substances. Consumption of adulterated honey may cause several health modifications such as weight gain, diabetes, liver, and kidney dysfunction. A standard protocol to develop honey toxicity in zebrafish is still uncertain due to unpredictable factors. So, in this study, an optimized protocol was developed to investigate honey toxicity and metabolite fingerprinting in zebrafish, embryo and adult. Therefore, the aims of this study were: 1) to determine the lethal concentration (LC_{50}) of adulterated honey using zebrafish embryo, 2) to elucidate toxicology of selected adulterated honey based on lethal dose (LD_{50}) using adult zebrafish, 3) to determine the effects of adulterated honey on histological changes of zebrafish and, 4) to screen the metabolites profile of adulterated honey by using zebrafish blood serum. Hence, two types of honey were collected from the acacia environment (*Heterotrigona itama* and *Apis mellifera).* Pure *H.itama* was adulterated by different types of sugar such as light corn sugar, cane sugar, inverted sugar, and palm sugar (in the proportion of 1-3% (w/w) from the total volume). On the other hand, acid adulterants such as acetic acid, citric acid, and tamarind were added to *A.mellifera* honey in the proportion of 3, 5, and 7% (w/w). The LC_{50} was determined by the toxicological assessment of honey samples on zebrafish embryos in different exposure concentrations in 24, 48, 72, and 96 hours' post-fertilization (hpf). Pure *A.mellifera* and *H.itama* honey represent the LC_{50} of 31.10±1.63 (mg/ml) and 34.40±1.84 (mg/ml) at 96 hpf, respectively. Acetic acid has the lowest LC_{50} (4.98±0.06 mg/ml) among acid adulterants while inverted sugar represents the lowest LC_{50} (5.03±0.92 mg/ml) among sugar adulterants. The highest concentration (7% for acids and 3% for sugar) of adulterants were used to study the toxicology of adulterated honey using adult zebrafish in terms of

acute, prolong-acute, and sub-acute test. The results of the LD_{50} from the sub-acute toxicity test of pure *A.mellifera* and *H.itama* honey were 2.18±0.45 (mg/ml) and 2.33±0.04 (mg/ml), respectively. The histological studies of internal organs show a lesion in the liver, kidney, and spleen of adulterated treated-honey groups compared to the control group that can be extrapolated to the human tissue alteration. Furthermore, the LC-MS/MS method was used for metabolite profiling from the zebrafish's blood serum which were force-fed by pure and adulterated *A.mellifera* and *H.itama* honey. Chemometrics analysis was performed by correlating the metabolites detected with toxicity of pure and adulterated honey samples using orthogonal partial least square discriminant analysis (OPLS-DA) model. These results revealed six endogenous metabolites in both pure and adulterated honey treated group as follow: (1) Xanthotoxol, (2) S-Cysteinosuccinic acid, (3) 2,3-Diphosphoglyceric acid, (4) Cysteinyl-Tyrosine, (5) 16-Oxoandrostenediol, and (6) 3,5-Dicaffeoyl-4-succinoylquinic acid.

The zebrafish toxicity test could be a standard method for assessing the potential toxicity of honey toxicology. According to this study, all studied adulterants have health disadvantages toward human health based on their LC_{50}, LD_{50} value and internal organ toxicology. According to a significant ($p \leq 0.05$) increase of mortality rate (%) of zebrafish- embryo and adult- in both sugar and acid adulterated honey, it proved that food additives, may not be beneficial toward human health all the time. The kidney, liver and spleen are the main organs that fail due to the consumption of adulterated honey by *in vivo* histological examination. This study also successfully identified endogenous metabolites that were responsible for the toxic impact of adulterated honey using LC-MS/MS based metabolomics integrated with chemometrics analysis. Considering all approaches, these results might be a promising candidate for early diagnostic biomarkers that can prevent the developing of metabolic diseases such as diabetes (type 1 and 2). The information gained from this research will permit an evaluation of the potential risk associated with the consumption of adulterated as compared to pure honey.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN GULA DAN PEMALSUAN ASID TERHADAP KESELAMATAN MADU YANG DIHASILKAN OLEH LEBAH *Apis mellifera* LINNAEUS DAN *Heterotrigona itama* COCKERELL

Oleh

RAFIEH FAKHLAEI

Jun 2021

Pengerusi: Professor Jinap Selamat, PhD
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Madu adalah bahan pemanis semula jadi yang dihasilkan oleh lebah madu daripada rembesan nektar tumbuhan atau rembesan serangga penghisap tumbuhan, melalui aktiviti enzim, regurgitasi, dan penyejatan air. Madu, telah digunakan untuk anti-penuaan, meningkatkan libido dan sistem imunisasi, merawat kahak di dalam bronkus, dan melegakan sakit tekak, batuk, dan selsema. Madu mempunyai pelbagai khasiat farmakologi dan manfaat kesihatan seperti anti-radang, antioksidan, dan anti-barah. Oleh itu, madu cenderung dipalsukan melalui pelabelan yang tidak sahih dan pemalsuan melalui pencampuran dengan madu yang murah dan berkualiti rendah, gula, dan bahan lain. Pengambilan madu yang telah dipalsukan boleh menyebabkan beberapa masalah kesihatan seperti kenaikan berat badan, diabetes, komplikasi hati, dan disfungsi buah pinggang. Protokol standard untuk membangunkan ketoksikan madu di dalam *zebrafish* masih belum dapat dipastikan kerana faktor yang tidak dapat diramalkan. Oleh itu, dalam kajian ini, protokol optima telah dibangunkan untuk menyiasat ketoksikan madu dan cap jari metabolit di dalam zebrafish, embrio dan induk. Oleh itu, tujuan kajian ini adalah: 1) untuk menentukan kepekatan mematikan (LC_{50}) madu yang telah dipalsukan dengan menggunakan embrio zebrafish, 2) untuk menjelaskan toksikologi madu yang telah dipalsukan berdasarkan dos yang mematikan (LD_{50}) menggunakan induk zebrafish dewasa, 3) untuk menentukan kesan madu yang telah dipalsukan pada perubahan-perubahan histologi zebrafish dan, 4) untuk menyaring profil metabolit-metabolit madu yang telah dipalsukan dengan menggunakan serum darah zebrafish. Oleh itu, dua jenis madu dikumpulkan dari persekitaran akasia (*Heterotrigona itama* dan *Apis mellifera*). *H.itama* tulen telah dipalsukan melalui pencampuran pelbagai jenis gula seperti gula jagung ringan, gula tebu, gula sonsang, dan gula aren (dalam perkadaran 1-3% (b/b) daripada jumlah keseluruhan). Selain itu, bahan pemalsuan berasaskan asid seperti asid asetik, asid sitrik, dan asam jawa ditambahkan pada madu *A.mellifera* dalam kadar 3, 5, dan 7% (b/b). LC_{50} ditentukan melalui penilaian toksikologi sampel madu terhadap embrio *zebrafish* yang didedahkan dalam kepekatan madu yang berbeza dalam 24, 48, 72, dan 96 jam selepas persenyawaan (hpf). Madu *A.mellifera* dan *H.itama* yang tulen masing-masing merekodkan nilai LC_{50} pada 31.10 ± 1.63 (mg/ml) dan 34.40 ± 1.84 (mg/ml) bagi 96 hpf. Asid asetik mempunyai nilai LC_{50} terendah (4.98 ± 0.06 mg/ml) di

antara bahan pemalsuan berasaskan asid manakala gula sonsang merekodkan nilai LC_{50} terendah (5.03 ± 0.92 mg / ml) berbanding gula tambahan yang lain. Kepekatan tertinggi untuk setiap bahan pemalsuan (7% untuk asid dan 3% untuk gula) digunakan untuk mengkaji toksikologi madu yang yang telah dipalsukan menggunakan induk *zebrafish* dewasa melalui ujian akut, akut yang berpanjangan, dan sub-akut. Keputusan yang diperoleh daripada ujian ketoksikan sub-akut LD_{50} untuk madu *A.mellifera* dan *H.itama* tulen adalah masing-masing 2.18 ± 0.45 (mg/ml) dan 2.33 ± 0.04 (mg/ml). Kajian histologi organ dalaman menunjukkan luka pada hati, ginjal, dan limpa bagi kumpulan *zebrafish* yang telah dirawat dengan madu yang telah dipalsukan berbanding kumpulan kawalan yang mana dapat diekstrapolasi terhadap perubahan tisu manusia. Selanjutnya, kaedah LC-MS/MS digunakan untuk mendapatkan profil metabolit di dalam serum darah zebrafish yang telah diberi makan madu *A.mellifera* dan *H.itama* tulen dan yang telah dipalsukan secara paksaan. Hasil keputusan menunjukkan enam metabolit endogen dalam kumpulan yang dirawat dengan madu tulen dan madu yang telah dipalsukan seperti berikut: (1) Xanthotoxol, (2) asid S-Cysteinosuccinic, (3) asid 2,3-Diphosphoglyceric, (4) Cysteinyl-Tyrosine, (5) 16-Oxoandrostenediol, dan (6) asid 3,5-Dicaffeoyl-4-succinoylquinic.

