DIFFERENTIATION OF GELATINE (BOVINE, PORCINE AND FISH) BY MAILLARD REACTION USING E-TONGUE AND E-NOSE COMBINED WITH CHEMOMETRICS METHOD

BY

ISMARTI MUHAMMAD SOHIEB

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International Institute for Halal Research and Training
International Islamic University Malaysia

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ABSTRACT

Food fraud and adulteration are the global issues, currently. One critical issue is about gelatine which comes from a variety of animal sources. Therefore, analytical method for gelatine must be developed. The objectives of this study were to improve the Maillard reaction, which produces flavour compounds in gelatine. A bovine gelatine-xylose model was used for optimisation. Furthermore, this research also aimed to evaluate the ability of E-tongue and E-nose in differentiating gelatine based on its sources and to investigate volatile and non-volatile compounds of gelatine and the Maillard reaction products (MRPs). There were five instruments employed in this research. The first instrument used was Ultra-Violet spectroscopy to determine the browning intensities of gelatine-xylose model's MRPs. The second was E-tongue, which has 16 membrane lipid sensors, and the third was an E-nose with eight metal oxide semiconductors gas sensors. Next, HPLC was used to analysis free amino acids (FAA) as non-volatile chemicals. Lastly, a SPME-GC-MS used to evaluate the volatile organic molecules. This investigation used gelatine standards from bovine, fish, and porcine bought from Sigma Aldrich. MANOVA and ANOVA statistical tests using SPSS software were carried out. Data were also analyzed using a chemometrics that included Principal Component Analysis and Linear Discriminant Analysis. The initial pH, reaction temperature, and heating time had a modest effect on the browning intensity of MRPs and affected the development of brown colour of MRPs. With an initial pH of 10.9, a temperature of 140 °C, and a heating time of 37.28 minutes, the best reaction conditions were established. Additionally, the result showed that E-tongue and E-nose, aided by the Maillard reaction paired with LDA, may be used to differentiate gelatine based on the sources with a percentage of accuracy greater than 95%. Also, the differentiation attained for gelatine samples without Maillard process had an accuracy ranging from 93% to 98%. Eleven amino acids detected for gelatine in various level concentration namely arginine, lysine, isoleucine, leucine, tyrosine, valine, glutamic acid, aspartic acid, threonine, serine, and alanine. Two undetected amino acids in gelatine namely methionine and phenylalanine were detected in the MRPs samples. The diversity of FAA in gelatine and the MRPs induces various E-tongue sensor responses and influences overall sensory qualities. Furthermore, 67 volatile compounds also detected in different concentration level. Among them, furfural, acetic acid, nonanone, dimethyl disulphide, and decanone were considered as the important volatiles in gelatine due to its abundance. In the Maillard reaction products, furfural, 1-(2-furanylmethyl)-1H-pyrrole, 1-(2-furanyl)-ethanone, acetic acid, and 2,2'-bifuran were predominant. Finally, heptanol, octanal, nonanal, nonanone, dimethyl disulphide, and dimethyl trisulphide could be considered as important compounds due to its low odour threshold value. They had a direct impact on the overall flavour of samples assessed using E-nose sensors. All these findings indicate that the proposed extension was successful in meeting the study's objectives.

Keyword: halal authentication, gelatine-xylose, Maillard reaction, E-tongue, E-nose, Principal Component Analysis, Linear Discriminant Analysis.

