METABOLITE PROFILING, IDENTIFICATION AND QUANTIFICATION OF PECTORALIS MAJOR AND SERUM FROM DIFFERENT CHICKEN BREEDS

By

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Chair : Prof. Jinap Selamat, PhD
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In Malaysia, local village chicken, known as “Ayam kampung”, is a premium chicken meat sold at a higher price than other chicken breeds. Food fraud involving the sale of colored broiler chicken as village chicken is motivated by a three to four-fold increase in profit due to their similar appearances and size. A previous study of chicken and beef products in Malaysia's local markets reported that approximately 78% of these products were associated with meat substitution and mislabelling. The objectives of this study are to distinguish four chicken breeds and further identify potential distinguishing metabolites corresponding to each chicken breed using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy with an untargeted metabolomics approach. Four types of chicken breeds were obtained from commercial farms, comprising of local village chicken, broiler chicken (Cobb), spent laying chicken (Dekalb), and colored broiler chicken (Hubbard). All pectoralis major and serum samples of four chicken breeds were analyzed using GC-MS and NMR. The multivariate analysis was applied through principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) assessments for GC-MS and NMR results. For GC-MS, all four chicken breeds were successfully separated into three distinct groups, with 30 and 40 characteristic metabolites identified for the pectoralis major and serum, respectively. Similarly, for NMR, all four chicken breeds were separated into three main groups, with 14 and 14 characteristic metabolites identified for the pectoralis major and serum, respectively. Both pectoralis major and serum were characterized by an abundance of metabolites comprising amino acids (alanine, glutamic acid, glycine, threonine, serine), fatty acid (linoleic acid), nucleotides (inosine monophosphate, nicotinamide adenine dinucleotide), organic acids (lactic acid, succinic acid and 3-hydroxybutyric acid), peptide (anserine), sugars (allose and glucose), sugar alcohol (myo-inositol), and other compounds under both analytical platforms. From the multivariate analysis, local village chicken was successfully differentiated from other commercial breeds by pectoralis major and serum under GC-MS and NMR, respectively. The present

GC-MS results for pectoralis major showed that local village chicken was not separated from colored broiler (Hubbard). In conclusion, the present results proved that an untargeted metabolomics approach coupled with GC-MS and NMR is suitable for differentiating different chicken breeds from commercial farms. The identified potential metabolites characterized for respective chicken breeds derived from multivariate analysis provide basic information on discriminating metabolites and references in chicken meat authentication for related regulatory authorities.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMPROFILAN, IDENTIFIKASI DAN KUANTIFIKASI METABOLIT DALAM PEKTORALIS MAJOR DAN SERUM DARIPADA AYAM YANG BERBEZA BAKA

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Di Malaysia, ayam kampung tempatan yang dikenali sebagai “ayam kampung” merupakan daging ayam permium yang dijual pada harga yang lebih tinggi berbanding dengan baka ayam yang lain. Penipuan yang melibatkan penjualan ayam pedaging berwarna sebagai ayam kampung adalah didorong oleh peningkatan keuntungan dari tiga sehingga empat kali ganda disebabkan oleh rupa dan saiz yang sama. Satu kajian terdahulu bagi produk ayam dan daging yang terdapat dalam pasaran tempatan di Malaysia telah melaporkan bahawa kira-kira 78% daripada produk-produk ini adalah dikaitkan dengan penggantian daging dan perlabelan yang salah. Objektif kajian ini adalah untuk membezakan empat baka ayam dan seterusnya mengenalpasti metabolit pembezaan berpotensi yang sepadan dengan setiap baka ayam dengan menggunakan kromatografi gas spektrometri jisim (GC-MS) dan spektoskopi resonans magnetik nuklear (NMR) menerusi pendekatan metabolomik yang tidak bersasar. Empat jenis baka ayam telah diperolehi daripada ladang komersial, terdiri daripada ayam kampung tempatan, ayam pedaging (Cobb), ayam penelur tua (Dekalb) dan ayam pedaging berwarna (Hubbard). Semua sampel pektoralis major dan serum bagi empat jenis baka ayam telah dianalisa dengan menggunakan GC-MS dan NMR. Analisis multivariat telah digunakan menerusi penilaian analisis komponen utama (PCA) dan analisis diskriminasi kuasa dua terkecil separa ortogon (OPLS-DA) bagi keputusan GC-MS dan NMR. Untuk GC-MS, keempat-empat baka ayam telah berjaya diasingkan kepada tiga kumpulan yang berbeza, dengan pengenalpastian 30 dan 40 metabolit ciri masing-masing untuk pektoralis major dan serum. Begitu juga dengan NMR, keempat-empat baka ayam telah diasingkan kepada tiga kumpulan utama, dengan pengenalpastian 14 dan 14 metabolit ciri masing-masing dalam pektoralis major dan serum. Kedua-dua pektoralis major dan serum telah