Ujian ketoksikan zebrafish boleh dijadikan kaedah standard untuk penilaian potensi ketoksikan toksikologi madu. Mengikut kajian ini, semua bahan pemalsuan yang telah dikaji mempunyai kelemahan terhadap kesihatan manusia berdasarkan nilai LC_{50}, LD_{50} dan toksikologi organ dalaman. Mengikut pertambahan kadar kematian (%) embrio zebrafish dan induk yang signifikan ($p \leq 0.05$) di dalam madu yang dipalsukan dengan gula dan asid, ianya membuktikan aditif makanan, berkemungkinan tidak memberi kelebihan terhadap kesihatan manusia sepanjang masa. Buah pinggang, hati dan limpa adalah organ utama yang gagal berfungsi disebabkan oleh pengambilan madu yang telah dipalsukan mengikut pemeriksaan histologi secara *in vivo*. Kajian ini juga berjaya mengenalpasti metabolit endogen yang bertanggungjawab kepada impak toksik madu yang telah dipalsukan menggunakan kaedah metabolomik berasaskan LC-MS/MS yang disatukan dengan analisis kimometrik. Mempertimbangkan semua pendekatan, keputusan ini berkemungkinan menjadi calon yang sesuai untuk diagnostik biomarker secara awal yang dapat mencegah berlakunya penyakit metabolik seperti diabetes (jenis 1 dan 2). Maklumat yang diperoleh dalam kajian ini akan membenarkan evaluasi risiko berpotensi yang berkait dengan pengambilan madu yang telah dipalsukan berbanding dengan madu tulen.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 09 September 2021

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I hereby confirm that:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- The research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
°F	Degree Fahrenheit
µg	Microgram
µL	Microliter
µM	Micromolar
µm	Micrometre
2,3 DPG	2,3-diphosphoglyceric acid
5-HMF	%-Hydroxymethylfurfural
ADIOL	Androstenediol
AKI	Acute kidney injury
ANOVA	Analysis of variance
bpm	Heartbeats per minutes
BW	Bodyweight
Ca	Calcium
CCD	Colony collapse disorder
CKD	Chronic kidney diseases
cm	Centimetre
Cr	Chromium
CRDS	Cavity ring-down spectroscopy
Cu	Copper
DART	Direct analysis in real-time
EU	European union
Fe	Iron
FRAP	Ferric reducing antioxidant power
g	Gram
GC-MS	Gas chromatography-mass spectrometry
GS	Glucose syrup
h	Hour
H&E	Hematoxylin and eosin
HFCS	High-fructose corn syrup

HFD	High fructose diet
HPAEC-PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
hpf	Hours post-fertilization
IC_{50}	Free radical scavenging activity
IHC	International honey commission
IRMS	Isotope ratio mass spectrometry
IS	Inverted syrup
K	Potassium
kg	Kilogram
KPD	Koperasi pembanguna desa
LC_{50}	Median lethal concentration
LC-ECD	Liquid chromatography-electrochemical detection
LC-MS	Liquid chromatography-mass spectrometry
LC-QTOF-MS	Liquid chromatography-quadrupole-time-of-flight-mass spectrometry
LD_{50}	Median lethal dose
LOD	Limit of detection
LDL	Low-density lipoprotein
LOQ	Limit of quantification
M	Molar
m/z	Mass to charge ratio
MARDI	Malaysian agricultural research and development institute
MAVP	Malaysian Association of Veterinary Pathology
Mg	Magnesium
mm	Millimetre
Mn	Manganese
mS	Millisecond
MS-MS	Liquid chromatography-tandem mass spectrometry
NA	Not available
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate
ND	Not detected

NMR	Nuclear magnetic resonance
OPLS	Orthogonal partial least square
OPLS-DA	Orthogonal projection to latent structure discriminant analysis
P	Phosphorus
PG	Prostaglandin
PLS-LDA	Partial least square-linear discrimination analysis
PLSR	Partial least squares regression
PTR-MS	Proton transfer reaction mass spectroscopy
QAE	Quarcetin acid equivalent
RM	Ringgit Malaysia
ROS	Reactive oxygen species
RSA	Radical scavenging activity
SCIRA	Stable carbon isotope ratio analysis
SD	Standard deviation
Se	Selenium
TFC	Total flavonoids content
TPC	Total phenolic contents
TSE	Tamarind seed extract
UHPLC/Q-TOF-MS	Ultrahigh-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry
UPM	Universiti putra malaysia
USD	United States dollar
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
Z	Zink

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Honey is flower nectar that has been collected, stored and dehydrated by honey bees in order to improve the nutritional properties and become consumable by a human (Akhmazillah, Farid, & Silva, 2013). Honey can be consumed not only as a sweetener, but also have a therapeutic effect on human health.

Meanwhile, honey is well known high nutritional value food, it can also be toxic naturally by transferring plant toxin such as pyrrolizidine alkaloids or by adulterants which added into the pure honey by mankind for economic profits (Y.-Z. Zhang et al., 2019). Furthermore, honey is subjected to adulteration due to its limited availability. Adulterants are referring to any substances, which are added to the original and pure product. Honey can be adulterated directly (addition of adulterants) or indirectly (bee-feeding). Cheap sweeteners such as cane sugar, corn syrup, maltose, and high fructose syrup are being used commonly to adulterate the honey (Puscas, Hosu, & Cimpoiu, 2013).

Since the stingless bee (*Heterotrigona itama*) honey has a higher price in the market, honeybee (*Apis mellifera*) honey has been adulterated with weak acid in order to manipulate its sour taste and sold as *H. itama*. The increasing adulteration trend is worrisome as most of the honey adulteration with a high concentration of sugars can lead to serious health risks, especially in diabetic patients who unintentionally consume these products (Salvador et al., 2019). Therefore, investigating the toxicity of pure and adulterated honey must be considered in order to prevent health issues among consumers.

The animal model study is a promising step in food safety researches. This animal model should share similarity to the human. Zebrafish (*Danio rerio*) is an animal model which has become an applicable tool especially in the toxicity filed due to high transparency, early life stage development and cost reduction. In addition, the zebrafish has a 70% physiological similarity to the human body rather than other mammalians (Dayal et al., 2016). While the early life stage of zebrafish (embryonic stage) can provide primary information, acute and chronic test on adult zebrafish allow the observation of any sign regarding toxicity. To this aim, histological analysis should be employed to observe the more in-depth microscopic scale of cellular composition and tissue structure. Changes in various zebrafish's organs such as; liver, kidney and spleen would be used as a key biomarker in toxicology (dos Santos et al., 2018).

Metabolomics analysis in food authentication is a major concern in recent decades in order to assess the safety and quality of food. While various techniques have been applied, new developed analytical approaches such as mass spectroscopy (MS) and advanced statistical and chemometric tools have gained interest in metabolomics studies (Cubero-Leon, Penalver, & Maquet, 2014).

1.2 Significance of The Study

The tropical climate of Malaysia provides suitable habitat for stingless bee (*H. itama)* farming that makes it beneficial to Malaysian bee farmers. *H. itama* honey has been reported to possess stronger antioxidant activity, higher phenolic and nutrient content compared to other local honey species. In addition, it has the potential to be used as a therapeutic reagent owing to its anti-bacterial, anti-fungal, anti-cancer and anti-diabetic properties. Unfortunately, consumers can distinguish *H. itama* honey only by its sour taste. Thus, some unfaithful farmers adulterate cheaper honey such as *A. mellifera* honey with adulterants to manipulate the *H. itama* honey taste. The *A. mellifera* honey is sweeter, cheaper and contains fewer nutrients compare to *H. itama* honey. On the other hand, some other farmers add cheap sugar adulterants to *H. itama* honey in order to increase their profits and make it more acceptable by consumer's taste. Regrettably, using those adulterants has harmful side effects on human health that have to be studied.

In Malaysia, the production of honey by honeybees, such as *A. mellifera*, has not been profoundly successful due to the *Varroa dectructor* mite outbreak of 1996. Therefore, those farmers who adulterate the *A. mellifera* honey, have to import it from other countries and adulterate it with low-quality adulterants to manipulate the taste and sell it as *H. itama* honey, which has a higher market price. Pure *H. itama* honey has a higher price than pure *A. melifera* honey, RM 120 and RM 80 per kg, respectively. On the other hand, low-quality sweetener adulterants were added to pure *H. itama* honey in order to increase the volume, which reducing the price while selling as pure *H. itama* honey in the market. As a result of this fraud, wholesalers gain much of the profits. Meanwhile, consumers who are seeking low price and economical products, attract by cheap and low-quality honey. Adulteration has an adverse impact on consumer's health which may lead to increase blood sugar, followed by the release of insulin hormone and type II diabetes, abdominal weight gain and obesity, raise in the level of blood lipid and high blood pressure (Soares, Amaral, Oliveira, and Mafra (2017). Thus, it is essential to investigate the health impact of honey consumption and promote public awareness regarding honey adulteration and its harmful effects on consumer health.