خلاصة البحث

تعتبر قضية التزييف وتخليط اﻷطعمة من القضايا العاملية، ومن أهمها الجيالتين إذ أنه يتم الحصول عليه من الحيوانات بشتى أنواعها. ولذلك، فال بد من تطوير طريقة تحليل الجيالتين.ويستهدفهذا البحثلتطوير تفاعل ميالرد الذي ينتج مركبات النكهة من الجيالتين. تم استخدام نموذج جيالتين البقر مع الزيلوز من أجل عملية التحسين .وبالتالي،يستهدفهذا البحثأيضا لتقييم قدرةاللساناإللكتروني واﻷنفاإللكتروني فيتمييزالجيالتينحسباملصدر اﻷلصلي، وتحقيق املركبات املتطايرة وغير املتطايرةمن الجيالتين ومنتجات تفاعل ميالرد(MRPs) املستمدة من نموذج الجيالتين معالزيلوز. تم استخدام خمس أدوات رئيسيةفي هذا البحث: اﻷلداةاألولى هيمطيافيةاﻷشعةفوق البنفسجي لقياسشدةاللونالبنيمنمنتجاتتفاعلميالردلنموذجالجيالتينالزيلوز. واﻷلداةالثانية هياللسان اإللكتروني بحيث تتوفر له 16 مستشعر الغشاء الدهني. واﻷلداة الثالثة هي اﻷنف اإللكتروني بثمانية مستشعرات للغاز املصنوعة من أشباه املوصالت ألكسيد املعادن. واﻷلداة الرابعة هي استشراب السائل لرفيع اإلنجاز(HPLC) املستعملة لتعيين اﻷحماض اﻷمينية الحرة كمركبات غير متطايرة في العينات. واﻷلداة اﻷخيرة هي أداة االستخراج الدقيق للحالة الصلبة –االستشراب الغازي–مطيافية الكتلة(MS-GC-SPME) لتعيين املركبات العضوية املتطايرة في العينات. هذا البحث يستخدم ثالثةمعايير جيالتينيةمن اﻷبقارواﻷسماك والخنازير التي تم شراؤها من شركة سيجما الدريتش.وتم إجراء االختبارات اإلحصائية MANOVA و ANOVA باستخدام برنامج. SPSS وتم تحليل املعطيات أيضا بالقياسات الكيميائية املتكونة من تحليل العنصر الرئيس ي(PCA) وتحليل التمييز الخطي.(LDA) فبناء على نتائج البحث، كان الرقم الهيدروجيني اﻷولي ودرجة حرارة التفاعل ومدة التسخين لها تأثير معنوي في كثافة اللون البني ملنتجات تفاعل ميالرد وأثرتعلىتكويناللونالبنيفيمنتجاتتفاعلميالرد.وتم الحصولعلىالظروفاملثلىللتفاعلعند الهيدروجينياﻷولي 9.10،ودرجةالحرارة 140،°Cووقتالتسخين 28.37 دقيقة. ومن ثم، واستكشف هذا البحث أن اللسان اإللكتروني واﻷنفاإللكتروني بمساعدةتفاعل امليالردمعتحليلاالتمييزالخطي

يمكناستخدامهما لتمييزالجيالتينحسباملصدر بنسبة دقة أعلى من 95.٪وإضافةإلىذلك، تتم املاحظةباختالفعينات الجيالتين بدون تفاعل ميالردوبدقةنسبية تراوحت بين 93-98 ا فيالجيالتين بمستوياتمختلفةمن التركيزاتوهيأرجينين، ا أميني ٪ .تم اكتشافأحد عشر حمض وليسين، وآيزولوسين، وليسين، وتيروسين، وحمض الفالين، وحمض الجلوتاميك، وحمض أالسبارتيك، وثريونين، وسيرين، وأالنين. وتم اكتشاف نوعين من أال حماض أالمينية غير املكتشفة في الجيالتين وهما ميثيونين وفينيل أالنين في عينات منتجات تفاعل ميالرد. يؤدي تنوع أالحماض أالمينية الحرة في العينات إلى تمييز استجابات مستشعر اللسان إللكتروني ويؤثر في جميع الصفات الحسية للعينات. ومن ثم، تم اكتشاف 67 ا متطا مركب ا ب ا أيض ير تركيزات مختلفة، من بينهافورفورال،وحمضالخليك،ونونانون،وديميتيل ديسولفيدا،والديكانون،و تعتبر من املواد املتطايرة الهامة في الجيالتين بسبب وفرتها. وفي أمر تفاعل امليالرد، كان فورفورال، و1(-2-فوران ميتيل H1-)فيرول، و1(-2-فورانيل -)أيتانون،وخمض أسيتات،و2-2-بيفوران تعتبرمن املركبات السائدة.وفي النهاية، يمكن اعتبار هيبتانول،وأوكتانال، ونونانال، ونونانوني، وديميتيل تريسلفيد من املركبات املهمة ا لقيمتها املنخفضةللرائحة نظر ، حيثكان لها تأثير مباشر على النكهة الكلية للعينات التي تم تقييمها باستخدام مستشعرات أالنف إللكترونية. كل هذه البيانات من نتائج البحث ا فيتحقيق أهدافهذا البحث.

