EFFECTS OF PALM FATS ON PHYSICOCHEMICAL PROPERTIES OF MYOFIBRILLAR PROTEIN IN THE PRODUCTION OF LOW-FAT SAUSAGE

NURUL SHAEERA BINTI SULAIMAN

FACULTY OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2023

EFFECTS OF PALM FATS ON PHYSICOCHEMICAL PROPERTIES OF MYOFIBRILLAR PROTEIN IN THE PRODUCTION OF LOW-FAT SAUSAGE

NURUL SHAEERA BINTI SULAIMAN

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF FOOD SCIENCE AND NUTRITION
UNIVERSITI MALAYSIA SABAH
2023

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL : **EFFECTS OF PALM FATS ON PHYSICOCHEMICAL PROPERTIES OF MYOFIBRILLAR PROTEIN IN THE PRODUCTION OF LOW-FAT SAUSAGE**

IJAZAH : **SARJANA SAINS**

BIDANG : **TEKNOLOGI MAKANAN**

Saya **NURUL SHAEERA BINTI SULAIMAN**, Sesi **2021-2023**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak milik Universiti Malaysia Sabah
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
[ ]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[/]	TIDAK TERHAD	

Disahkan Oleh,

NURUL SHAEERA BINTI SULAIMAN
MN2011013T

(Tandatangan Pustakawan)

Tarikh : 30 Jun 2023

(Prof. Madya Dr. Wolyna Pindi)
Penyelia Utama

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries, and references, which have been duly acknowledged.

31 March 2023

Nurul Shaeera binti Sulaiman
MN2011013T

CERTIFICATION

NAME : **NURUL SHAEERA BINTI SULAIMAN**

MATRIC NUM. : **MN2011013T**

TITLE : **EFFECTS OF PALM FATS ON PHYSICOCHEMICAL PROPERTIES OF MYOFIBRILLAR PROTEIN IN THE PRODUCTION OF LOW-FAT SAUSAGE**

DEGREE : **MASTER OF SCIENCE**

FIELD : **FOOD TECHNOLOGY**

VIVA DATE : **31 MARCH 2023**

CERTIFIED BY;

Signature

SUPERVISOR
Assoc. Prof. Dr. Wolyna Pindi

ACKNOWLEDGEMENT

First and foremost, I am thankful to Allah for giving me the strength, knowledge, ability, and opportunity to undertake this study and complete it satisfactorily.

I want to acknowledge and thank my respected supervisor, Assoc. Prof. Dr. Wolyna Pindi, who made this work possible. Her guidance and advice carried me through all the stages of my research. I would also like to thank Dr. Md. Jahurul Haque Akanda and Dr. Mohd Dona Sintang, whose worthy comments and suggestions are appreciable in completing this dissertation. Not to mention the Dean, lecturers, and staff of the Faculty of Food Science and Nutrition, who helped me in one way or another as I worked to finish my thesis.

Additionally, I would want to express my gratitude to my parents, Sulaiman Liman and Dayang Muyong, as well as my entire family for their unwavering support and understanding as I undertook my study. Your prayer for me was what sustained me this far.

I want to sincerely thank Biasiswa Kerajaan Negeri Sabah for making my academic path possible. I was thrilled to learn of my selection for this scholarship, and I am deeply appreciative of your support. I would also like to extend my appreciation to the Malaysian Palm Oil Board for providing the palm fats needed in this research. Finally, I want to express my gratitude to my friends for their help and encouragement during this research.