In this study, four different sugar adulterants and three different acid adulterants that have been used commonly in honey adulteration have been investigated. While similar studies focused on detection techniques, in here the impact of adulterated honey consumption is the focus point of the research. Moreover, zebrafish animal model is the other key novel scope of this study. By using the zebrafish animal model, the LC_{50}, LD_{50}, hatching rate, mortality rate, and histological analysis have been approached. Finally, by using the LC MS/MS analysis, the synthesized metabolites in related to the consumption of the adulterated honey have been discovered and investigated. Therefore, a clear and fundamental platform for better understanding and studying the honey adulterants has been developed.

1.3 Scope of Study

There are four main stages in this work: Adulteration, Toxicity test (embryo and adult), Histopathology and Metabolite analysis which presented in Table 1.1.

Table 1.1 : Overview of research scopes

Stage	Research Scope
Stage 1: Zebrafish embryo toxicity test	• Adulteration of pure *H. itama* and *A. mellifera* honey with different concentration of sugars and acids • Median lethal concentration (LC_{50}) analysis • Hatching rate determination • Heart rate determination
Stage 2: Zebrafish adult toxicity test	• Force feed adult zebrafish • Median lethal dose (LD_{50}) determination • Acute, prolong acute and sub-acute toxicity test
Stage 3: Histopathology	• Histopathological analysis of liver, kidneys and spleen of zebrafish
Stage 4: Metabolomics shifting	• Cardiac blood withdraw • Serum collection • LC/MS-MS analysis

1.4 Objectives

The specific objectives of this study are as follow:

1. To determine the LC_{50} of pure and adulterated *H. itama* and *A. mellifera* honey in zebrafish (*Danio rerio*) embryos
2. To determine the LD_{50} of pure and adulterated *H. itama* and *A. mellifera* honey by force-feeding of adult zebrafish (*Danio rerio*)
3. To examine the histological examination of adult zebrafish after treatment with pure and adulterated *H. itama* and *A. mellifera* honey
4. To identify the LC-MS/MS based metabolite of pure and adulterated *H. itama* and *A. mellifera* honey in the serum of adult zebrafish.

1.5 Organization of Thesis

This dissertation adopts a University Putra Malaysia style guide to the presentation, logically aimed and systematically rendered to enhance the understanding of this research. The thesis is divided into seven chapters as follow:

Chapter one highlights the background of the study, the significance of the study, which builds the motivation for this project, and the objectives of this research.
Chapters two represent the literature review, which covers the type, regulation and toxicity of honey, histopathology of zebrafish and finally metabolomics. However, no research has been published regarding the *in-vivo* toxicity assessment of pure and adulterated *H. itama* and *A. mellifera* honey.

Chapter three determines the toxicity of pure and adulterated *H. itama* and *A. mellifera* honey using the zebrafish embryos effect on median lethal concentration (LC_{50}), hatching rate and heart rate.

Chapter four evaluates the median lethal dose (LD_{50}) of pure and adulterated *H. itama* and *A. mellifera* honey on adult zebrafish during acute, prolong-acute and sub-acute toxicity test.

Chapter five illustrates the histopathological study of the zebrafish force-feeds by adulterated honey.

Chapter six analyses the LC-MS-based metabolite shifting in the serum of adult zebrafish after treatment with pure and adulterated *H. itama* and *A. mellifera* honey.

Chapter seven demonstrates the comprehensive conclusion along with recommendations for future work.

REFERENCES

A Kosenko, E., Aliev, G., & G Kaminsky, Y. (2016). Relationship between chronic disturbance of 2, 3-diphosphoglycerate metabolism in erythrocytes and Alzheimer disease. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), 15*(1), 113-123.

Adams, J., & Wong, M. S. (1968). A correlation between urinary steroid metabolites and pathways of steroidogenesis in human breast-tumour tissue. *The Lancet, 292*(7579), 1163-1166.

Adenan, M. N. H., Yazan, L. S., Christianus, A., Hashim, N. F. M., Harun, A. R., Halim, N. H. A., . . . Rahim, K. A. (2018). Effects of Kelulut honey from Trigona sp. on zebrafish (Danio rerio) embryo that mimics human embryonic development. *Journal of Cell and Animal Biology, 12*(2), 5-14.

Afroz, R., Tanvir, E., Zheng, W., & Little, P. (2016). Molecular pharmacology of honey. *Clin Exp Pharmacol, 6*(212), 2161-1459.1000.

Agency., E. C. (2010). Evaluation of New Scientific Evidence Concerning the Restrictions Contained in Annex XVII to Regulation (EC) No. 1907/2006 (REACH): Review of New Available Information for Di-'isononyl'Phthalate (DINP).

Ajibola, A. (2013). *Effects of dietary supplementation with pure natural honey on metabolism in growing Sprague-Dawley rats.*

Ajibola, A., Chamunorwa, J. P., & Erlwanger, K. H. (2013). Dietary supplementation with natural honey promotes growth and health of male and female rats compared to cane syrup. *Scientific Research and Essays, 8*(14), 543-553.

Akhmazillah, M., Farid, M., & Silva, F. (2013). High pressure processing (HPP) of honey for the improvement of nutritional value. *Innovative Food Science & Emerging Technologies, 20*, 59-63.

Al-Waili, N., Salom, K., Al-Ghamdi, A., & Ansari, M. J. (2012). Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. *The Scientific World Journal, 2012*.

Alimentarius, C. (2020). General standard for food additives, CODEX STAN 192-1995. Codex Alimentarius Commission, last revision 2018. The Food and Agriculture Organization of the United Nations (FAO), Rome. *The World Health Organization (WHO), Geneva. Available http://www. fao. org/gsfaonline/docs/CXS_192e. pdf. Accessed, 8*.

Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., & Giampieri, F. (2014). The composition and biological activity of honey: a focus on Manuka honey. *Foods, 3*(3), 420-432.

Ariefdjohan, M. W., Martin, B. R., Lachcik, P. J., & Weaver, C. M. (2008). Acute and chronic effects of honey and its carbohydrate constituents on calcium absorption in rats. *Journal of agricultural and food chemistry, 56*(8), 2649-2654.

Aries, E., Burton, J., Carrasco, L., De Rudder, O., & Maquet, A. (2016). Scientific support to the implementation of a coordinated control plan with a view to establishing the prevalence of fraudulent practices in the marketing of honey. *European Commission, Belgium*, 38.

Arroyo-Manzanares, N., García-Nicolás, M., Castell, A., Campillo, N., Viñas, P., López-García, I., & Hernández-Córdoba, M. (2019). Untargeted headspace gas chromatography–Ion mobility spectrometry analysis for detection of adulterated honey. *Talanta, 205*, 120123.

Awashti, S. (2014). Alteration in the Properties of Aloe vera due to Fungal Infection.

Azeredo, L. d. C., Azeredo, M., De Souza, S., & Dutra, V. (2003). Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins. *Food chemistry*.

Bagul, M. B., Sonawane, S. K., & Arya, S. S. (2018). Bioactive characteristics and optimization of tamarind seed protein hydrolysate for antioxidant-rich food formulations. *3 Biotech, 8*(4), 218.

Bakar, N. A., Sata, N. S. A. M., Ramlan, N. F., Ibrahim, W. N. W., Zulkifli, S. Z., Abdullah, C. A. C., . . . Amal, M. N. A. (2017). Evaluation of the neurotoxic effects of chronic embryonic exposure with inorganic mercury on motor and anxiety-like responses in zebrafish (Danio rerio) larvae. *Neurotoxicology and teratology, 59*, 53-61.

Basic histology and histopathology of fish. (2019). (A. Salleh Ed.): UPM Press.

Belanger, S. E., Rawlings, J. M., & Carr, G. J. (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. *Environmental toxicology and chemistry, 32*(8), 1768-1783.

Benchoula, K., Khatib, A., Quzwain, F., Che Mohamad, C. A., Wan Sulaiman, W. M. A., Abdul Wahab, R., . . . Alajmi, M. F. (2019). Optimization of hyperglycemic induction in zebrafish and evaluation of its blood glucose level and metabolite fingerprint treated with Psychotria malayana jack leaf extract. *Molecules, 24*(8), 1506.

Bett, C. K. (2017). Factors Influencing Quality Honey Production. *International Journal of Academic Research in Business and Social Sciences, 7*(11), 281-292.