الكلمات المفتاحية: المصادقة الحلالية، الجيلاتين الزيلوز، تفاعل ميلارد، اللسان الإلكتروني، الأنف الإلكتروني، تحليل العنصر الرئيسي، تحليل التمييز الخطي

APPROVAL PAGE

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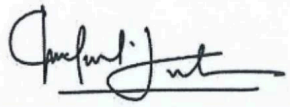

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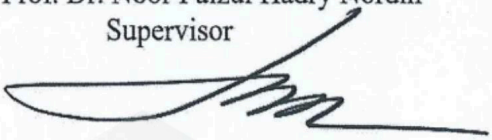

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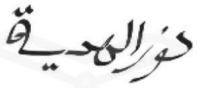

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Co-Supervisor

Prof. Dr. Irwandi Jaswir
Internal Examiner

Assoc. Prof. Dr. Md. Monowarul Mobin Siddique
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I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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This thesis is dedicated to Islam and Muslims.

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infra-Red
BEHS	Bis(2-ethylhexyl) Sebacate
BG	Bovine Gelatine
BGX	Bovine Gelatine-Xylose
BPNN	Back Propagation Neural Network
BPPA	Bis (1-buthylpenthyl) Adipate
CAGR	Compound Annual Growth Rate
CCD	Central Composite Design
CP	Conductive Polymer
DAQ	Data Acquisition
DNA	Deoxyribonucleic Acid
DOE	Design of Expert
DSC	Differential Scanning Calorimetry
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAA	Free Amino Acid
FG	Fish Gelatine
FGX	Fish Gelatine-Xylose
FTIR	Fourier Transform Infra-Red
GA	Gallic Acid
GCMS	Gas Chromatography-Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
LCMS	Liquid Chromatography-Mass Spectroscopy
LDA	Linear Discriminant Analysis
LRI	Linear retention Indices
MANOVA	Multivariate Analysis of Variance
MLR	Multiple Linear Regression
MOS	Metal Oxide Semiconductor
MOSFET	Metal Oxide Semiconductor Field Effect Transistors
MRPs	Maillard Reaction Products
MTAC	Methyl Tri-octyl Ammonium Chloride
NIRS	Near Infra-Red Spectroscopy
NPOE	2-Nitrophenyl Octyl Ether
OA	Oleic Acid
OFAT	One Factor Analysis at Time
OPA	Orto Phtalaldehyde
OT	Odour Threshold
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PG	Porcine Gelatine
PGX	Porcine Gelatine-Xylose
PLS	Partial Least Square
PVC	Polyvinyl Chloride
RT	Retention Time

RBD	Refined Bleached Deodorized
SAW	Surface Acoustic Wave
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis
SPME	Solid Phase Micro Extraction
TDAB	Tetra Dodecyl Ammonium Bromide
TGS	Taguchi Gas Sensor
THF	Tetra Hydro Furan
TMR	Transparency Market Research
TOF-MS	Time of Flight Mass Spectroscopy
UV	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Food authentication and adulteration detection are huge concern in the food ecosystem, not only for producers but also for consumers and government. Technological advancement in food, pharmaceutical, and cosmetics industries has increased the possibility of counterfeiting practices. Pig and pig derivatives such as pork, lard, and gelatine are not only used as an additive but also as the main material in the manufacture of food and pharmaceutical products. For this reason, the identification of pig derivatives in food and pharmaceuticals is essential.