dicirikan oleh banyak metabolit yang terdiri daripada asid amino (alanina, asid glutamat, glisina, treonina, serina), asid lemak (asid linoleik), nukleotida (inosin monofosfat, nikotinamida adenina dinukleotida), asid organik (asid laktik, asid suksinik dan asid 3-hidrosibutirik), peptida (anserin), gula (allosa dan glukosa), gula alkohol (myo-inositol), dan sebatian lain di bawah kedua-dua platform analisis. Berdasarkan analisis multivariat, ayam kampung tempatan berjaya dibezakan daripada baka komersial yang lain oleh pektoralis major dan serum masing-masing dengan menggunakan GC-MS and NMR. Keputusan GC-MS bagi pektoralis major dalam kajian ini menunjukkan bahawa ayam kampung tempatan tidak dapat dibezakan daripada ayam pedaging berwarna (Hubbard). Secara kesimpulan, keputusan dalam kajian ini telah membuktikan bahawa pendekatan metabolomik yang tidak bersasar berasaskan GC-MS dan NMR adalah sesuai digunakan untuk membezakan baka ayam yang berlainan daripada ladang komersial. Metabolit berpotensi yang dikenalpasti dan dicirikan dengan baka ayam berkenaan yang diperolehi daripada analisis multivariat telah memberikan maklumat asas terhadap metabolit yang membezakan baka ayam dan menjadi rujukan dalam pengesahan daging ayam bagi kegunaan agensi penguatkuasaan yang berkaitan.

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LIST OF ABBREVIATIONS

^{1}H NMR	Proton Nuclear Magnetic Resonance
1D	One dimensional
2D	Two dimensional
AOAC	Association Officials of Analytical Chemistry
ANOVA	Analysis of variance
ATP	Adenosine monophosphate
CE-MS	Capillary electrophoresis mass spectrometry
CRM	Certified reference material
CV	Coefficient of variation
DVS	Department of Veterinary Services, Malaysia
dd	Double of doublet
d	Doublet
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
FTIR	Fourier-transform infrared spectrometry
FSA	Food Standards Agency
GC-MS	Gas chromatography mass spectrometry
g	Gravity
HMDB	Human Metabolome Database
ICH	International Council of Harmonization
IMP	Inosine monophosphate

ISO	International Organization for Standardization
ISTD	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantitation
MeCN	Acetonitrile
MeOH	Methanol
MVA	Multivariate data analysis
ms	Mass spectrometry
m	Multiplet
NAD^+	Nicotinamide adenine dinucleotide
NIST	National Institute of Standards and Technology
NMR	Nuclear magnetic resonance
OLS	Ordinary least square
OPLS-DA	Orthogonal partial least squares discriminant analysis
PCA	Principal component analysis
PCC	Per capita consumption
PC	Principal component
PLS-DA	Partial least squares discriminant analysis
pH	Negative logarithmic scale value of hydrogen ion concentration
$PRSD_r$	Predicted relative standard deviation of repeatability
$PRSD_R$	Predicted Relative standard deviation of reproducibility
RSD_r	Relative standard deviation of repeatability
RSD_R	Relative standard deviation of reproducibility
R^2	Correlation of coefficient

rpm	Revolutions per minute
SD	Standard deviation
SSR	Self-sufficiency ratio
s	Singlet
TIC	Total ion chromatogram
TSP	Sodium-3-trimethylsilylpropanoic acid
t	Triplet
QC	Quality control
VIP	Variable in projection value
α	Alpha
β	Beta
°C	Degree Celsius
et al.	And other authors
Σ	Sum