Bhatti, F., Asad, S., Khan, Q. M., Mobeen, A., Iqbal, M. J., & Asif, M. (2019). Risk assessment of genetically modified sugarcane expressing AVP1 gene. *Food and Chemical Toxicology, 130*, 267-275.

Bhusari, S., Muzaffar, K., & Kumar, P. (2014). Effect of carrier agents on physical and microstructural properties of spray dried tamarind pulp powder. *Powder Technology, 266*, 354-364.

Biemar, F., Argenton, F., Schmidtke, R., Epperlein, S., Peers, B., & Driever, W. (2001). Pancreas development in zebrafish: early dispersed appearance of endocrine hormone expressing cells and their convergence to form the definitive islet. *Developmental biology, 230*(2), 189-203.

Biluca, F. C., Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *Journal of Food Composition and Analysis, 50*, 61-69.

Black, C., Haughey, S. A., Chevallier, O. P., Galvin-King, P., & Elliott, C. T. (2016). A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. *Food chemistry, 210*, 551-557.

Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American College of Nutrition, 27*(6), 677-689.

Bogdanov, S., Lüllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., . . . Piro, R. (1999). Honey quality and international regulatory standards: review by the International Honey Commission. *Bee world, 80*(2), 61-69.

Bonnefont-Rousselot, D. (2002). Glucose and reactive oxygen species. *Current Opinion in Clinical Nutrition & Metabolic Care, 5*(5), 561-568.

Bouacha, M., Ayed, H., & Grara, N. (2018). Honey bee as alternative medicine to treat eleven multidrug-resistant bacteria causing urinary tract infection during pregnancy. *Scientia pharmaceutica, 86*(2), 14.

Bronte, V., & Pittet, M. J. (2013). The spleen in local and systemic regulation of immunity. *Immunity, 39*(5), 806-818. Retrieved from https://www.cell.com/immunity/pdf/S1074-7613(13)00470-6.pdf

Callao, M. P., & Ruisánchez, I. (2018). An overview of multivariate qualitative methods for food fraud detection. *Food Control, 86*, 283-293.

Cavanna, D., Righetti, L., Elliott, C., & Suman, M. (2018). The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach. *Trends in Food Science & Technology, 80*, 223-241.

Chakrabarti, S. K., & Denniel, C. (1996). S-[(1 and 2)-phenyl-2-hydroxyethyl]-cysteine-induced cytotoxicity to rat renal proximal tubules. *Toxicology and applied pharmacology, 137*(2), 285-294.

Chen, H., Yuan, B., Miao, H., Tan, Y., Bai, X., Zhao, Y.-Y., & Wang, Y. (2015). Urine metabolomics reveals new insights into hyperlipidemia and the therapeutic effect of rhubarb. *Analytical Methods, 7*(7), 3113-3123.

Chen, Q., Qi, S., Li, H., Han, X., Ouyang, Q., & Zhao, J. (2014). Determination of rice syrup adulterant concentration in honey using three-dimensional fluorescence spectra and multivariate calibrations. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 131*, 177-182.

Chuttong, B., Chanbang, Y., Sringarm, K., & Burgett, M. (2016). Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South east Asia (Thailand). *Food Chemistry, 192*, 149-155.

Çinar, S. B., Ekşi, A., & Coşkun, İ. (2014). Carbon isotope ratio (13C/12C) of pine honey and detection of HFCS adulteration. *Food chemistry, 157*, 10-13.

Ciulu, M., Solinas, S., Floris, I., Panzanelli, A., Pilo, M. I., Piu, P. C., . . . Sanna, G. (2011). RP-HPLC determination of water-soluble vitamins in honey. *Talanta, 83*(3), 924-929.

Collymore, C., Rasmussen, S., & Tolwani, R. J. (2013). Gavaging adult zebrafish. *JoVE (Journal of Visualized Experiments)*(78), e50691.

Commission, C. A. (2001). Revised codex standard for honey. *Codex Stan*, 12-1981.

Commission, C. A., Commission, J. F. W. C. A., & Programme, J. F. W. F. S. (2001). *Codex alimentarius* (Vol. 9): Bernan Assoc.

Cordella, C., Militao, J. S., Clément, M.-C., Drajnudel, P., & Cabrol-Bass, D. (2005). Detection and quantification of honey adulteration via direct incorporation of sugar syrups or bee-feeding: preliminary study using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and chemometrics. *Analytica Chimica Acta, 531*(2), 239-248.

Cortés, M. E., Vigil, P., & Montenegro, G. (2011). The medicinal value of honey: a review on its benefits to human health, with a special focus on its effects on glycemic regulation. *International Journal of Agriculture and Natural Resources, 38*(2), 303-317.

Cubero-Leon, E., Penalver, R., & Maquet, A. (2014). Review on metabolomics for food authentication. *Food Research International, 60*, 95-107.

Dahme, T., Katus, H. A., & Rottbauer, W. (2009). Fishing for the genetic basis of cardiovascular disease. *Disease models & mechanisms, 2*(1-2), 18-22.

Dayal, N., Thakur, M., Soparkar, A., Doctor, M., Patil, P., & Joshi, D. (2016). Effective method to deliver test substance in adult zebrafish. *Int J Adv Res, 4*(7), 543-551.

de Souza, L. P., Alseekh, S., Naake, T., & Fernie, A. (2019). Mass Spectrometry-Based Untargeted Plant Metabolomics. *Current protocols in plant biology, 4*(4).

Deshpande, S. S. (2002). *Handbook of Food Toxicology*.

Devine, S. M., Cobbs, C., Jennings, M., Bartholomew, A., & Hoffman, R. (2003). Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood, The Journal of the American Society of Hematology, 101*(8), 2999-3001.

dos Santos, I. V. F., de Souza, G. C., Santana, G. R., Duarte, J. L., Fernandes, C. P., Keita, H., . . . Carvalho, H. O. (2018). Histopathology in Zebrafish (Danio rerio) to Evaluate the Toxicity of Medicine: An Anti-Inflammatory Phytomedicine with Janaguba Milk (Himatanthus drasticus Plumel). *Histopathology: An Update*, 39.

Du, B., Wu, L., Xue, X., Chen, L., Li, Y., Zhao, J., & Cao, W. (2015). Rapid screening of multiclass syrup adulterants in honey by ultrahigh-performance liquid chromatography/quadrupole time of flight mass spectrometry. *Journal of Agricultural and Food Chemistry, 63*(29), 6614-6623.

Eames, S. C., Philipson, L. H., Prince, V. E., & Kinkel, M. D. (2010). Blood sugar measurement in zebrafish reveals dynamics of glucose homeostasis. *Zebrafish, 7*(2), 205-213.

Ehrsson, H., Eksborg, S., & Wallin, I. (1978). Metabolism of 8-methoxypsoralen in man: Identification and quantification of 8-hydroxypsoralen. *European Journal of Drug Metabolism and Pharmacokinetics, 3*(2), 125-128.

Esteki, M., Shahsavari, Z., & Simal-Gandara, J. (2019). Food identification by high performance liquid chromatography fingerprinting and mathematical processing. *Food Research International*.

Eteraf-Oskouei, T., & Najafi, M. (2013). Traditional and modern uses of natural honey in human diseases: a review. *Iranian journal of basic medical sciences, 16*(6), 731.

Fakhlaei, R., Selamat, J., Khatib, A., Razis, A. F. A., Sukor, R., Ahmad, S., & Babadi, A. A. (2020). The Toxic Impact of Honey Adulteration: A Review. *Foods, 9*(11), 1538. Retrieved from https://www.mdpi.com/2304-8158/9/11/1538

FAO. (2001). Food and Agriculture Organization of the United Nations. Revised codex standard for honey (No. CODEX STAN 12–1981). Retrieved from www.fao.org/input/download/standards/310/cxs_012e.pdf

FAO. (2014). Compendium of food additive specifications. Retrieved from http://www.fao.org/3/a-i4144e.pdf

Fogo, A. B., Lusco, M. A., Najafian, B., & Alpers, C. E. (2017). AJKD Atlas of Renal Pathology: Osmotic Tubular Injury. *American Journal of Kidney Diseases, 69*(2), e11-e12.

Fu, L.-l., Ding, H., Han, L.-f., Jia, L., Yang, W.-z., Zhang, C.-x., . . . Guo, D.-a. (2019). Simultaneously targeted and untargeted multicomponent characterization of

Erzhi Pill by offline two-dimensional liquid chromatography/quadrupole-Orbitrap mass spectrometry. *Journal of Chromatography A, 1584*, 87-96.

Fuhrman, J. (2018). The hidden dangers of fast and processed food. *American journal of lifestyle medicine, 12*(5), 375-381.

Garcia, J. S., Vaz, B. G., Corilo, Y. E., Ramires, C. F., Saraiva, S. A., Sanvido, G. B., . . . Zacca, J. J. (2013). Whisky analysis by electrospray ionization-Fourier transform mass spectrometry. *Food Research International, 51*(1), 98-106.

