

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

BY

ISMARTI MUHAMMAD SOHIEB

**A thesis submitted in fulfilment of the requirement for the
degree of Doctor of Philosophy in Halal Industry**

**International Institute for Halal Research and Training
International Islamic University Malaysia**

JUNE 2022

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

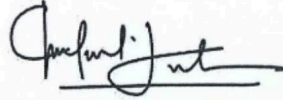
تعتبر قضية التزييف وتخليط الأطعمة من القضايا العالمية، ومن أهمها الجيالتين إذ أنه يتم الحصول عليه من الحيوانات بشتى أنواعها. ولذلك، فال بد من تطوير طريقة تحليل الجيالتين. ويستهدف هذا البحث تطوير تفاعل ميالرد الذي ينتج مركبات النكهة من الجيالتين. تم استخدام نموذج جيالتين البقر مع الزيلوز من أجل عملية التحسين. وبالتالي، يستهدف هذا البحث أيضا لتقييم قدرة اللسان الإلكتروني وألنف الإلكتروني في تمييز الجيالتين حسب المصدر الأصلي، وتحقيق المركبات الملتطيرة وغير الملتطيرة من الجيالتين ومنتجات تفاعل ميالرد (MRPs) المستمدة من نموذج الجيالتين مع الزيلوز. تم استخدام خمس أدوات رئيسية في هذا البحث: الأداة الأولى هي مطيافية الأشعة فوق البنفسجي لقياس شدة اللون البنين من منتجات تفاعل ميالرد لنموذج الجيالتين الزيلوز. والأداة الثانية هي اللسان الإلكتروني بحيث تتوفر له 16 مستشعر الغشاء الدهني. والأداة الثالثة هي أنف الإلكتروني بثمانية مستشعرات للغاز المصنوعة من أشباه الموصلات أكسيد الميالدن. والأداة الرابعة هي استشراب السائل لرفع الإنجاز (HPLC) الملتعملة لتعيين الأحماض الأمينية الحرة كمركبات غير متطيرة في العينات. والأداة الأخيرة هي أداة الاستخراج الدقيق للحالة الصلبة - الاستشراب الغازي - مطيافية الكتلة (MS-GC-SPME) لتعيين المركبات العضوية الملتطيرة في العينات. هذا البحث يستخدم ثلاثة معايير جيالتينية من الأبقار والأسماك والخنازير التي تم شراؤها من شركة سيجما الدريتش. وتم إجراء التجارب الإحصائية MANOVA و ANOVA باستخدام برنامج SPSS وتم تحليل الملعطيات أيضا بالقياسات الكيميائية الملتكونة 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 لمنتجات تفاعل ميالرد وأثر تعلق كوينالون البنين في منتجات تفاعل ميالرد. وتم الحصول على الظروف المثلثة لتفاعل عند الهيدروجين الأولي 9.10، ودرجة الحرارة 140°C، ووقت التسخين 28.37 دقيقة. ومن ثم، واستكشف هذا البحث أن اللسان الإلكتروني وألنف الإلكتروني بمساعدة تفاعل الميالدن مع تحليل التمييز الخطي

يمكن استخدامها لتمييز الجيالاتين بحسب المصدر بنسبة دقة أعلى من 95%. وإضافة لذلك، تتم ملاحظة باختلاف العينات الجيالاتين بدون تفاعل ميلرد بدقة نسبية تراوحت بين 93-98 في الجيالاتين بمستويات مختلفة من التركيز أو هياكل جينين، أميني % . تم اكتشاف أحد عشر حمض و ليسين، وأيزولوسين، و ليسين، وتيروسين، وحمض الفالين، وحمض الجلوتاميك، وحمض ألسبارتيك، و ثريونين، وسيرين، وألنين. وتم اكتشاف نوعين من أأل حمض أألينية غير أملكشفة في الجيالاتين وهما ميثيونين وفينيل أألين في عينات منتجات تفاعل ميلرد. يؤدي تنوع أألماض أألينية أألرة في العينات إلى تمييز استجابات مستشعر اللسان أألكتروني ويؤثر في جميع الصفات أألسية للعينات. ومن ثم، تم اكتشاف 67 أمتطأ مركب أ ب أ أيضا ير تركيزات مختلفة، من بينها فورفورال، وحمض أألليك، ونونانون، وديميتيل ديسولفيدا، وألديكانون، و تعتبر من أملواد أملتطأيرة أألأمة في الجيالاتين بسبب وفرأها. وفي أألر تفاعل أمليلارد، كان فورفورال، و 1(-)2- فوران ميتيل (H1-) فيرول، و 1(-)2- فورانيل (-) أألانون، وحمض أألينات، و 2-2- ييفوران تعتبر من أملركبات السائدة. وفي أألنهاية، يمكن أألبار هيلتانول، وأوكتانال، ونونانال، ونونانوني، وديميتيل تريسلفيد من أملركبات أألأمة أألقيمتها أملنخفضة للرائحة نظر ، حيث كان لها أألأثير مباشر على أألأمة أأللية للعينات التي تم أألقيمتها بأستخدام مستشعرات أألنف أألكترونية. كل هذه البيانات من نتائج البحث أألتي أأل تحقيق أألأهداف هذا البحث.