Currently, gelatine remains a major concern among consumers since its source of origins is deemed unacceptable from the aspect of culture, health, and religion. A report released by Transparency Market Research (TMR) stated that the global gelatine market in 2011 had a production capacity of 348.9-kilo tons and is estimated to reach a production capacity of 450.7-kilo tons by 2018. Another source reinforced that the global gelatine market demand was 620.6-kilo tons in 2019 and is projected to expand at a volume-based CAGR of 5.9% from 2020 to 2027. In contrast, halal gelatine in the global market only less than 2% of the total gelatine production. Most of gelatine is derived from porcine skin (80%), followed by bovine hide (15%) and porcine bone, cattle bone, and fish (5%), respectively (Tongdeesoontorn & Rawdkuen, 2019). Nowadays, many researchers have paid increasing attention on an alternative source of gelatine, such as fish and poultry processing by-products (Abdullah et al., 2016; Abedinia et al., 2017; Khiari et al., 2013; Monsur et al., 2014; Silva, Bandeira, & Pinto, 2014).

Gelatine is a pure protein and has a functional role in living organisms. In this age, gelatine has been widely applied in foods, pharmaceuticals, neutraceuticals, and cosmetics products. The classical food, photographic, cosmetic and pharmaceutical application of gelatine is based mainly on its gel-forming and thickening properties (Gomez-Guillen et al., 2011; Mariod & Adam, 2013). It has been used as an emulsifier, foaming agents, colloid stabilizers, biodegradable film-forming materials, micro-

encapsulating agents, and the source of bioactive peptides (Chung, 2020; Gomez-Guillen et al., 2011).

Species-specific detections of animal protein in food have been reported in various techniques such as chromatography (Bargen et al., 2013), spectroscopy (Mandrile et al., 2017; Rahmania et al., 2015), and also Polymerase Chain Reaction (PCR) (Maryam et al., 2016; Rahmawati et al., 2016). With regards to gelatine authentication, some techniques, such as electrophoretic, chromatography-based techniques, spectroscopy techniques, Enzyme-Linked Immuno-Sorbent Assay (ELISA), thermal analysis, chemical reaction, and PCR have been presented as well. Chromatography-based technique offers a reliable tool for separation and quantitative analysis, and thus, it is mostly employed for differentiation purposes. In addition, the FTIR spectroscopy technique was reported as simple, rapid, and accurately effective in differentiating gelatine sources, while PCR was deemed as an ideal technique to be used for the detection of porcine DNA in gelatine due to the higher stability of DNA compared to protein (Sepminarti et al., 2016). However, the existing methods are mostly in need of modern-technology instruments, which requires high skill level to successfully operate the technology, and high monetary cost. On this basis, it is pivotal to develop an efficient and cheaper alternative method, such as array sensor systems combined with artificial intelligence like electronic tongue (E-tongue) and electronic nose (E-nose), which are deemed promising for this purpose.

Some reports find that UV-spectroscopy techniques have been successfully applied in the differentiation of bovine and porcine gelatine (Tan et al., 2012; Hamid et al., 2019). A chemical reaction, namely the Maillard reaction that was able to develop the browning effect in gelatine, was also introduced as an authentication technique. The differentiation of bovine and porcine gelatine was obtained by the different browning intensities of the Maillard Reaction Products (MRPs) of gelatine after reaction by reducing sugar. However, there was no report about other aspects of the gelatine-MRPs, such as aroma and taste. Therefore, this study aimed to elaborate the possibility to use gelatine-MRPs flavour and taste active component as indicators for differentiation of gelatine sources and to establish the simple, rapid, and accurate method for differentiation of gelatine based on the flavour and taste by using lab-made potentiometric E-tongue and E-nose. Chemometric techniques provide an opportunity for classifying or discriminating materials with respect to their similarities since these

techniques have the capability to extract distinctive properties from the complex data generated from the instruments (Cebi et al., 2019). Additionally, several studies (Dong et al., 2017; Hidayat et al., 2019; Nurjuliana et al., 2011) suggest that the application of E-tongue and E-nose combined with chemometrics has great potential to solve adulteration and authentication problems.