García, N. L. (2018). The current situation on the international honey market. *Bee world, 95*(3), 89-94.

Gehlawat, J. (2001). New technology for invert sugar and high fructose syrups from sugarcane.

Ghobadian, M., Nabiuni, M., Parivar, K., Fathi, M., & Pazooki, J. (2017). Histopathological evaluation of zebrafish (Danio rerio) larvae following embryonic exposure to MgO nanoparticles. *Iranian Journal of Fisheries Sciences, 17*(3), 959-969.

Gittes, G. K. (2009). Developmental biology of the pancreas: a comprehensive review. *Developmental biology, 326*(1), 4-35.

Gomes, S., Dias, L. G., Moreira, L. L., Rodrigues, P., & Estevinho, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food and Chemical Toxicology, 48*(2), 544-548.

Guideline, P.-B. T. (2001). OECD guideline for the testing of chemicals. *The Hershberger, 601*, 858.

Guijarro-Díez, M., Nozal, L., Marina, M. L., & Crego, A. L. (2015). Metabolomic fingerprinting of saffron by LC/MS: novel authenticity markers. *Analytical and bioanalytical chemistry, 407*(23), 7197-7213.

Guo, N., Zhao, L., Zhao, Y., Li, Q., Xue, X., Wu, L., . . . Peng, W. (2020). Comparison of the Chemical Composition and Biological Activity of Mature and Immature Honey: An HPLC/QTOF/MS-Based Metabolomic Approach. *Journal of agricultural and food chemistry, 68*(13), 4062-4071.

Gupta, R. K. (2014). Taxonomy and distribution of different honeybee species. In *Beekeeping for poverty alleviation and livelihood security* (pp. 63-103): Springer.

Haendel, M. A., Tilton, F., Bailey, G. S., & Tanguay, R. L. (2004). Developmental toxicity of the dithiocarbamate pesticide sodium metam in zebrafish. *Toxicological Sciences, 81*(2), 390-400.

Haffner, S. M., Valdez, R. A., Hazuda, H. P., Mitchell, B. D., Morales, P. A., & Stern, M. P. (1992). Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes, 41*(6), 715-722.

Haq, M., Gonzalez, N., Mintz, K., Jaja-Chimedza, A., De Jesus, C. L., Lydon, C., . . . Berry, J. P. (2016). Teratogenicity of ochratoxin A and the degradation product, ochratoxin α, in the zebrafish (Danio rerio) embryo model of vertebrate development. *Toxins, 8*(2), 40.

Hara, Y., & Watanabe, N. (2015). Fatigue Alleviation Mechanism of Citric Acid Determined by Gene Expression Analysis in the Mouse Liver. *Food and Nutrition Sciences, 6*(12), 1095.

Hirschmann, H., de Courcy, C., Levy, R. P., & Miller, K. L. (1960). Adrenal precursors of urinary 17-ketosteroids. *Journal of Biological Chemistry, 235*(10), PC48-PC49.

Hivert, M., Perng, W., Watkins, S., Newgard, C., Kenny, L., Kristal, B., . . . Oken, E. (2015). Metabolomics in the developmental origins of obesity and its cardiometabolic consequences. *Journal of developmental origins of health and disease, 6*(2), 65-78.

Honore, P. M., De Bels, D., Preseau, T., Redant, S., & Spapen, H. D. (2018). Citrate: How to get started and what, when, and how to monitor? *Journal of translational internal medicine, 6*(3), 115-127.

Hurkova, K., Rubert, J., Stranska-Zachariasova, M., & Hajslova, J. (2017). Strategies to document adulteration of food supplement based on sea buckthorn oil: A case study. *Food Analytical Methods, 10*(5), 1317-1327.

Iskandar, I., Setiawan, F., Sasongko, L., & Adnyana, I. (2017). Six-month chronic toxicity study of tamarind pulp (Tamarindus indica L.) water extract. *Scientia pharmaceutica, 85*(1), 10.

Iskandar, I., Setiawan, F., Sasongko, L. D., & Adnyana, I. K. (2017). Six-month chronic toxicity study of tamarind pulp (Tamarindus indica L.) water extract. *Scientia pharmaceutica, 85*(1), 10.

Ismail, M. M. (2014). *Competitiveness of beekeeping industry in Malaysia*: Universiti Putra Malaysia Press.

Ismail, M. M., & Ismail, W. I. W. (2018). *Development of stingless beekeeping projects in Malaysia.* Paper presented at the E3S Web of Conferences.

Ito, N., Phillips, S. E., Stevens, C., Ogel, Z. B., McPherson, M. J., Keen, J. N., . . . Knowles, P. F. (1991). Novel thioether bond revealed by a 1.7 Å crystal structure of galactose oxidase. *Nature, 350*(6313), 87-90.

Ivanisevic, J., & Want, E. J. (2019). From Samples to Insights into Metabolism: Uncovering Biologically Relevant Information in LC-HRMS Metabolomics Data. *Metabolites, 9*(12), 308.

Jalil, A. H. (2014). *Beescape for Meliponines: Conservation of Indo-Malayan Stingless Bees*: Partridge Publishing Singapore.

Jensen, T., Abdelmalek, M. F., Sullivan, S., Nadeau, K. J., Green, M., Roncal, C., . . . Kang, D.-H. (2018). Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. *Journal of hepatology, 68*(5), 1063-1075.

Johnson, R. (2014). Food fraud and economically motivated adulteration of food and food ingredients. In: Congressional Research Service Washington DC.

Johnson, R. J., Sanchez-Lozada, L. G., & Nakagawa, T. (2010). The effect of fructose on renal biology and disease. *Journal of the American society of nephrology, 21*(12), 2036-2039.

Jurczyk, A., Roy, N., Bajwa, R., Gut, P., Lipson, K., Yang, C., . . . Phillips, N. (2011). Dynamic glucoregulation and mammalian-like responses to metabolic and developmental disruption in zebrafish. *General and comparative endocrinology, 170*(2), 334-345.

Kalogiouri, N. P., Aalizadeh, R., Dasenaki, M. E., & Thomaidis, N. S. (2020). Application of High Resolution Mass Spectrometric methods coupled with chemometric techniques in olive oil authenticity studies-A review. *Analytica Chimica Acta*.

Kalogiouri, N. P., Alygizakis, N. A., Aalizadeh, R., & Thomaidis, N. S. (2016). Olive oil authenticity studies by target and nontarget LC–QTOF-MS combined with advanced chemometric techniques. *Analytical and bioanalytical chemistry, 408*(28), 7955-7970.

Kayacier, A., & Karaman, S. (2008). Rheological and some physicochemical characteristics of selected Turkish honeys. *Journal of Texture Studies*, *39*(1), 17-27.

Khan, F. R., & Alhewairini, S. S. (2018). Zebrafish (Danio rerio) as a model organism. In *Current Trends in Cancer Management*: IntechOpen.

Khedkar, P. M., Jagdale, D. M., & Dhande, S. R. (2018). Acute toxicity testing of synthesized pyrazoline derivatives in adult zebrafish. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*, *9*(1), 277-281.

Kim, H., Kosinski, P., Kung, C., Dang, L., Chen, Y., Yang, H., . . . Liu, G. (2017). A fit-for-purpose LC–MS/MS method for the simultaneous quantitation of ATP and 2, 3-DPG in human K2EDTA whole blood. *Journal of Chromatography B, 1061*, 89-96.

Kinkel, M. D., Eames, S. C., Philipson, L. H., & Prince, V. E. (2010). Intraperitoneal injection into adult zebrafish. *JoVE (Journal of Visualized Experiments)*(42), e2126.

Kiss, N., & Hamar, P. (2016). Histopathological evaluation of contrast-induced acute kidney injury rodent models. *BioMed Research International, 2016*.

Kondo, T., Kishi, M., Fushimi, T., & Kaga, T. (2009). Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *Journal of agricultural and food chemistry, 57*(13), 5982-5986.

Kovács, R., Bakos, K., Urbányi, B., Kövesi, J., Gazsi, G., Csepeli, A., . . . Horváth, Á. (2016). Acute and sub-chronic toxicity of four cytostatic drugs in zebrafish. *Environmental Science and Pollution Research, 23*(15), 14718-14729.

Küçük, M., Kolaylı, S., Karaoğlu, Ş., Ulusoy, E., Baltacı, C., & Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry, 100*(2), 526-534.

Kuru, P. (2014). Tamarindus indica and its health related effects. *Asian Pacific Journal of Tropical Biomedicine, 4*(9), 676-681.

Lee, K. Y., Jang, G. H., Byun, C. H., Jeun, M., Searson, P. C., & Lee, K. H. (2017). Zebrafish models for functional and toxicological screening of nanoscale drug delivery systems: promoting preclinical applications. *Bioscience reports, 37*(3).