الكلمات المفتاحية: المصادقة أألألية، أأليلاتين أأليلوز، تفاعل ميلارد، اللسان أألكتروني، أألنف أألكتروني، أألليل أألنصر أألأسي، أألليل أألأميز أألطي

APPROVAL PAGE

The thesis of Ismarti Muhammad Sohieb has been approved by the following:




Assoc. Prof. Dr. Noor Faizul Hadry Nordin
Supervisor



Prof. Dr. Eng. Kuwat Triyana, M. Si.
Co-Supervisor



Prof. Hamzah M. Salleh
Co-Supervisor



Dr. Nurrulhidayah A. Fadzillah
Co-Supervisor

Prof. Dr. Irwandi Jaswir
Internal Examiner

Assoc. Prof. Dr. Md. Monowarul Mobin Siddique
External Examiner

Prof. Dr. Othman Omran Khalifa
Chairman

DECLARATION

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.

(Ismarti Muhammad Sohieb)



Signature

Date: June 20th, 2022



COPYRIGHT PAGE

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

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

I declare that the copyright holders of this thesis are jointly owned by the student and IIUM.

Copyright © 2022 (Ismarti Muhammad Sohieb) and International Islamic University Malaysia. All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgment.
2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieved system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by (Ismarti Muhammad Sohieb)

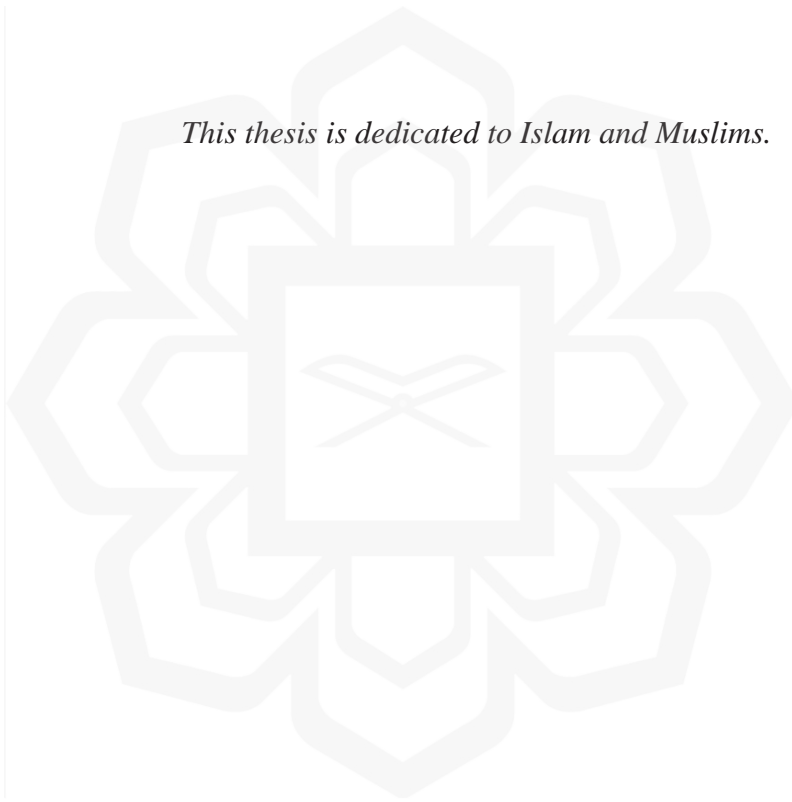


Signature

June 20th, 2022

Date

This thesis is dedicated to Islam and Muslims.