Nurul Shaeera binti Sulaiman
31 March 2023

ABSTRACT

This research was conducted to better understand the impacts of lipid type on the gel formation by chicken myofibrillar proteins (MP) in low-fat sausage. This study provides new insights to overcome challenges in producing reduced-fat meat products by examining the potential application of refined palm oil (RPO) and palm stearin (ST) as chicken fat (CF)substitutes. The MP gelation influenced by three lipids: CF (MP-CF), RPO (MP-RPO), and ST (MP-ST), at high-fat levels, was examined through physicochemical and thermal analyses. RPO was chosen as the best animal fat substitute because it has the strongest elastic gel properties with the highest thermal stability ($p < 0.05$) throughout the cooling and heating stages. It also provides the highest water holding capacity (WHC) and gel strength ($p < 0.05$). When the effects of RPO addition were tested at low-fat concentrations [1.5% (RPO1.5), 2.0% (RPO2.0), 2.5% (RPO2.5), and 3.0% (RPO3.0)], it was found that the gels appeared to be softer than the high-fat gel. Among the low-fat gels, RPO3.0 had the highest WHC (55.39%) and gel strength (8.62 g). Furthermore, only RPO3.0 showed a loss factor of less than 0.1, indicating a strong solid-like structure, although all gels displayed elastic properties with storage modulus greater than loss modulus values. Then, low-fat sausages were formulated using RPO at 1.5% (LFS1.5), 2.0% (LFS2.0), 2.5% (LFS2.5), and 3.0% (LFS3.0) per 100 g and compared with high-fat sausage containing 20% chicken fat (HFS). All treated samples showed higher moisture ($p < 0.05$), higher protein ($p < 0.05$), and lower fat content ($p < 0.05$) than the HFS sample. However, there was no significant difference in the ash content ($p > 0.05$) among the samples. Sample LFS1.5 and LFS2.0 showed lower WHC and higher cooking loss ($p < 0.05$) than the control. Furthermore, adding RPO at concentrations higher than 2.0% resulted in the low-fat sausage that had higher hardness and chewiness as well as greater storage and loss modulus. In terms of colour, low-fat sausage appeared to be lighter and more yellow than high-fat sausage. Notably, low-fat sausages showed significantly lower ($p < 0.05$) TBARS values than the HFS control during 28 days of storage. According to the sensory panellists, LFS3.0 received the highest overall acceptance score owing to its excellent aroma, taste, and juiciness. However, all the treated samples received overall acceptance score above 6.3 which indicated that the products were considered acceptable. In conclusion, RPO could mimics the function of animal fat in terms of physicochemical properties and sensory attributes to produce low-fat emulsified meat products, as shown in LFS1.5, that comply with the regulation and meet the demands of health-conscious consumers.

