EFFECT OF MECHANICAL WORK AND SELECTED PLANT PROTEASES IN THE PRODUCTION OF BUFFALO MEAT PATTIES

By

MOHAMAD AFIFI ISMAIL

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Chairman : Ismail Fitry Mohammad Rashedi, PhD
Faculty : Food Science and Technology

Buffalo meat has been used widely in meat processing due to its good binding properties with high protein and low-fat content. However, the major problem rise in the industry is associate with the toughness of the buffalo meat. Therefore, several attempts had been done to improve the palatability of the tough meat by using physical and enzymatic treatment. The lack of studies that explain the relationship of mechanical process and plant protease incorporation on the physicochemical and sensory of buffalo meat patties has led to this study. The objectives of the present work were (i) to evaluate the physicochemical, sensorial properties and microstructural of buffalo meat patties produced using different mixing equipment (bowl cutter, universal mixer, and meat mixer), (ii) to determine the protease activities in selected plant extracts (ambarella, candlenut, carambola, ginger, jujube, kiwifruit and lemon) and their marination effects on the buffalo meats, and (iii) to evaluate the effects of mechanical work of mixing using the universal mixer and selected plant proteases (kiwifruit and candlenut) on the physicochemical, sensorial and microstructural properties of buffalo meat patties. In the first objective, the textural and sensorial properties of patties produced using the universal mixer were in between the patties produced using the bowl cutter and meat mixer. Besides that, scanning electron microscopy revealed slightly less homogeneity with a large number of pores, and less disruption of patties using the universal mixer. Therefore, the universal mixer was selected to be used for the third objective based on the results obtained. In the second objective, candlenut possessed the highest protease activities at 8.19 U/mL while kiwifruit and ginger showed similar protease activity in the range between 1.41 to 1.67 U/mL. The treatment using plant extract had decreased the water holding capacity of buffalo meat except for ginger. All treatments had increased the Trichloroacetic acid (TCA) soluble peptides content except carambola. Candlenut and jujube treatment had increased the

total soluble protein by 38.08% and 32.11%, and salt soluble protein by 7.16% and 8.07%, meanwhile kiwifruit treated meats had the highest collagen solubility at 31.74%. A significant improvement in Warner-Bratzler shear force value was observed in kiwifruit, candlenut and lemon treated samples by 59.74%, 28.07% and 18.95%, respectively. Overall, kiwifruit showed a higher tenderizing effect while candlenut possessed higher protease activity; therefore, both kiwifruit and candlenut were selected to be used for the third objective. In the third objective, marinating buffalo meat with kiwifruit and candlenut extract resulted in increases in the gel strength by 56.81% and 48.13%, and a significant improvement in binding properties. Meanwhile, the addition of kiwifruit and candlenut extract had increased cooking loss by 22.43% and 24.32%, as well as a higher total soluble protein at 79.09 mg/g and 79.40 mg/g raw meat, respectively. The buffalo meat patties added with kiwifruit extract had the lowest hardness value at 46.64, gumminess value at 32.27 and chewiness value at 27.60. The addition of kiwifruit also produced the highest score for tenderness at 7.56. Treatment of protease regardless by addition or marination had disintegrated the protein structure which was revealed by scanning electron microscopy. Overall, using the universal mixer was found to be the most effective in providing a good binding effect, improved sensorial properties and less disruption in microstructural of buffalo meat patties. In enzymatic treatment, kiwifruit and candlenut gave a high tenderizing effect and higher protease activity, respectively. Lastly, the application of the universal mixer together with kiwifruit and candlenut plant protease have led to an improved microstructural, physicochemical properties and palatability of buffalo meat patties. Hence, the present study shows that the application of protease combined with mechanical work of mixing can be a new potential method in producing meat products such as patties.