ACKNOWLEDGEMENTS

Praise be to Allah (SW), the Most Beneficial and Most Merciful, the Lord of all creation. Mercy and Peace be upon the Prophet Muhammad (PBUH). Gratefully, I thank Allah (SW) for giving me health, strength, determination and the ability to write my work.

My profound gratitude goes to my supervisors, Assoc. Prof. Dr. Noor Faizul Hadry Nordin (Main supervisor) and Prof. Dr. Eng. Kuwat Triyana, M.Si. (Co-supervisor) for spending a lot of time on advising and giving constant encouragements, contributions, supports, and deep insight regardless of their occupied responsibilities. To Prof. Hamzah M. Salleh (Co-supervisor) and Dr. Nurrulhidayah A. Fadzillah (Co-supervisor) who with faith and patients have given significant comments, clarification, suggestion, and encouragement throughout the completion of this thesis. To them, I convey the unforgettable motivation for giving me time to enable to complete the work.

I wish to express my appreciation and thanks to my husband, Agus Sulistyو for his prayer and continuous support, my children: Hasna Mazaya, Irsyad Abdirrohman, Abdulloh Umar, Utsman and also baby Rosyid Abdurrozzaq for allowing me to absent in their time growing up. Special thanks to my father (Muhammad Sohieb rahimahullah), who passed away when I'm not beside him, and my mother Suryanah rahimahallah, who struggles with her sick and allowing me to be able in this intellectual journey, and finally also passed away on September 2021, for that, I will be forever grateful.

Finally, For Indonesia Endowment Fund for Education (LPDP), thank you for financial support during my PhD study. For all my teachers, my friends, those who provided their time, effort and support for this project. To the members of my thesis committee, thank you for sticking with me.

Once again, praise be to Allah for His endless mercy on us one of which is enabling us to successfully round off the efforts of writing this thesis. Alhamdulillah.

TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval Page	v
Declaration	vi
Copyright	vii
Acknowledgements	ix
Table of Contents	x
List of Tables	xiv
List of Figures	xvi
List of Abbreviation	xviii
CHAPTER ONE: INTRODUCTION	1
1.1 BACKGROUND OF THE STUDY	1
1.2 STATEMENT OF THE PROBLEM.....	3
1.3 PURPOSE OF THE STUDY	4
1.4 RESEARCH QUESTIONS	4
1.5 RESEARCH OBJECTIVES	5
1.6 RESEARCH HYPOTHESIS	5
1.7 SIGNIFICANCE OF THE STUDY	6
1.8 LIMITATION OF THE STUDY	6
1.9 CHAPTER SUMMARY	6
CHAPTER TWO: LITERATURE REVIEW	8
2.1 INTRODUCTION	8
2.2 HALAL AND THAYYIB ASPECT IN FOOD PRODUCTION	8
2.2.1 Basic Concept of Halal Food	8
2.3 GELATINE.....	9
2.3.1 Sources of Gelatine	9
2.3.2 Structure and Characteristics of Gelatine	10
2.3.3 Methods for Gelatine Authentication	12
2.4 SIGNIFICANCE OF THE MAILLARD REACTION IN FOOD PRODUCTION	17
2.4.1 Introduction to the Maillard Reaction.....	17
2.4.2 The Factors that Affect the Maillard Reaction	18
2.4.3 Maillard Reaction Products	20
2.4.3.1 Coloured Polymeric Maillard Reaction Products	20
2.4.3.2 Flavour Compounds.....	21
2.5 SENSORY ANALYSIS FOR FOOD DETECTION AND CLASSIFICATION	24
2.5.1 Introduction to Sensory Analysis.....	24
2.5.2 Artificial Sensor for Food Authentication	25
2.5.2.1 Electronic Nose	26
2.5.2.2 Electronic Tongue.....	28
2.5.3 Application of E-nose and E-tongue in Halal Authentication ..	31
2.6 CHEMOMETRICS	33

2.6.1 Introduction to Chemometrics	33
2.6.2 The Utilization of PCA and LDA in Halal Authentication	34
2.7 CHAPTER SUMMARY	39