ABSTRAK

KESAN LEMAK SAWIT TERHADAP SIFAT FIZIKOKIMIA PROTEIN MIOFIBRIL DALAM PEMBUATAN SOSEJ RENDAH LEMAK

Kajian ini dijalankan untuk lebih memahami kesan jenis lemak pada pembentukan gel oleh protein miofibril ayam (MP) dalam sosej rendah lemak. Kajian ini memberikan pemahaman yang lebih mendalam untuk mengatasi masalah dalam pembuatan produk daging rendak lemak dengan mengkaji potensi minyak sawit bertapis (RPO) dan stearin (ST) sebagai pengganti lemak ayam (CF). Proses pembentukan gel menggunakan tiga jenis lemak pada kandungan tinggi; CF (MP-CF), RPO (MP-RPO), dan ST (MP-ST) telah dikaji melalui analisis fizikokimia dan terma. RPO dipilih sebagai pengganti lemak haiwan terbaik kerana ia menunjukkan sifat elastik gel yang kuat dengan kestabilan terma tertinggi ($p < 0.05$) sewaktu penyejukan dan pemanasan. RPO juga mempunyai kapasiti memegang air (WHC) dan kekuatan gel tertinggi ($p < 0.05$). Apabila kesan penambahan RPO diuji pada kepekatan lemak yang rendah [1.5% (RPO1.5), 2.0% (RPO2.0), 2.5% (RPO2.5), dan 3.0% (RPO3.0)], gel yang dihasilkan adalah lebih lembut berbanding gel tinggi lemak. RPO3.0 menunjukkan WHC (55.39%) dan kekuatan gel (8.62 g) tertinggi berbanding gel rendah lemak yang lain. Tambahan pula, hanya RPO3.0 mempunyai nilai tan δ kurang daripada 0.1, menandakan pembentukan struktur pepejal yang kuat. Walau bagaimanapun, setiap sampel gel rendah lemak menunjukkan sifat-sifat elastik dengan nilai modulus elastik (G') yang lebih tinggi daripada modulus likat (G"). Kemudian, sosej rendah lemak dihasilkan menggunakan RPO pada kepekatan 1.5% (LFS1.5), 2.0% (LFS2.0), 2.5% (LFS2.5), dan 3.0% (LFS3.0) setiap 100 g dan dibandingkan dengan sosej tinggi lemak (HFS) yang mengandungi 20% lemak ayam. Semua sampel sosej rendah lemak menunjukkan kandungan kelembapan dan protein yang lebih tinggi ($p < 0.05$) serta kandungan lemak yang lebih rendah ($p < 0.05$) daripada HFS. Namun begitu, kandungan abu dalam semua sampel tidak menunjukkan sebarang perbezaan yang signifikan ($p > 0.05$). Formulasi LFS1.5 dan LFS2.0 mempunyai WHC yang lebih rendah dan kehilangan memasak yang lebih tinggi ($p < 0.05$) berbanding HFS. Selain itu, menambah RPO pada kepekatan lebih tinggi daripada 2.0% menghasilkan sosej dengan kekerasan dan kekenyalan yang lebih tinggi serta modulus elastik dan likat yang lebih besar. Dari segi warna, sosej rendah lemak kelihatan lebih cerah dan lebih kuning berbanding sosej tinggi lemak. Di samping itu, sosej rendah lemak menunjukkan nilai TBARS yang lebih rendah ($p < 0.05$) daripada HFS selama 28 hari penyimpanan. Berdasarkan analisis penilaian deria, LFS3.0 mendapat skor penerimaan keseluruhan yang tertinggi kerana aroma dan rasa yang boleh diterima dengan baik. Namun, semua sampel sosej rendah lemak menerima skor penerimaan keseluruhan melebihi 6.3 yang menandakan produk tersebut boleh diterima. Kesimpulannya, RPO boleh menggantikan fungsi lemak haiwan berdasarkan sifat fizikokimia dan deria untuk menghasilkan produk daging emulsi rendah lemak seperti yang ditunjukkan dalam LFS1.5 yang mematuhi garis panduan dan memenuhi permintaan pengguna yang mementingkan kesihatan.

LIST OF CONTENTS

CHAPTER 3: METHODOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MP	-	Myofibrillar protein
WHO	-	World Health Organization
PO	-	Palm oil
CVD	-	Cardiovascular disease
TAGs	-	Triacylglycerols
TBARS	-	Thiobarbituric acid reactive substances
GA	-	Gum Arabic
CMC	-	Carboxymethyl cellulose
PF	-	Pomegranate flour
WSG	-	Whey protein isolate – sodium dodecyl sulphate gel
CO	-	Canola oil
CPO	-	Crude palm oil
PKO	-	Palm kernel oil
RPO	-	Refined palm oil
POP	-	1-palmitoyl-2-oleoyl-3-palmitoyl-sn-glycerol
POO	-	1-palmitoyl-2-oleoyl-3-oleoyl-sn-glycerol
IV	-	Iodine value
SV	-	Saponification value
POl	-	Palm olein
ST	-	Palm stearin
PMF	-	Palm mid-fraction
SMP	-	Steep melting point
SFC	-	Solid fat content
SFA	-	Saturated fatty acids
USFA	-	Unsaturated fatty acids
FFA	-	Free fatty acids