Letertre, M. P., Dervilly, G., & Giraudeau, P. (2020). Combined nuclear magnetic resonance spectroscopy and mass spectrometry approaches for metabolomics. *Analytical Chemistry*.

Li, K., Wu, J.-Q., Jiang, L.-L., Shen, L.-Z., Li, J.-Y., He, Z.-H., . . . He, M.-F. (2017). Developmental toxicity of 2, 4-dichlorophenoxyacetic acid in zebrafish embryos. *Chemosphere, 171*, 40-48.

Li, L., He, M., Xiao, H., Liu, X., Wang, K., & Zhang, Y. (2018). Acetic acid influences BRL-3A cell lipid metabolism via the AMPK signalling pathway. *Cellular Physiology and Biochemistry, 45*(5), 2021-2030.

Li, S., Shan, Y., Zhu, X., Zhang, X., & Ling, G. (2012). Detection of honey adulteration by high fructose corn syrup and maltose syrup using Raman spectroscopy. *Journal of Food Composition and Analysis, 28*(1), 69-74.

Li, S., Zhang, X., Shan, Y., Su, D., Ma, Q., Wen, R., & Li, J. (2017). Qualitative and quantitative detection of honey adulterated with high-fructose corn syrup and maltose syrup by using near-infrared spectroscopy. *Food Chemistry, 218*, 231-236.

Lillicrap, A. (2009). The use of zebrafish embryos as an alternative approach for ecotoxicity testing.

Luis, G., Rubio, C., Gutiérrez, A., Hernández, C., González-Weller, D., Revert, C., . . . Hardisson, A. (2012). Palm tree syrup; nutritional composition of a natural edulcorant. *Nutricion hospitalaria, 27*(2), 548-552.

Macdonald, R. (1977). Red cell 2, 3-diphosphoglycerate and oxygen affinity. *Anaesthesia, 32*(6), 544-553.

Machado De-Melo, A. A., Almeida-Muradian, L. B. d., Sancho, M. T., & Pascual-Maté, A. (2018). Composition and properties of Apis mellifera honey: A review. *Journal of Apicultural Research, 57*(1), 5-37.

Maddison, L. A., & Chen, W. (2017). Modeling pancreatic endocrine cell adaptation and diabetes in the zebrafish. *Frontiers in endocrinology, 8*, 9.

Mahmoudi, R., Ghojoghi, A., & Ghajarbeygi, P. (2016a). Honey safety hazards and public health.

Mahmoudi, R., Ghojoghi, A., & Ghajarbeygi, P. (2016b). Honey safety hazards and public health. *Journal of Chemical Health Risks, 6*(4).

Malaysian Standard. (2017). Kelulut (Stingless bee) honey-specification. Retrieved from https://www.scribd.com/document/398215369/Kelulut-Stingless-bee-honey-Specification

Marghitas, L. A., Dezmirean, D. S., Pocol, C. B., Marioara, I., Bobis, O., & Gergen, I. (2010). The development of a biochemical profile of acacia honey by identifying biochemical determinants of its quality. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38*(2), 84-90.

Marinho, J. F. U., Correia, J. E., de Castro Marcato, A. C., Pedro-Escher, J., & Fontanetti, C. S. (2014). Sugar cane vinasse in water bodies: Impact assessed by liver histopathology in tilapia. *Ecotoxicology and environmental safety, 110*, 239-245.

McKee, R. A., & Wingert, R. A. (2015). Zebrafish renal pathology: Emerging models of acute kidney injury. *Current pathobiology reports, 3*(2), 171-181.

Mehryar, L., & Esmaiili, M. (2011). *Honey and honey adulteration detection: A review.* Paper presented at the Proceedings of 11th International Congress on Engineering and Food.

Michener, C. D. (2013). The meliponini. In *Pot-honey* (pp. 3-17): Springer.

Miguel, M., Antunes, M., & Faleiro, M. L. (2017). Honey as a complementary medicine. *Integrative medicine insights, 12*, 1178633717702869.

Mijanur Rahman, M., Gan, S. H., & Khalil, M. (2014). Neurological effects of honey: current and future prospects. *Evidence-Based Complementary and Alternative Medicine, 2014*.

Mohorko, N., Petelin, A., Jurdana, M., Biolo, G., & Jenko-Pražnikar, Z. (2015). Elevated serum levels of cysteine and tyrosine: early biomarkers in asymptomatic adults at increased risk of developing metabolic syndrome. *BioMed Research International, 2015*.

Moniruzzaman, M., Khalil, M. I., Sulaiman, S. A., & Gan, S. H. (2013). Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera. *BMC Complementary and Alternative Medicine, 13*(1), 43.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PloS one, 5*(3), e9754.

Muraoka-Cook, R., Shin, I., Yi, J., Easterly, E., Barcellos-Hoff, M., Yingling, J., . . . Arteaga, C. (2006). Activated type I TGFβ receptor kinase enhances the survival of mammary epithelial cells and accelerates tumor progression. *Oncogene, 25*(24), 3408.

Mustafa, M. Z., Yaacob, N. S., & Sulaiman, S. A. (2018). Reinventing the honey industry: opportunities of the stingless bee. *The Malaysian journal of medical sciences: MJMS, 25*(4), 1.

Ni, H., Peng, L., Gao, X., Ji, H., Ma, J., Li, Y., & Jiang, S. (2019). Effects of maduramicin on adult zebrafish (Danio rerio): Acute toxicity, tissue damage and oxidative stress. *Ecotoxicology and environmental safety, 168*, 249-259.

Ninness, M. M., Stevens, E. D., & Wright, P. A. (2006). Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (Oncorhynchus mykiss) embryos. *Journal of experimental biology, 209*(10), 1874-1882.

Nunes, B., Antunes, S., Gomes, R., Campos, J., Braga, M., Ramos, A., & Correia, A. (2015). Acute effects of tetracycline exposure in the freshwater fish Gambusia holbrooki: antioxidant effects, neurotoxicity and histological alterations. *Archives of environmental contamination and toxicology, 68*(2), 371-381.

Nur sapinah binti mat zin. (2018). *Database development for prediction of acid and sugar adulteration in honey and stingless bee honey*. (Unpublished master's dissertation). University putra malaysia, Malaysia.,

Oecd. (1994). *OECD Guidelines for the Testing of Chemicals*: Organization for Economic.

OECD. (2012). Test No.TG210: Fish, Early-life Stage Toxicity Test, OECD Guideline for testing of chemicals, Adopted by the Council on 17th July 1992.

OECD, G. (1992). 203—for testing of chemicals. In: OECD.

Oka, T., Nishimura, Y., Zang, L., Hirano, M., Shimada, Y., Wang, Z., . . . Tanaka, T. (2010). Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. *BMC physiology, 10*(1), 21.

Olivares-Pérez, A., Mejias-Brizuela, N., Grande-Grande, A., & Fuentes-Tapia, I. (2012). Corn syrup holograms. *Optik, 123*(5), 447-450.

Olsen, A. S., Sarras Jr, M. P., & Intine, R. V. (2010). Limb regeneration is impaired in an adult zebrafish model of diabetes mellitus. *Wound repair and regeneration, 18*(5), 532-542.

Ouchemoukh, S., Louaileche, H., & Schweitzer, P. (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys. *Food control, 18*(1), 52-58.

Pangilinan, N. C., Ivie, G. W., Clement, B. A., Beier, R. C., & Uwayjan, M. (1992). Fate of [14 C] xanthotoxin (8-methoxypsoralen) in laying hens and a lactating goat. *Journal of chemical ecology, 18*(2), 253-270.

Paradkar, M., & Irudayaraj, J. (2002). Discrimination and classification of beet and cane inverts in honey by FT-Raman spectroscopy. *Food Chemistry, 76*(2), 231-239.

Parichy, D. M., Elizondo, M. R., Mills, M. G., Gordon, T. N., & Engeszer, R. E. (2009). Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Developmental dynamics, 238*(12), 2975-3015.

Paris-Palacios, S., Biagianti-Risbourg, S., & Vernet, G. (2000). Biochemical and (ultra) structural hepatic perturbations of Brachydanio rerio (Teleostei, Cyprinidae) exposed to two sublethal concentrations of copper sulfate. *Aquatic Toxicology, 50*(1-2), 109-124.

Pastor, K., Ačanski, M., Vujić, D., Bekavac, G., Milovac, S., & Kravić, S. (2016). Rapid method for small grain and corn flour authentication using GC/EI–MS and multivariate analysis. *Food Analytical Methods, 9*(2), 443-450.

Paulo, D., Fontes, F., & Flores-Lopes, F. (2012). Histopathological alterations observed in the liver of Poecilia vivipara (Cyprinodontiformes: Poeciliidae) as a tool for the environmental quality assessment of the Cachoeira River, BA. *Brazilian Journal of Biology, 72*(1), 131-140.