CHAPTER THREE: RESEARCH METHODOLOGY 40

3.1 INTRODUCTION	40
3.2 MATERIALS	40
3.3 INSTRUMENTATION	41
3.3.1 Electronic Tongue.....	41
3.3.2 Electronic Nose.....	42
3.4 EXPERIMENT PROCEDURES	44
3.4.1 Screening of Variables Influence the Browning Intensity of MRPs.....	44
3.4.1.1 Effect of Gelatine Solution Concentration.....	44
3.4.1.2 Effect of Xylose Concentration	44
3.4.1.3 Effect of Initial pH.....	45
3.4.1.4 Effect of Reaction Temperature.....	45
3.4.1.5 Effect of Heating Time	45
3.4.1.6 Measurement of Browning Intensity	45
3.4.2 Optimisation of Maillard Reaction Model.....	46
3.4.2.1 Design of Experiment	46
3.4.2.2 Measurement of Browning Intensity	47
3.4.2.3 Validation of The Model.....	47
3.4.3 Characterisation of Gelatine from Different Sources	47
3.4.3.1 Amino Acid Analysis using LC-MS.....	47
3.4.4 E-tongue Sensor Preparation and Testing.....	48
3.4.4.1 Sensor Preparation	48
3.4.4.2 Preparation of Basic Standard Taste Solution	48
3.4.4.3 Sensor Testing with Standard Tastes Solution.....	48
3.4.5 Differentiation of Gelatine Sources using E-tongue.....	49
3.4.5.1 Sample Preparation	49
3.4.5.2 Measurement of Samples.....	49
3.4.6 Differentiation of Gelatine Sources using E-nose	50
3.4.6.1 Sample Preparation	50
3.4.6.2 Measurement of Samples.....	50
3.4.7 Investigation of Active Sensory Compounds of Gelatine and MRPs.....	51
3.4.7.1 Non-Volatile Analysis Using HPLC.....	51
3.4.7.2 Volatile Analysis using SPME-GCMS.....	52
3.5 DATA ANALYSIS	53
3.5.1 Effect of Variable Reaction to the Browning Intensity	53
3.5.2 Optimisation of Maillard Reaction Model.....	53
3.5.3 Characterisation of Gelatine from Different Sources	54
3.5.4 E-tongue Data Pre-Processing and Analysis	54
3.5.5 E-nose Data Pre-Processing and Analysis	55
3.5.6 Investigation of Active Sensory Compounds	55
3.5.6.1 Non-Volatile Compounds Analysis	55
3.5.6.2 Volatile Compounds Analysis	56
3.5.7 Multivariate Statistic for Data Analysis	56

3.6 CHAPTER SUMMARY	57
CHAPTER FOUR: OPTIMISATION OF MAILLARD REACTION MODEL OF BOVINE GELATINE-XYLOSE MODEL SYSTEM USING RESPONSE SURFACE METHOD	59
4.1 INTRODUCTION	59
4.2 EFFECT OF VARIABLE REACTION TO THE BROWNING INTENSITY OF MRPS	59
4.2.1 Gelatine Solution Concentration.....	60
4.2.2 Xylose Concentration	61
4.2.3 Initial pH.....	62
4.2.4 Reaction Temperature.....	64
4.2.5 Heating Time	66
4.3 OPTIMISATION OF MAILLARD REACTION MODEL IN GELATINE-XYLOSE SYSTEM	68
4.3.1 Optimisation of The Model	68
4.3.2 Validation of the Model.....	72
4.4 CHAPTER SUMMARY	73
CHAPTER FIVE: DIFFERENTIATION OF BOVINE, PORCINE AND FISH GELATINE BY MAILLARD REACTION USING E- TONGUE COMBINED WITH CHEMOMETRICS	74
5.1 INTRODUCTION	74
5.2 CHARACTERISATION OF GELATINE FROM DIFFERENT ANIMAL SOURCES	74
5.2.1 Amino Acid Composition of Gelatine	74
5.3 E-TONGUE SENSOR'S PREPARATION AND TESTING	78
5.3.1 Potentiometric Signal Profile Data for Sensor Testing.....	78
5.3.2 Principle Component Analysis for Sensor Testing.....	83
5.4 DIFFERENTIATION OF GELATINE ASSISTED BY MAILLARD REACTION USING E-TONGUE	86
5.4.1 Potentiometric Signal Profiles Data Gelatine and The MRPs ..	86
5.4.2 Principle Component Analysis for E-tongue Data	94
5.4.3 Linear Discriminant Analysis for E-tongue Data	97
5.5 CHAPTER SUMMARY	99
CHAPTER SIX: DIFFERENTIATION OF BOVINE, PORCINE, AND FISH GELATINE BY MAILLARD REACTION USING E-NOSE COMBINED WITH CHEMOMETRICS	101
6.1 INTRODUCTION	101
6.2 DIFFERENTIATION OF GELATINE USING E-NOSE.....	101
6.2.1 Signal Processing Data E-nose	101
6.2.2 Principle Component Analysis for E-nose Data.....	117
6.2.3 Linear Discriminant Analysis for E-nose Data.....	120
6.3 CHAPTER SUMMARY	124
CHAPTER SEVEN: ACTIVE SENSORY COMPOUNDS OF BOVINE, PORCINE AND FISH GELATINE AND THE MRPs	125
7.1 INTRODUCTION	125