ppm	-	Parts per million
MHC	-	Myosin heavy chains
ATP	-	Adenosine triphosphate
ROS	-	Reactive oxygen species
EAI	-	Emulsifying activity index
WHC	-	Water holding capacity
LMWA	-	Law of matching water affinities
DF	-	Dietary fibre
IDF	-	Insoluble dietary fibre
RC	-	Regenerated cellulose
CPI	-	Chickpea protein isolate
SPI	-	Soy protein isolate
CF	-	Chicken fat
AOAC	-	Association of Official Analytical Chemists
SDS-PAGE	-	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Over the past 50 years, the world's meat consumption has grown substantially by 62%, with a three-fold increase among developing nations since 1963 (Bostami *et al.*, 2017). Meat, a nutrient-dense food, is necessary for the human diet because it contains high-quality protein, essential amino acids, B-group vitamins, and minerals (Cofrades *et al.*, 2017). Meat proteins are more easily digested and absorbed by the human body than legumes and grain proteins (Chen *et al.*, 2017). Depending on protein solubility, meat proteins are classified into salt-soluble myofibrillar proteins, water-soluble sarcoplasmic proteins, and insoluble extracellular matrix proteins (Zhang *et al.*, 2022b). Myofibrillar proteins (MP), accounting for 50 – 60% of total meat proteins, are divided into contractile proteins, e.g. myosin and actin, and regulatory proteins, e.g. tropomyosin, troponin, titin, desmin, and Z-disk (Matarneh *et al.*, 2023).

In the production of emulsified meat products such as sausage, animal fat has been widely used because of its technical benefits; thus, meat products unquestionably contribute to the consumption of saturated fats (Paglarini *et al.*, 2022). As a precautionary measure and to reduce the global burden of these metabolic risk factors, several health organizations have lately released in-depth reports to promote dietary saturated fat reduction as part of healthy eating methods. In this regard, the 2020 Dietary Guidelines for Americans state that a healthy eating pattern should limit daily consumption of saturated fats and recommends that consumers choose packaged foods lower in saturated fats and higher in polyunsaturated and monounsaturated fats (US Department of Health and Human

Services, 2019). Meanwhile, the World Health Organization underlined that to achieve a healthy diet, total fat intake should be less than 30%, and saturated fat intake should be reduced to lower than 10% of total energy (World Health Organization, 2019).

The rising awareness among consumers prompts manufacturers to focus on creating healthier meat products by cutting back on animal fat or using fat replacers that replicate the qualities of saturated fat (Kumar, 2021). Several strategies, such as the addition of dietary fibres, protein isolates, and hydrocolloids, have been proposed to improve lipid profiles in the formulations (Utama *et al.*, 2018). Indeed, fat plays an essential role in the mouthfeel and juiciness of meat products, so the direct reduction of animal fat may result in products with less desirable quality (de Oliveira Fagundes *et al.*, 2017).

The use of unsaturated vegetable oils as fat substitute is an interesting strategy for developing low-fat meat products. Those reformulation process focus on incorporating plant oil to reduce saturated fat and cholesterol while increasing monounsaturated and polyunsaturated fatty acids (Nieto & Lorenzo, 2021). Previous studies have looked at how several vegetable oils, such as olive oil (Câmara *et al.*, 2020b), canola oil (Alejandre *et al.*, 2019), soybean oil (Ferrer-González *et al.*, 2019), sunflower oil (Pereira *et al.*, 2020), and sesame oil (Kang *et al.*, 2017), affected the final qualities of emulsified meat products.

Palm oil (PO) accounted for nearly 35% of total vegetable oils consumption and is also the first in global oil production. The world's leading and second largest palm oil producers, Indonesia and Malaysia produced more than 85% of the total PO supply (Absalome *et al.*, 2020). PO is extracted from the mesocarp of palm fruits (*Elaeis guineensis*) and can be further fractionated into solid and liquid components known as palm stearin and palm olein, respectively. PO contains phytonutrients including carotenoids and vitamin E, as well as roughly equal amounts of saturated and unsaturated fatty acids (Absalome *et al.*, 2020).

Due to its high saturated fat content and semi-solid structure (Urugo *et al.*, 2021), palm oil may substitute animal fat in the reformulation strategy to fulfil consumers' demand for healthier meat products. Theoretically, the properties of emulsified meat products are highly dependent on the gel-forming ability of MP. Myofibrillar proteins are principally responsible for the functionality of processed meat products by providing the structure of heat-induced gels through the formation of three-dimensional matrices, which may be accountable for retaining water and oil (Zhao *et al.*, 2021). The gel-forming ability of MP determined the sensory properties, juiciness, and product yield and eventually affected consumer satisfaction (Chen *et al.*, 2018). Therefore, it is crucial to understand the impact of substituting animal fat with PO on the physicochemical properties of MP to develop an acceptable low-fat meat product.