Peng, J., Xie, W., Jiang, J., Zhao, Z., Zhou, F., & Liu, F. (2020). Fast quantification of honey adulteration with laser-induced breakdown spectroscopy and chemometric methods. *Foods, 9*(3), 341.

Pinu, F. R., Beale, D. J., Paten, A. M., Kouremenos, K., Swarup, S., Schirra, H. J., & Wishart, D. (2019). Systems biology and multi-omics integration: Viewpoints from the metabolomics research community. *Metabolites, 9*(4), 76.

Prandi, B., Lambertini, F., Faccini, A., Suman, M., Leporati, A., Tedeschi, T., & Sforza, S. (2017). Mass spectrometry quantification of beef and pork meat in highly processed food: Application on Bolognese sauce. *Food control, 74*, 61-69.

Prasad, K., & Dhar, I. (2014). Oxidative stress as a mechanism of added sugar-induced cardiovascular disease. *International Journal of Angiology, 23*(04), 217-226.

Prassas, I., & Diamandis, E. P. (2008). Novel therapeutic applications of cardiac glycosides. *Nature reviews Drug discovery, 7*(11), 926.

Puscas, A., Hosu, A., & Cimpoiu, C. (2013). Application of a newly developed and validated high-performance thin-layer chromatographic method to control honey adulteration. *Journal of Chromatography A, 1272*, 132-135.

Ramli, N. Z., Chin, K.-Y., Zarkasi, K. A., & Ahmad, F. (2019). The beneficial effects of stingless bee honey from Heterotrigona itama against metabolic changes in rats fed with high-carbohydrate and high-fat diet. *International journal of environmental research and public health, 16*(24), 4987.

Ranneh, Y., Akim, A. M., Ab Hamid, H., Khazaai, H., Fadel, A., & Mahmoud, A. M. (2019). Stingless bee honey protects against lipopolysaccharide induced-chronic subclinical systemic inflammation and oxidative stress by modulating Nrf2, NF-κB and p38 MAPK. *Nutrition & metabolism, 16*(1), 15.

Rao, P. V., Krishnan, K. T., Salleh, N., & Gan, S. H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. *Revista Brasileira de Farmacognosia, 26*(5), 657-664.

Ratiu, I. A., Al-Suod, H., Bukowska, M., Ligor, M., & Buszewski, B. (2020). Correlation Study of Honey Regarding their Physicochemical Properties and Sugars and Cyclitols Content. *Molecules, 25*(1), 34.

Razali, M. T. A., Zainal, Z. A., Maulidiani, M., Shaari, K., Zamri, Z., Mohd Idrus, M. Z., . . . Rui, L. L. (2018). Classification of Raw Stingless Bee Honeys by Bee Species Origins Using the NMR-and LC-MS-Based Metabolomics Approach. *Molecules, 23*(9), 2160.

Rodrigues, S., Antunes, S., Nunes, B., & Correia, A. (2017). Histological alterations in gills and liver of rainbow trout (Oncorhynchus mykiss) after exposure to the antibiotic oxytetracycline. *Environmental toxicology and pharmacology, 53*, 164-176.

Ruiz-Matute, A. I., Rodríguez-Sánchez, S., Sanz, M. L., & Martínez-Castro, I. (2010). Detection of adulterations of honey with high fructose syrups from inulin by GC analysis. *Journal of Food Composition and Analysis, 23*(3), 273-276.

Sabaliauskas, N. A., Foutz, C. A., Mest, J. R., Budgeon, L. R., Sidor, A. T., Gershenson, J. A., . . . Cheng, K. C. (2006). High-throughput zebrafish histology. *Methods, 39*(3), 246-254.

Saiful Yazan, L., Zali, M., Shyfiq, M. F., Mohd Ali, R., Zainal, N. A., Esa, N., . . . Gopalsamy, B. (2016). Chemopreventive properties and toxicity of Kelulut honey in Sprague Dawley rats induced with Azoxymethane. *BioMed research international, 2016*.

Salvador, L., Guijarro, M., Rubio, D., Aucatoma, B., Guillén, T., Vargas Jentzsch, P., . . . Vásquez, L. (2019). Exploratory monitoring of the quality and authenticity of commercial honey in Ecuador. *Foods, 8*(3), 105.

Samaee, S.-M., Rabbani, S., Jovanović, B., Mohajeri-Tehrani, M. R., & Haghpanah, V. (2015). Efficacy of the hatching event in assessing the embryo toxicity of the nano-sized TiO2 particles in zebrafish: a comparison between two different classes of hatching-derived variables. *Ecotoxicology and environmental safety, 116*, 121-128.

Samarghandian, S., Farkhondeh, T., & Samini, F. (2017). Honey and health: A review of recent clinical research. *Pharmacognosy research, 9*(2), 121.

Samat, S., Enchang, F. K., Abd Razak, A., Hussein, F. N., & Ismail, W. I. W. (2018). Adulterated honey consumption can induce obesity, increase blood glucose level and demonstrate toxicity effects. *Sains Malays, 47*(2), 353-365.

Samat, S., Kanyan Enchang, F., Nor Hussein, F., & Wan Ismail, W. I. (2017). Four-week consumption of Malaysian honey reduces excess weight gain and improves obesity-related parameters in high fat diet induced obese rats. *Evidence-Based Complementary and Alternative Medicine, 2017*.

Santen, R. (1986). Determinants of tissue oestradiol levels in human breast cancer. *Cancer surveys, 5*(3), 597-616.

Santos, M. D. d., Chen, G., Almeida, M. C., Soares, D. M., de Souza, G. E. P., Lopes, N. P., & Lantz, R. C. (2010). Effects of caffeoylquinic acid derivatives and C-flavonoid from Lychnophora ericoides on in vitro inflammatory mediator production. *Natural product communications, 5*(5), 1934578X1000500512.

Saxena, S., Gautam, S., & Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food chemistry, 118*(2), 391-397.

Schievano, E., Stocchero, M., Morelato, E., Facchin, C., & Mammi, S. (2012). An NMR-based metabolomic approach to identify the botanical origin of honey. *Metabolomics, 8*(4), 679-690.

Schnell, R., Sandalova, T., Hellman, U., Lindqvist, Y., & Schneider, G. (2005). Siroheme-and [Fe4-S4]-dependent NirA from Mycobacterium tuberculosis is a sulfite reductase with a covalent Cys-Tyr bond in the active site. *Journal of Biological Chemistry, 280*(29), 27319-27328.

Scholz, M. B. d. S., Quinhone Júnior, A., Delamuta, B. H., Nakamura, J. M., Baudraz, M. C., Reis, M. O., . . . Bianchini, F. P. (2020). Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. *Journal of Food Science and Technology, 57*(5), 1896-1903. doi:10.1007/s13197-019-04225-3

Se, K. W., Ghoshal, S. K., Wahab, R. A., Ibrahim, R. K. R., & Lani, M. N. (2018). A simple approach for rapid detection and quantification of adulterants in stingless bees (Heterotrigona itama) honey. *Food Research International, 105*, 453-460.

Segner, H. (2004). Cytotoxicity assays with fish cells as an alternative to the acute lethality test with fish. *Alternatives to Laboratory Animals, 32*(4), 375-382.

Sethi, O., Anand, K., & Gulati, O. (1992). Evaluation of xanthotoxol for central nervous system activity. *Journal of ethnopharmacology, 36*(3), 239-247.

Shamsudin, S., Selamat, J., Sanny, M., Abd. Razak, S.-B., Jambari, N. N., Mian, Z., & Khatib, A. (2019). Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. *International Journal of Food Properties*, *22*(1), 239-264.

Shamsudin, S., Selamat, J., Sanny, M., AR, S. B., Jambari, N. N., & Khatib, A. (2019). A Comparative Characterization of Physicochemical and Antioxidants Properties of Processed Heterotrigona itama Honey from Different Origins and Classification by Chemometrics Analysis. *Molecules, 24*(21), 3898.

Siddiqui, A. J., Musharraf, S. G., & Choudhary, M. I. (2017). Application of analytical methods in authentication and adulteration of honey. *Food Chemistry, 217*, 687-698.

Simmons, C. R., Liu, Q., Huang, Q., Hao, Q., Begley, T. P., Karplus, P. A., & Stipanuk, M. H. (2006). Crystal structure of mammalian cysteine dioxygenase a novel mononuclear iron center for cysteine thiol oxidation. *Journal of Biological Chemistry, 281*(27), 18723-18733.

Singh, I., & Singh, S. (2018). Honey moisture reduction and its quality. *Journal of food science and technology, 55*(10), 3861-3871.

Singh, S. (2020). Entomophagy: Insects as Human Food. *AGRICULTURE & FOOD*, 76.