7.2 NON-VOLATILE COMPOUNDS ANALYSIS OF GELATINE AND THE MRPS USING HPLC.....	125
7.3 VOLATILE COMPOUND ANALYSIS OF GELATINE AND ITS MRPS USING SPME-GCMS	132
7.4 CHAPTER SUMMARY	145
CHAPTER EIGHT: CONCLUSION AND RECOMMENDATION	146
8.1 CONCLUSION AND IMPLICATION	146
8.2 RECOMMENDATION.....	148
REFERENCES.....	149
APPENDIX A: MANOVA FOR AMINO ACID ANALYSIS OF STANDARD GELATINE.....	167
APPENDIX B: VARIABLE SCREENING OF MAILLARD REACTION MODEL	173
APPENDIX C: DOE RESULT FOR OPTIMIZATION MAILLARD REACTION GELATINE-XYLOSE MODEL.....	178
APPENDIX D: FEATURE EXTRACTION DATA AND SUMMARY MULTIVARIATE ANALYSIS OF POTENTIOMETRIC SIGNAL PROFILE OF E-TONGUE FOR SENSOR TESTING	185
APPENDIX E: FEATURE EXTRACTION DATA AND SUMMARY MULTIVARIATE ANALYSIS OF POTENTIOMETRIC SIGNAL PROFILE OF E-TONGUE FOR GELATINE SAMPLES	202
APPENDIX F: FEATURE EXTRACTION DATA AND SUMMARY MULTIVARIATE ANALYSIS OF POTENTIOMETRIC SIGNAL PROFILE OF E-NOSE	242
APPENDIX G: FREE AMINO ACID ANALYSIS USING HPLC	286
APPENDIX H: VOLATILE COMPOUND ANALYSIS USING SPME-GC- MS	306
APPENDIX I: LIST OF PUBLICATION	310
INDEX.....	318

LIST OF TABLES

Table 2.1	Effectiveness and limitation of various analytical techniques for gelatine authentication	16
Table 2.2	Utilization of PCA and LDA in halal authentication	36
Table 3.1	Composition of the lipid-polymeric membrane of each sensor	42
Table 4.1	ANOVA for effect of gelatine solution concentration to pH	61
Table 4.2	ANOVA for effect of xylose concentration to browning intensity	62
Table 4.3	ANOVA for the effect of initial pH to browning intensity	63
Table 4.4	ANOVA for effect of reaction temperature to the browning intensity	65
Table 4.5	ANOVA for effect of heating time to the browning intensity	67
Table 4.6	Central composite design for measurement of browning intensity	69
Table 4.7	ANOVA for response surface linear model	70
Table 4.8	ANOVA for response surface reduced linear model	71
Table 4.9	Result of predicted and actual data at optimum condition for browning intensity	73
Table 5.1	Amino acid composition in standard gelatine (w/w)	75
Table 5.2	MANOVA for the amino acid composition of gelatine	76
Table 5.3	Test between the subjects affected by amino acids in gelatine	77
Table 5.4	Descriptive statistic for sensor response of standard basic taste solution	79
Table 5.5	MANOVA for standard basic tastes solution	81
Table 5.6	Between subject analysis using ANOVA for standard basic taste solutions	82
Table 5.7	Extracted Eigen-value of the correlation matrix for sensor responses of the standard basic taste solutions	84
Table 5.8	Descriptive statistics data of sensor response for gelatine and the MRPs	87
Table 5.9	MANOVA test for gelatine	91
Table 5.10	MANOVA test for MRPs	91
Table 5.11	Test of Between-subjects effects for gelatine analysis	92