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

KESAN PENGGUNAAN KAEDAH MEKANIKAL DAN PROTEASE TERPILIH DALAM MENGHASILKAN BURGER DAGING KERBAU

Oleh

MOHAMAD AFIFI ISMAIL

Julai 2021

Pengerusi : Ismail Fitry Mohammad Rashedi, PhD
Fakulti : Sains dan Teknologi Makanan

Daging kerbau telah digunakan secara meluas dalam pemprosesan daging kerana ciri pengikatnya yang baik dengan kandungan protein yang tinggi dan rendah lemak. Walau bagaimanapun, terdapat masalah utama yang berlaku dalam industri iaitu disebabkan oleh daging kerbau yang keras. Oleh itu, beberapa usaha telah dilakukan bagi menambahbaik ciri daging yang keras tersebut melalui kaedah fizikal dan enzimatik. Kurangnya kajian yang menjelaskan hubungkait proses mekanikal dan penggunaan protease tumbuhan terhadap fizikokimia dan sensori burger daging kerbau telah mendorong kepada kajian ini. Objektif kajian ini adalah (i) untuk menilai ciri fizikokimia, sensori dan mikrostruktur burger daging kerbau yang dihasilkan menggunakan mesin pengadun yang berbeza (mangkuk pengadun pemotong daging, pengadun serbaguna, dan pengadun daging), (ii) untuk menentukan aktiviti protease dalam ekstrak tumbuhan terpilih (kedondong, buah keras, belimbing, halia, kurma merah, buah kiwi dan lemon) dan kesan perapannya terhadap daging kerbau, dan (iii) untuk menilai kesan penggunaan kaedah pengadunan mekanikal (mesin pengadun serbaguna) dan protease tumbuhan yang terpilih (buah kiwi dan buah keras) terhadap ciri fizikokimia, sensori dan mikrostruktur burger daging kerbau. Untuk objektif pertama, ciri tekstur dan sensori burger yang dihasilkan menggunakan mesin pengadun serbaguna berada pada pertengahan antara burger yang dihasilkan menggunakan mangkuk pengadun pemotong daging dan mesin pengadun daging. Selain itu, melalui pengimbasan elektron mikroskopi menunjukkan kurang keseragaman serta terdapat banyak rongga, dan kurang kehancuran pada burger yang dihasilkan menggunakan mesin pengadun serbaguna. Oleh itu, mesin pengadun serbaguna telah dipilih untuk digunakan pada objektif ketiga berdasarkan hasil yang diperolehi. Untuk objektif kedua, buah keras memiliki aktiviti protease paling tinggi pada nilai 8.19 U/mL manakala buah kiwi dan halia

menunjukkan aktiviti protease yang sama dalam lingkungan antara 1.41 hingga 1.67 U/mL. Perapan menggunakan ekstrak tumbuhan telah mengurangkan keupayaan mengekalkan air bagi sampel daging kerbau kecuali ekstrak halia. Kesemua perapan telah meningkatkan kandungan peptida larut asid Trichloroacetic (TCA) kecuali ekstrak belimbing. Perapan buah keras dan kurma merah telah meningkatkan jumlah kelarutan protein sebanyak 38.08% dan 32.11%, dan kelarutan protein yang larut dalam garam sebanyak 7.16% dan 8.07%, sementara itu sampel daging yang diperap dengan ekstrak buah kiwi memiliki kelarutan kolagen paling tinggi sebanyak 31.74%. Peningkatan yang ketara pada nilai daya ricih Warner-Bratzler telah dicatatkan bagi sampel daging yang diperap dengan buah kiwi, buah keras dan lemon masing-masing sebanyak 59.74%, 28.07% dan 18.95%. Secara keseluruhan, buah kiwi menunjukkan kesan melembutkan daging yang lebih tinggi manakala buah keras mempunyai aktiviti protease yang lebih tinggi; oleh itu, buah kiwi dan buah keras dipilih untuk digunakan dalam objektif ketiga. Untuk objektif ketiga, perapan daging kerbau dengan ekstrak buah kiwi dan buah keras telah meningkatkan kekuatan gel sebanyak 56.81% dan 48.13%, dan mencatatkan peningkatan yang ketara dari segi daya pengikat. Manakala penambahan ekstrak buah kiwi dan buah keras telah meningkatkan kadar penurunan berat selepas masak sebanyak 22.43% dan 24.32%, serta jumlah kelarutan protein yang lebih tinggi masing-masing pada 79.09 mg/g dan 79.40 mg/g daging mentah. Burger daging kerbau yang ditambahkan dengan ekstrak buah kiwi mempunyai nilai kekerasan terendah pada 46.64, nilai keanjalan pada 32.27 dan nilai kekenyalan pada 27.60. Penambahan ekstrak buah kiwi juga menghasilkan skor kelembutan tertinggi iaitu 7.56. Rawatan protease samada melalui kaedah penambahan atau perapan telah menjejaskan struktur protein yang terbukti melalui pengimbasan elektron mikroskopi. Secara keseluruhan, penggunaan mesin pengadun serbaguna didapati paling berkesan dalam memberikan kesan daya pengikat yang baik, meningkatkan nilai sensori dan mengurangkan kehancuran pada mikrostruktur burger daging kerbau. Dalam rawatan enzimatik, buah kiwi dan buah keras memberikan kesan kelembutan yang tinggi dan aktiviti protease yang lebih tinggi. Akhir sekali, penggunaan mesin pengadun serbaguna bersama dengan protease buah kiwi dan buah keras telah menghasilkan peningkatan dari segi ciri mikrostruktur, fizikokimia dan sensori burger daging kerbau. Oleh itu, kajian ini menunjukkan bahawa penggunaan protease yang digabungkan dengan pengadunan mekanikal mampu menjadi satu kaedah terbaru yang berpotensi dalam menghasilkan produk daging seperti burger.