Soares, S., Amaral, J. S., Oliveira, M. B. P., & Mafra, I. (2017). A comprehensive review on the main honey authentication issues: Production and origin. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 1072-1100.

Sobrino-Gregorio, L., Vargas, M., Chiralt, A., & Escriche, I. (2017). Thermal properties of honey as affected by the addition of sugar syrup. *Journal of food engineering, 213*, 69-75.

Sole, S. S., & Srinivasan, B. (2012). Aqueous extract of tamarind seeds selectively increases glucose transporter-2, glucose transporter-4, and islets' intracellular calcium levels and stimulates β-cell proliferation resulting in improved glucose homeostasis in rats with streptozotocin-induced diabetes mellitus. *Nutrition research, 32*(8), 626-636.

Srinivasan, T., & Rao, P. V. (2014). Free acidity measurement–A review. *Talanta, 118*, 162-171.

Stanstrup, J., Broeckling, C. D., Helmus, R., Hoffmann, N., Mathé, E., Naake, T., . . . Salek, R. M. (2019). The metaRbolomics Toolbox in Bioconductor and beyond. *Metabolites, 9*(10), 200.

Sulieman, A. M. E., Alawad, S. M., Osman, M. A., & Abdelmageed, E. A. (2015). Physicochemical characteristics of local varieties of tamarind (Tamarindus indica L), Sudan. *International Journal of Plant Research, 5*(1), 13-18.

Tagawa, N., Takano, T., Fukata, S., KUMA, K., Tada, H., Izumi, Y., . . . AMINO, N. (2001). Serum concentration of androstenediol and androstenediol sulfate in patients with hyperthyroidism and hypothyroidism. *Endocrine journal, 48*(3), 345-354.

Taleuzzaman, M., Kala, C., & Gilani, S. J. (2020). Validation, Chemical Composition, and Stability of Honey from Indian Himalayas. In *Therapeutic Applications of Honey and its Phytochemicals* (pp. 81-100): Springer.

Thavarajah, R., Mudimbaimannar, V. K., Elizabeth, J., Rao, U. K., & Ranganathan, K. (2012). Chemical and physical basics of routine formaldehyde fixation. *Journal of oral and maxillofacial pathology: JOMFP, 16*(3), 400.

Uchenna, U. E., Shori, A. B., & Baba, A. S. (2017). Tamarindus indica seeds improve carbohydrate and lipid metabolism: An in vivo study. *Journal of Ayurveda and integrative medicine*.

Union, E. (2012). Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. *Off J Eur Union L, 167*, 1-116.

Vaclavik, L., Schreiber, A., Lacina, O., Cajka, T., & Hajslova, J. (2012). Liquid chromatography–mass spectrometry-based metabolomics for authenticity assessment of fruit juices. *Metabolomics, 8*(5), 793-803.

Veana, F., Flores-Gallegos, A. C., Gonzalez-Montemayor, A. M., Michel-Michel, M., Lopez-Lopez, L., Aguilar-Zarate, P., . . . Rodríguez-Herrera, R. (2018). Invertase: An Enzyme with Importance in Confectionery Food Industry. In *Enzymes in Food Technology* (pp. 187-212): Springer.

Venkatarajulu, V., & Sundaram, K. (2017). Induced Developmental Toxicity Studies With Mercuric Chloride and Polychlorinated Biphenyls on Danio Rerio (Zebrafish) Embryo. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 7*(2), 39.

Virgiliou, C., Kanelis, D., Pina, A., Gika, H., Tananaki, C., Zotou, A., & Theodoridis, G. (2020). A targeted approach for studying the effect of sugar bee feeding on the metabolic profile of Royal Jelly. *Journal of Chromatography A, 1616*, 460783.

Wang, J., Xue, X., Du, X., Cheng, N., Chen, L., Zhao, J., . . . Cao, W. (2014). Identification of acacia honey adulteration with rape honey using liquid chromatography–electrochemical detection and chemometrics. *Food analytical methods, 7*(10), 2003-2012.

Wang, S., Guo, Q., Wang, L., Lin, L., Shi, H., Cao, H., & Cao, B. (2015). Detection of honey adulteration with starch syrup by high performance liquid chromatography. *Food Chemistry, 172*, 669-674.

Wei, G.-x., HUANG, J.-k., & Jun, Y. (2012). Honey safety standards and its impacts on China's honey export. *Journal of Integrative Agriculture, 11*(4), 684-693.

Weir, M. R., & Dzau, V. J. (1999). The renin-angiotensin-aldosterone system: a specific target for hypertension management. *American journal of hypertension, 12*(S9), 205S-213S.

Wilson, J., George, B., & Umukoro, G. (2011). Effects of honey on the histology of liver in adult Wistar rats. *Biology and Medicine, 3*(1), 1-5.

Wu, L., Du, B., Vander Heyden, Y., Chen, L., Zhao, L., Wang, M., & Xue, X. (2017). Recent advancements in detecting sugar-based adulterants in honey–A challenge. *TrAC Trends in Analytical Chemistry, 86*, 25-38.

Xiang, B., Cheng, C., Xia, J., Tang, L., Mu, J., & Bi, Y. (2020). Simultaneous identification of geographical origin and grade of flue-cured tobacco using NIR spectroscopy. *Vibrational Spectroscopy, 111*, 103182. doi:https://doi.org/10.1016/j.vibspec.2020.103182

Yadata, D. (2014). Detection of the electrical conductivity and acidity of honey from different areas of Tepi. *Food Science and Technology, 2*(5), 59-63.

Yamada, T., Iida, T., Takamine, S., Hayashi, N., & Okuma, K. (2015). Safety evaluation of rare sugar syrup: single-dose oral toxicity in rats, reverse mutation assay, chromosome aberration assay, and acute non-effect level for diarrhea of a single dose in humans. *Shokuhin eiseigaku zasshi. Journal of the Food Hygienic Society of Japan, 56*(5), 211-216.

Yu, Z., Kastenmüller, G., He, Y., Belcredi, P., Möller, G., Prehn, C., . . . Ceglarek, U. (2011). Differences between human plasma and serum metabolite profiles. *PloS one, 6*(7).

Zábrodská, B., & Vorlová, L. (2015). Adulteration of honey and available methods for detection–a review. *Acta Veterinaria Brno, 83*(10), 85-102.

Zaefarian, F., Abdollahi, M. R., Cowieson, A., & Ravindran, V. (2019). Avian liver: the forgotten organ. *Animals, 9*(2), 63.

Zakbah, M. (2011). Beekeeping in pineapple smallholdings: a case of Apis mellifera. *Acta horticulturae*.

Zhang, J., Chen, H., Fan, C., Gao, S., Zhang, Z., & Bo, L. (2020). Classification of the botanical and geographical origins of Chinese honey based on 1H NMR profile with chemometrics. *Food Research International, 137*, 109714. doi:https://doi.org/10.1016/j.foodres.2020.109714

Zhang, W., Zhao, H., Zhang, J., Sheng, Z., Cao, J., & Jiang, W. (2019). Different molecular weights chitosan coatings delay the senescence of postharvest nectarine fruit in relation to changes of redox state and respiratory pathway metabolism. *Food chemistry, 289*, 160-168.

Zhang, Y.-Z., Wang, S., Chen, Y.-F., Wu, Y.-Q., Tian, J., Si, J.-J., . . . Hu, F.-L. (2019). Authentication of Apis cerana Honey and Apis mellifera Honey Based on Major Royal Jelly Protein 2 Gene. *Molecules, 24*(2), 289.

Zhong, R., Chen, Y., Ling, J., Xia, Z., Zhan, Y., Sun, E., . . . Song, J. (2019). The Toxicity and Metabolism Properties of Herba Epimedii Flavonoids on Laval and Adult Zebrafish. *Evidence-Based Complementary and Alternative Medicine, 2019*.

Zhu, L., Wang, Z., Wong, L., He, Y., Zhao, Z., Ye, Y., . . . Lin, G. (2018). Contamination of hepatotoxic pyrrolizidine alkaloids in retail honey in China. *Food Control, 85*, 484-494.

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LIST OF PUBLICATIONS

Fakhlaei, R., Selamat, J., Khatib, A., Razis, A. F. A., Sukor, R., Ahmad, S., & Babadi, A. A., The Toxic Impact of Honey Adulteration: A Review. Foods, IF: 3.189, 2020, 9(11), 1538. Retrieved from https://www.mdpi.com/2304-8158/9/11/1538

Fakhlaei, R., Selamat, J., Razis, A. F. A., Sukor, R., Ahmad, S., Amani Babadi, A., & Khatib, A., In Vivo Toxicity Evaluation of Sugar Adulterated Heterotrigona itama Honey Using Zebrafish Model. Molecules, IF: 4.411, 2021, 26(20), 6222. Retrieved from https://www.mdpi.com/1420-3049/26/20/6222.

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