1.2 **STATEMENT OF THE PROBLEM**

The unclear information and labelling regarding gelatine source in the market has increased consumers' concern over its halal authenticity. In concern with food safety, an analytical laboratory approach needs to be done in order to know chemically additive substances that the food product may contain. Currently, several methods have been developed for halal authentication purposes in gelatine. Chromatography, spectroscopy, DNA, and protein-based methods such as PCR and ELISA, respectively, are the most commonly used methods due to their high success rate, accuracy and preciseness. However, the disadvantages of these methods are that it tends to be time-consuming and destructive, and thus requires high-skilled experts to perform the studies. Worse yet, toxic chemicals are used for destruction and sample extraction (Cebi et al., 2019). These challenges reveal a necessity to develop rapid, in-expensive, and effective techniques to determine the sources of gelatine as raw and processed ingredients in food products.

On the other hand, artificial sensing techniques using portable E-nose and E-tongue in conjunction with chemometrics was widely used as sensitive and fast techniques for authentication and quality analysis of a wide range of food. Meat differentiation based on the aroma profile was highlighted in some previous studies. Furthermore, volatile compounds in meat and meat processed foods made from different animals have been investigated by using solid-phase microextraction–gas chromatography–mass spectrometry (SPME/GC–MS). Other studies also revealed about amino acids and protein characterisation based on the taste profile. However, no study has been done on gelatine differentiation, as well as based on the aroma and its taste profiles. In addition, very limited literature addressed about flavour compounds of

gelatine. Therefore, research needs to be conducted to investigate the possibility of gelatine authentication based on the flavour compounds.

1.3 **PURPOSE OF THE STUDY**

Chemical analysis using the Maillard reaction has been revealed to successfully differentiate bovine and porcine gelatine based on the colour intensity of melanoidins. However, no previous study has been reported about the flavour of gelatine-MRPs as well as its aroma and taste. Thus, this study was conducted to investigate the differentiation of gelatine based on the flavour compounds of gelatine and gelatine-MRPs. This research sought to establish a rapid method for gelatine authentication based on the flavour characteristics for a different source of origins by focusing on porcine, bovine, and fish gelatine.

1.4 **RESEARCH QUESTIONS**

The research question in this study as follow:

1. What are the optimum conditions of Maillard reaction for gelatine-xylose model for gelatine authentication?
2. How is the capability of potentiometric E-tongue in differentiation of gelatine based on the sources?
3. How is the capability of E-nose in the differentiation of gelatine based on the sources?
4. What are the volatile and non-volatile compounds in the gelatine-xylose model MRPs which can be used as marker compounds in the differentiation of gelatine?

1.5 **RESEARCH OBJECTIVES**

The present study aimed to achieve the following objectives:

1. to use the MRPs of gelatine-xylose model for gelatine authentication
2. to evaluate the capability of potentiometric E-tongue in the differentiation of gelatine based on the sources of origin;
3. to evaluate the capability of E-nose in the differentiation of gelatine based on the sources of origin; and
4. to verify the volatile and non-volatile compounds of MRPs from the gelatine-xylose model as the marker compounds in authentication.

1.6 **RESEARCH HYPOTHESIS**

Key aroma compounds in food are present only in trace concentrations of 1µg/kg to 1mg/kg. Nevertheless, they contribute to the respective flavour because of their low odour perception threshold. When a peptide/amino acid and sugar mixture is heated and undergoes Maillard reaction to produce flavour compounds, amino acids contribute differently toward an aroma and taste. The aroma profile can be investigated using E-nose, while the taste profile using E-tongue.

Since the amino acid composition of gelatine varies with its origin, it is possible that flavour compounds, when subjected to Maillard reaction, will vary. The differences will be the key principle in halal authentication of gelatine in this study. The hypotheses are as follows:

H1 pH, temperature, and heating time have a positive effect on the MRPs of the gelatine-xylose model.

H2 E-tongue combined with chemometric tools could be applied to differentiate gelatine and MRPs based on the origin sources.

H3 E-nose combined with chemometric tools could be applied to differentiate gelatine and MRPs based on the origin sources.

H4 Different volatile and non-volatile compounds of MRPs from gelatine-xylose models could be used as marker compounds for authentication.