1.2 Problem statement

Emulsified meat products are made through secondary meat processing and include 20–30% fat. According to Food Regulations 1985, manufactured meat shall be the meat product prepared from meat, whether cut, chopped, minced or comminuted and shall not contain more than 30% of fat. The saturated animal fat particles play a critical role in the cooking loss rate, emulsion formation, hardness, juiciness, flavor, and appearance of meat products. However, consumers are becoming increasingly concerned about animal fat usage, particularly regarding nutritional issues and negative perceptions about the calorific value and cholesterol content. Moreover, saturated fat intake is often associated with a higher occurrence of cardiovascular diseases (CVD) and several harmful health implications such as diabetes and obesity, among other physiological disorders.

Developing good low-fat meat products is laborious because fat provides desirable quality attributes. Fat is also one of the factors for the perception of flavor after food mastication. For these reasons, substituting animal fat with unsaturated oils usually leads to technological challenges, including deteriorated texture and lower heating stability. Moreover, the problem of using vegetable oils is that these oils cannot form solid structures at room temperature and commonly need hydrogenation process. A high saturation level in animal fat results in more solid materials, which assemble into crystalline structures of triacylglycerols. The solid fat also provides desired texture, stability, and functionality in processed meat products. In this sense, reducing and replacing the saturated animal fat in meat processing is not easily achieved without negatively affecting the quality of final products.

Currently, the high-fat content of meat products is incompatible with the idea of a healthy diet. The main issue with reformulating meat products is that it degrades the stability of gel emulsions. Emulsified meat production depends heavily on fat modifications such as concentration and lipid source. Therefore, consumers become less likely to purchase reduced-fat meat products because it may affect taste profiles. In this sense, MP play a significant role as the main protein in meat and serves as an emulsifier in products like sausage. However, there is insufficient information on the ability of MP to gel in high-protein, low-fat meat products that use vegetable oil as the fat substitute.

1.3 Research objectives

This research aims to develop a low-fat chicken sausage using palm oil as animal fat substitute. The objectives of this research are:

1. To determine the effect of palm oils addition on the physicochemical properties and thermal stability of myofibrillar protein gel.
2. To evaluate the chemical properties of myofibrillar protein gel at low concentrations of refined palm oil.
3. To investigate the effect of refined palm oil addition at low-fat concentrations on the physicochemical and sensory properties of sausage.

1.4 Importance of study

The study might offer different insights on using palm oil as an animal fat alternative in the production of sausage. PO can potentially substitute partially hydrogenated fats as PO is semi-solid in nature and does not require any hydrogenation process, which often results in the formation of trans-fatty acids (de Oliveira Fagundes *et al.*, 2017). According to previous research, PO is relatively stable to oxidative deterioration due to the oil being rich in tocopherols and tocotrienols (Dian *et al.*, 2017). Besides, the low level of polyunsaturated fatty acids helps PO overcome lipid oxidation in meat products without the use of synthetic antioxidants. Hence, the findings of this study may directly benefit consumers with meat products with better oxidative stability.

The research aims to assist the meat industry in overcoming technological challenges in low-fat meat products since PO has several distinctive properties, such as structural hardness, slow crystallization properties, and high and low melting triacylglycerols (TAGs) that provide a wide plastic range. In addition, the saturation level of PO is comparable to those of animal fat, which is 48% and 49%, respectively. As a result, it is possible to produce low-fat sausage with comparable qualities to those made with animal fat by examining the effects of PO on the gelation properties of myofibrillar protein.

