UNIVERSITI PUTRA MALAYSIA

*DEVELOPMENT OF PROTEOMIC TECHNIQUE FOR POTENTIAL
SPECIES-SPECIFIC PEPTIDE MARKER DISCOVERY OF COMMERCIAL
SEASONING CUBES*

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DEVELOPMENT OF PROTEOMIC TECHNIQUE FOR POTENTIAL SPECIES-SPECIFIC PEPTIDE MARKER DISCOVERY OF COMMERCIAL SEASONING CUBES

By

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DEVELOPMENT OF PROTEOMIC TECHNIQUE FOR POTENTIAL SPECIES-SPECIFIC PEPTIDE MARKER DISCOVERY OF COMMERCIAL SEASONING CUBES

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October 2020

Chairman : Professor Shuhaimi bin Mustafa, PhD
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Recent years, concerns regarding food product authentication particularly in meat-based product has been alarming. The development of proteomic technique in food authentication and their application to determine quality and safety issues of the ingredients has been progressively investigated by fellow researchers. The complexity of matrices in commercial seasoning cubes have given challenge in developing authentication method. Hence, the present study has been carried on developing protein extraction method on commercial seasoning cubes and generates electrophoretic profiles of chicken, beef, pork and anchovies commercial seasoning cubes and also to determine potential species-specific peptide marker in the commercial seasoning cubes using LC MS/MS analysis. Four flavors of seasoning cubes (Chicken, Beef, Anchovies and Pork) from three brands (T, K and M) were purchased. The efficacy of urea-thiourea extraction was studied using concentration of extracted protein from seasoning cubes. The electrophoretic profile of seasoning cubes of different species was analyzed using SDS-PAGE combined with principle component analysis (PCA). LC MS/MS analysis was developed to identify species-specific peptide markers in commercial seasoning cubes. Finding shows, the efficacy of urea-thiourea solvent extraction on commercial seasoning cubes was depending on species. Based on electrophoretic profile combined with PCA, seasoning cubes of four (flavors) species share similar protein molecular weight and group closely together. In this study, two porcine-specific peptide markers derived from hemoglobin subunit α and β, respectively have been successfully identified in pork commercial seasoning cubes. Also, one bovine-specific peptide marker derived from hemoglobin subunit α has been successfully identified in beef commercial seasoning cube. Meanwhile, two actinoperygii (bony fish)-specific peptide marker were identified in anchovies commercial seasoning cube and one aves (bird)-specific peptide marker was identified in chicken commercial seasoning cube. The methodology developed in this study has been proved to be successful in identifying species-specific peptide markers in seasoning cubes. Findings from this study can be a reference in validating of food authenticity especially in commercial seasoning products.

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

PEMBANGUNAN TEKNIK PROTEOMIK BAGI MENCARI POTENSI SPESIES-SPESIFIK PENANDA PEPTIDA DALAM KIUB PERASA KOMERSIAL

Oleh

UMMU NASUHA BINTI MOHD ASRI

Oktober 2020

Pengerusi : **Profesor Shuhaimi bin Mustafa, PhD**
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Dasawarsa ini, keprihatinan mengenai pengesahan makanan pada produk berasaskan daging semakin meningkat. Perkembangan teknik proteomik dalam pengesahan makanan dan menentukan kualiti dan keselamatan telah disiasat secara progresif oleh golongan penyelidik. Kerumitan matriks dalam kiub perasa komersial telah memberikan cabaran dalam mengembangkan kaedah pengesahan. Oleh itu, kajian ini bertujuan membangunkan teknik pengekstrakan protein dari kiub perasa komersial dan menjana profil elektroforesis bagi kiub perasa komersial dan seterusnya penemuan spesies-spesifik penanda peptida dalam kiub perasa komersial menggunakan analisis LC MS/MS. Kiub perasa komersial dari tiga jenama (T, K dan M) dan terdiri dari perasa (Ayam, Lembu, Ikan bilis dan Babi) telah dikumpulkan. Keberkesanan pengekstrakan urea-thiourea dikaji dengan menggunakan kepekatan protein yang diekstrak dari kiub perasa. Profil elektroforesis kiub perasa dari spesies yang berlainan dianalisis menggunakan SDS-PAGE yang digabungkan dengan analisis prinsip komponen(PCA). Analisis LC MS / MS diguna pakai untuk mengenal pasti spesies-spesifik penanda peptida dalam kiub perasa komersial. Kajian ini mendapati, keberkesanan penggunaan pelarut urea-thiourea pada kiub perasa komersial bergantung kepada spesies. Berdasarkan profil elektroforetik yang digabungkan dengan analisis PCA, kiub perasa dari empat (perisa) spesies berkongsi saiz molekul protein dan tergolong dalam kumpulan yg hampir sama. Dalam kajian ini, dua spesies-spesifik penanda peptida babi yang berasal dari subunit hemoglobin α dan β telah berjaya dikenal pasti. Satu penanda peptida spesifik lembu yang berasal dari subunit hemoglobin α berjaya dikenal pasti dalam kiub perasa komersial daging lembu. Sementara itu, dua penanda peptida spesifik actinoperygii (ikan bertulang) dikenal pasti dalam kiub perasa komersial ikan bilis dan satu penanda peptida spesifik aves (burung) dikenal pasti dalam kiub perasa komersial ayam. Metodologi yang dibangunkan dalam kajian ini telah terbukti berjaya mengenal pasti spesies-spesifik penanda peptida dalam kiub perasa. Penemuan dari kajian ini boleh menjadi rujukan dalam mengesahkan kesahihan makanan terutama dalam produk perasa komersial.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