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Poultry meat is considered one of the most efficient sources of protein. Due to the lower cost and healthy choice compared to red meat, readiness for further processing, and no religious or cultural prohibitions, the demand for poultry meat has increased worldwide in the past few decades (Boerboom et al., 2018). According to the Department of Statistics Malaysia (2020), poultry meat production in Malaysia has increased steadily from 2013-2019. The highest production of poultry meat was 1,611.1 million metric tonnes in 2016, followed by an estimation of 1,576.1 million metric tonnes in 2019. Besides that, the Self Sufficiency Ratio (SSR) and Per Capita Consumption (PCC) for poultry meat were recorded as 98.1% and 51.0 kg/year, respectively, in 2018. According to the Department of Veterinary Services (DVS), Malaysia (2020), the poultry population in Malaysia can be divided into four categories, including broiler, layer, breeder, and local fowl. The highest number of productions is broiler (171,980,685) followed by layer (62,162,143), breeder (15,473,432) and local fowl (11,210,061). Village chickens have shown a significant demand for the past few decades in the global poultry sector. In Malaysia, the demand for the village chicken is notably high due to its uniqueness of taste and better meat quality (Hakimi et al., 2019).

A reliable and exact detail regarding the foods we consume represents a significant demand for consumers, governments, and industries (Böhme et al., 2019). Food products are adulterated to maximize the volume and weight, masking inferior quality, and replacing authentic components with cheaper ones (Medina et al., 2019). For instance, a replacement with a comparable cheaper one of an original ingredient that is difficult for consumers to distinguish is the most common food fraud reported in publications (Abbas et al., 2018). Adulteration tends to occur in meat products due to unfair practices by the manufacturers and the high cost of animal protein to achieve economic benefits (Soares, 2013). Several studies in food authentication have been conducted to differentiate different species, breeds, and types of meats, including chicken, pork and beef (Ueda et al., 2018), different native chicken breeds in China (Xiao et al., 2019) and different muscle types (Mabuchi et al., 2018).

1.2 Problem statements

Food safety authorities and trade partners have significant concerns about the ability to demonstrate the authenticity of food products due to the increase in food fraud cases. In Malaysia, underage colored broiler has been claimed as

village chicken to deceive consumers due to its lower cost for economic gain, with approximately three to four times higher in price. A study by Chuah et al. (2016) found that approximately 78% of poultry and beef products in Malaysia's local markets were associated with meat substitution and mislabelling. Almost 100% of chicken, including marinated cut pieces of poultry meat sold as a village chicken in the Klang valley market, is a colored broiler (Azhar, 2019a; 2019b). There is no universal international standard for producing and labelling different types of chicken meat available in markets. Besides, local authorities have no regulation or affiliation with village chickens' claims. In chicken meat production, the commercial feeds fed to the chickens are closely related to metabolism changes and animal health conditions. The potential risks of misuse or excessive dosage of antimicrobial and feed additives in animal feed and also the cross-contamination of animal feeds during production and storage to public health and food safety issues in chicken meat production are unknown. The metabolites in chicken meat and serum associated with the commercial feeds which contain feed additives and antimicrobial are unknown.

1.3 Significance of the study

Up to this date, there have been very little data on the metabolite fingerprinting and profiling of the Malaysian indigenous chickens documented elsewhere. To the best of our knowledge, no such comparison using an untargeted metabolomics approach on different breeds of chicken available in the Malaysian market has been performed. According to the National Health and Morbidity Survey: Malaysian Adult Nutrition survey 2014, Volume III, Food Consumption Statistics of Total Population in Malaysia, the prevalence exposure for chicken is 94.46% and is considerably high (Institute for Public Health, 2014). However, there is a lack of established methods or systems for authentication of the safety and quality of chicken meat. Moreover, there is still no strategy to authenticate different chicken breeds in Malaysia. Hence, this study is designed to distinguish 4 chicken breeds in the market and identify the distinguishing metabolites affected mainly by commercial feeds using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) based metabolomic approach.

1.4 Hypothesis

The GC-MS and NMR-based metabolomics distinguishes four chicken breeds and identifies distinguishing metabolites that contributed to the respective chicken breeds.

1.5 Objectives of the study

The objectives of this study were as follows:

i) To distinguish four chicken breeds using GC-MS and NMR-based metabolomics.
ii) To identify the potential distinguishing metabolites of different chicken breeds using GC-MS and NMR-based metabolomics.
iii) To quantify / semi-quantify identified distinguishing metabolites that contributed to chicken breeds' separation using GC-MS and NMR-based metabolomics.

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