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Ismail Fitry bin Mohammad Rashedi, PhD
Senior Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Chong Gun Hean, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 November 2021

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Name and Matric Number: Mohamad Afifi Ismail

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Signature: _____
Name of Chairman of Supervisory Committee: Dr. Ismail Fitry Mohammad Rashedi

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Chong Gun Hean

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LIST OF ABBREVIATIONS

ºC	Degree celsius
kg	Kilogram
g	Gram
U	Enzyme unit
µmol	Micromole
ppm	Part per million
rpm	Revolution per minute
kDa	Kilo Dalton
L	Litre
mL	Millilitre
M	Molar
mm	Millimetre
cm	Centimetre
mm/s	Millimetre per second
min	Minutes
%	Per cent
GRAS	Generally recognized as safe
DMAB	Dimethylamino-benzaldehyde
LNPE	Nα-CBZ-*L*-lysine *p*-nitrophenyl ester
HCl	Hydrochloric acid
TCA	Trichloroacetic acid
SPP	Sodium pyrophosphate
STPP	Sodium tripolyphospate
$CaCl_2$	Calcium chloride
NaCl	Sodium chloride
BSA	Bovine serum albumin
WBSF	Warner-Bratzler shear force
CSA	Cross-sectional area
SEM	Scanning electron microscopy

MFI	Myofibrillar fragmentation index
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
WHC	Water holding capacity
TFR	Total fluid release
WR	Water release
FR	Fat release
IMCT	Intermuscular connective tissues
TSP	Total soluble protein
SSP	Salt soluble protein
WSP	Water-soluble protein

CHAPTER 1

GENERAL INTRODUCTION, PROBLEM STATEMENT AND RESEARCH OBJECTIVES

1.1 General Introduction

Consumption of meat products particularly patties had increased over the years. This kind of processed meat product is usually composed of a certain amount of meat protein and fat, and other non-meat ingredients (Heydari et al.,2016; Tornberg, 2013). Processing of patties requires meat protein with good binding properties to form a stable meat emulsion. In terms of good binding properties, buffalo meat was proven to have good emulsifying and water holding capacity (Devadason et al., 2014). Besides that, buffalo meat is also high in protein and low in fat content (Tateo et al., 2007). Therefore, buffalo meat is used widely in meat processing such as burgers, sausages and nuggets (Sachindra et al., 2005; Modiet al., 2004).