CO1	Cytochrome Oxidase 1
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked Immunosorbant Assay
LC-MS	Liquid Chromatography Mass Spectrometry
LC-TOF-MS	Liquid Chromatography Time-of-Flight Mass Spectrometry
MS	Mass Spectrometry
MAG	Monoacylglycerol
PFF	Peptide Fragmentation Fingerprinting
PMF	Peptide Mass Fingerprinting
PCR	Polymerase Chain Reaction
PTM	Post-Translational Modification
RFLP	Restriction Fragment Length Polymorphism
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
TAG	Triacylglycerol

CHAPTER 1

INTRODUCTION

Food seasonings are ingredients that are added to foods to enhance their flavor and taste. Enhancing the natural flavor and taste of food is part of the art of cooking. Everyone like a delicious and flavorful dishes either when they cook it themselves or visits a food vendor. Seasoning cube is one of the seasoning product and it is very well known and widely used in preparing food as its help to minimizes time. Seasoning cube or also known as bouillon cube. It is comprising of salt, sugar, flavor enhancer, herbs, spices and vegetable and it is made of mostly dry ingredient. Recent years, increases concerns regarding food product has been noticed particularly in meat-based product. As for seasoning cube, it has been claimed to have 2.5% of meat content and almost 97.5% is additive ingredients (Australia Patent No. WO 2006/063690 A1, 2006) Seasoning cube has been highly demanded worldwide. In order to persist in the highly competitive market and to obtain excessive profit, unfair labeling of Halal product is frequently happening (Yaakob, et. al, 2012). Besides, scientific intervention and packaging technologies have further made the identification of food components extremely difficult. Hence, a sensitive, easily performable, and reliable scientific method is highly anticipated to verify the ingredients in food product.

Food adulteration and fraud is driven by an increasingly fierce competition throughout the food industry (Li et al., 2018).Examples of common frauds are replacing key ingredients with cheaper alternatives and mislabeling of the animal species used in a food product. The non-declared introduction of food ingredients such as toxic or allergenic products may be harmful to consumer's health, thus representing a potential public health risk. Therefore, food safety and the assurance of the quality is becoming a crucial task in the international agenda of health organization. In order to overcome all these problems, the development of accurate and reliable food authentication is much needed.

Furthermore, sources of the products become significant concern when it deals with aspect of religion, health and diet. The usage of porcine derivatives is forbidden for Muslims and Jews while for Hindus, they are obliged not to consume cow as it is considered sacred to their custom. Moreover, it was also reported that most food allergic patients develop allergic reactions towards bovine and porcine derivatives, but do not react to fish derivatives (Muyonga, et. al, 2004). Hence, products sources must be labeled appropriately because once it has been manufactured and commercially trade it will be difficult to ensure its authentication whether it has been mixed or cross-contaminated.

Proteomics are one of the technologies that increasingly being used to profile expressed protein in food. The latest advances in proteomic technologies are in mass spectrometry, the creation of bioinformatics tools, spectral libraries and peptide database. These are

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proposing new chapter for the development of protein biomarkers (Peterman, 2017). During the many stages of food production, proteins can act as ideal indicators for many properties associated with food quality, composition, or origin. Proteome analysis can be applied for the systematic search for new marker proteins/peptides, thus accelerating the development of assays to detect adulteration and deceptive practices.

There are many different analytical methods have been used in food authentication such as chromatography, trace element analysis, genomic and proteomic. Recently, proteomic analysis has been successfully applied for the systematic search of new peptide markers. This might help the development of the database to detect adulteration and deceptive practices. There are several studies reported to be able to identify specific species peptide marker using proteomic analysis; Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) and Liquid Chromatography (Ballin, 2010; Montowska and Pospiech, 2013; Sarah et al., 2016). However, there is no study reported on proteomic technique authentication of animal species in commercial seasoning cubes. This might be due to the complexity of the food matrices. The studies regarding seasoning cubes were mostly focused on health concern and manufacturing process (Okeahialam and Bonifacenduru, 2015).

Hence, with the realization on the importance of the authentication of seasoning cubes, the general objective of this study was directed toward developing protein extraction method on commercial seasoning cubes and generating electrophoretic profiles of chicken, beef, pork and anchovies, and also determination of potential species-specific peptide marker in the seasoning cubes. It is hypothesized that denaturing buffer could efficiently extract proteomic-grade protein from commercial seasoning cubes. As well, LC-MS/MS would be able to identify potential species specific peptide marker in commercial food product.

Based on the above hypothesis the specific objective of the study in this thesis were to:

1. Develop method for protein extraction of commercial seasoning cubes.
2. Explore electrophoretic profiles via one dimensional SDS-PAGE and Principal Component Analysis (PCA).
3. Identify species-specific peptide marker of Chicken, Beef, Pork and Anchovies seasoning cubes via LC-MS/MS.

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