Table 5.12	Test of Between-subjects effects for the MRPs	93
Table 5.13	Accuracy of internal and external validation for each LDA model	99
Table 6.1	Descriptive statistics data for E-nose measurement using wet method	102
Table 6.2	MANOVA for gelatine under the wet method	106
Table 6.3	MANOVA for the MRPs under wet method	106
Table 6.4	Test of between- subjects effect of gelatine under wet method	107
Table 6.5	Test of between- subjects effect of the MRPs under wet method	108
Table 6.6	Descriptive statistics data for E-nose measurement using dry method	109
Table 6.7	MANOVA for gelatine under dry method	113
Table 6.8	MANOVA for the MRPs under dry method	114
Table 6.9	Test of between-subject effects for gelatine under dry method	115
Table 6.10	Test Between-subjects effects of the MRPs under dry method	116
Table 6.11	Accuracy of external and internal validations for each method using E-nose-LDA model	123
Table 7.1	Summary of standard amino acid analysis	126
Table 7.2	Free amino acid detected in different samples gelatine and MRPs	128
Table 7.3	MANOVA for FAA analysis of gelatine	130
Table 7.4	Test of between-subjects effect for the FAA of gelatine	130
Table 7.5	MANOVA for FAA analysis of the MRPs	131
Table 7.6	Test of between-subjects effects for the FAA of the MRPs	132
Table 7.7	Relative content of group of volatile compounds detected in gelatine and the MRPs	134
Table 7.8	Volatile compounds detected in gelatine and the MRPs from different sources of origin	137

LIST OF FIGURES

Figure 2.1	Scheme of the Maillard reaction	18
Figure 2.2	Scheme of flavour compounds formation and end products of Maillard reaction	22
Figure 2.3	Schematic diagram of E-nose	27
Figure 2.4	Schematic diagram of E-tongue	29
Figure 3.1	The E-tongue system components.	41
Figure 3.2	The E-nose system components	43
Figure 3.3	Sensor response of E-nose during the measurement process	51
Figure 4.1	pH of gelatine solution at various concentration	60
Figure 4.2	Browning intensity of MPRs at various xylose weights	61
Figure 4.3	Browning intensity of MPRs at variation initial pH	63
Figure 4.4	Browning intensity of MPRs at the variation reaction temperature	65
Figure 4.5	Browning intensity of MPRs at various heating times	66
Figure 4.6	Dependency of browning intensity toward the initial pH and temperature at a constant heating time	72
Figure 5.1	Sensor response for five basic standard taste solution	80
Figure 5.2	PCA plots for basic standard taste solutions	85
Figure 5.3	Radar plot for gelatine from E-tongue analysis	89
Figure 5.4	Radar plot for MPRs from E-tongue analysis	90
Figure 5.5	PCA plots of gelatine	95
Figure 5.6	PCA plots of MPRs	96
Figure 5.7	Linear Discriminant Analysis of gelatine for E-tongue analysis	98
Figure 5.8	Linear Discriminant Analysis of MPRs for E-tongue analysis	98
Figure 6.1	Radar plot for gelatine and the MPRs for wet method from E-nose analysis.	105
Figure 6.2	Radar plot for gelatine and MPRs for dry method from E-nose analysis..	113
Figure 6.3	PCA plots of (a) gelatine and (b) MPRs in wet method	118
Figure 6.4	PCA plot of (a) gelatine and (b) MPRs in dry method	119
Figure 6.5	LDA plots of (a) gelatine (b) MPRs using the wet method	121

Figure 6.6 LDA plots of (a) gelatine (b) MRPs using the dry method 122

Figure 7.1 Total number of volatile compounds detected in bovine, fish and porcine gelatine and its MRPs 133



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-buthylpentyl) 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 (Tongdeesontorn & 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, nutraceuticals, 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.