The processing of patties involves several steps starting from cutting, mincing, mixing and forming. Notably, these processing steps had a great influence on the properties of the final product, especially the mixing step. Lachowicz et al. (2003) found that mechanical work involves during the mixing could loose and disrupt the muscle fibres, thus improve the palatability. Besides that, the mixing also facilitated the extraction of myofibrillar protein, thus induced gelation and binding properties of processed meat products (Zhou et al., 2018; Xiong, 2005). Equipment such as bowl cutter, universal mixer or meat mixer are normally used for the mixing process. The mechanical work of the bowl cutter that consist of high-speed rotating knives produces very fine particles that is essential for protein solubilisation and the formation of stable emulsion (Zhou et al., 2018; Ducept et al., 2012). In contrast, a universal mixer operates based on the blade rotation that creates frictions between meat particles and other ingredients which had facilitate the protein solubilisation thus produce homogenised emulsion (Allais, 2010). Meanwhile, meat mixer provides gentler mixing process with vertical rotation of the mixing paddle, which eventually provided adequate time for protein solubilisation (Heinz & Hautzinger, 2007).

Over the years, the processing methods have been regularly modified to improve the quality attributes of meat products. One of the recent technique is the incorporation of exogenous protease to improve the palatability of tough meats. Abdel-Naeem and Mohamed (2016) found that the addition of ginger in camel meat burgers resulted in extensive fragmentation of myofibrils, increases collagen solubility and sensory properties. Meanwhile, Habib et al., (2018) reported that the incorporation of pomegranate rind powder in buffalo nuggets

had improved the emulsion stability and sensory attributes. Thus, the application of protease during processing could allow thorough distribution and maximize the tenderizing effect.

1.2 Problem Statement

1) Even though buffalo meat is used widely in meat processing, there is a problem associated with buffalo meat which is its toughness. The tough and coarse texture of buffalo meat is due to the collagen in connective tissue that becomes more complex and stronger when the animal age progresses (Kandeepan et al., 2009). In that case, several attempts had been done to improve the palatability of the tough meat mostly by using physical and enzymatic treatment. The mechanical work involves in meat processing such as cutting and grinding help to break down and reduce the connective tissue structure thus make it less intact (Zochowska-Kujawska et al., 2007). Meanwhile, mechanical work of mixing help to loosen the muscle fibers which subsequently facilitate protein solubilization (Lachowicz et al., 2003). Mixing equipment such as bowl cutter, universal and meat mixer provide different mechanical approaches. Therefore, various mechanical work of mixing using different equipment could lead to different patties attributes, however, the comparison in terms of quality and characteristics are yet to be tested scientifically.

2) Another technique used to reduce meat toughness is the treatment with exogenous protease which is also known as tenderizer (Pietrasik & Shand, 2011; Sullivan & Calkins, 2010). The meat is treated with exogenous protease either by infusion, injection or marination (Liu et al., 2011). The application of exogenous protease had improved meat tenderness by degrading myofibrillar protein and hydrolyzing connective tissue (Maqsood et al., 2018; Moon, 2018). Plant proteases such as papain, bromelain and ficin are widely available in the markets which had been used commercially in households and industries. Apart from these well-known proteases, several other plants show potential to be used for meat tenderization. Traditionally, plants such as ambarella, candlenut, carambola and jujube had been used as cooking ingredients of meat intentionally for flavouring as well as tenderizing the meat. The use of this plant in meat had been practiced for many years in some parts of the world, but not many reports were found. Thus, it is important to study the potential of these plants as meat tenderizers as a means of providing scientific evidence.

3) Most of the previous studies conducted were focusing on the utilization of proteolytic enzymes to tenderize the fresh meat as a pre-treatment. However, there are very limited studies on the use of these enzymes when incorporated as one of the raw ingredients in meat products during the processing. Until now, there are lack of studies that explain the relationship of mechanical process and plant protease incorporation on the physicochemical and sensory of buffalo meat patties.

1.3 Research Objectives

Therefore, the objectives of the present work were as follows:

1) To evaluate the physicochemical, sensorial and microstructural properties of buffalo meat patties produced using different mixing equipment (bowl cutter, universal mixer, and meat mixer)

2) To determine the protease activities in selected plant extracts (ambarella, candlenut, carambola, ginger, jujube kiwifruit and lemon) and their marination effects on the buffalo meats.

3) To evaluate the effects of mechanical work of mixing using the universal mixer and plant protease (kiwifruit and candlenut) application on the physicochemical, sensorial and microstructural properties of buffalo meat patties.

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