Additionally, studying the characteristics of MP and oil mixed gel can offer theoretical guidance for producing low-fat meat products. Simply substituting animal fats with unsaturated vegetable oils leads to a decrease in product quality because liquid oils lack the solid, elastic structure that animal fats exhibit at room temperature. Hence, this study focuses on utilizing palm oil, which is semi-solid in nature, to substitute the animal fat in meat products, forming a network structure via myofibrillar protein crosslinking. The interaction mechanisms of palm oil and MP can be used to formulate products with excellent sensory and physicochemical qualities, thus benefiting both consumers and the meat industry.

CHAPTER 2

LITERATURE REVIEW

2.1 Development of reduced-fat meat products

In 2018, 263 million tonnes of meat were produced worldwide, and by 2050, that number is expected to rise to 445 million tonnes (Srutee & Uday, 2021). In 2022 alone, the global meat production was 345.17 million tonnes, with poultry making up close to 40% of that amount (OECD, 2022). According to Salter (2018), from 2014 to 2016, the total meat consumption per capita worldwide was 34.1 kg/year. Most of the world's rising meat consumption is found in lower- and middle-income nations, like Indonesia and China, where it increased by 89% and 54%, respectively, between 1998 and 2018 (Whitnall & Pitts, 2019). In Malaysia, poultry consumption per person was greatest in 2021 (49.7 kg/year), followed by beef (5.5 kg/year) (OECD, 2022).

One of the most consumed meat products are sausages, whose prices and sensory properties are highly acceptable (Zhang *et al.*, 2022b). Emulsified meat product is made up of a dispersed phase (fat globules), a continuous phase (aqueous solution of salts), as well as soluble and insoluble proteins. During meat processing, raw meat proteins act as the emulsifying agents solubilised in a saline solution and often form network structures to exhibit various functional properties that determine the behaviour of proteins during storage and consumption (Cheng *et al.*, 2020).

Myofibrillar protein (MP) is principally responsible for the functional properties of proteins in meat products, which fall into three categories: protein-water interactions, protein-lipid interactions, and protein-protein interactions (Sun & Holley, 2011). Protein-water interactions impact protein solubilization and extraction, water retention, and viscosity, all of which influence the quality of the final product (Smith, 2010). Protein-lipid interactions cause structural changes in proteins via disulphide bonds, hydrophobic interactions, and hydrogen bonding (Shao *et al.*, 2015). Meanwhile, protein-protein interactions lead to the unfolding and aggregation of proteins, which creates a continuous, well-defined gel matrix (Smith, 2010).

Due to the significant amount of saturated fat in meat products, which are considered unhealthy, fat-reduced meat products have been formulated to offer healthier alternatives (Zhao *et al.*, 2019a). As a result of increased knowledge and awareness, consumers frequently prioritise the nutritional benefits of meat products over just the sensory and flavour ones (Kantono *et al.*, 2021). Thus, a potential market opportunity exists for processed meat products claiming specific health and ingredient benefits. However, in developing countries such as Malaysia, purchasing power and health claims are essential factors, in addition to the taste of meat products, which are prominently associated with consumers' purchasing behaviour (Font-i-Furnols & Guerrero, 2014). The global market for low-fat meat products is anticipated to increase by 25.5% annually from 1.8 billion USD in 2019 to 2.4 billion USD by 2027 (Zhang *et al.*, 2022d). According to the American Cancer Society, low-fat meat should have no more than 3 g of fat per 100 g of product, or a maximum of 30% of the calories should originate from fat (Duyff, 2006).

2.2 Fat replacers in meat products

Fat replacers are partially or entirely used in place of animal fat to manufacture low-fat meat products (Ospina-E *et al.*, 2012). A good fat replacer is a compound or a mixture of compounds that interact with proteins in meat and provides technological and functional properties in the absence or reduced fat content (Fuquay *et al.*, 2011). As presented in Figure 2.1, the ingredients used as fat replacers are categorized as fat mimetics and fat substitutes which consisted of carbohydrate-, protein- or lipid-based compounds (Kumar, 2021). However, the applications of these ingredients are not necessarily in a pure form and can be incorporated as powders, paste or emulsion gels (Asyrul-Izhar *et al.*, 2022).