LIPID OXIDATION AND PROTEIN CO-OXIDATION IN READY-TO-EAT LOW-MOISTURE SHREDDED (*SERUNDING*) BEEF AND CHICKEN

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Chair : Nazamid Saari, PhD
Faculty : Food Science and Technology

Ready-to-eat (RTE), low-moisture, high-lipid and high-protein meat products are convenient in emergencies like earthquakes, flash floods, and the global COVID-19 lockdown. Given the long storage times of these products, the lipid and protein components may be more susceptible to oxidation, which causes discolouration, shelf-life reduction, nutritional quality deterioration, lipid rancidity, and protein denaturation. This first objective of the research aimed to determine the effects of meat type, storage time and temperature on lipid oxidation and protein co-oxidation of shredded (*serunding*) beef and chicken during six months storage. The production of shredded (*serunding*) beef and chicken was done with desired moisture content (<7.5%) of 6.36% and 7.46% respectively. Immediate post-production analyses of shredded (*serunding*) beef and chicken include proximate composition, water activity, moisture content, fatty acid composition (FAC), reducing sugar, and scanning electron microscopy (SEM). Upon completion of the analyses, the storage study was instantly conducted to evaluate the physicochemical, lipid oxidation and protein co-oxidation of shredded (*serunding*) beef and chicken at 25, 40, and 60°C. Due to its higher polyunsaturated fatty acids (PUFA) content in shredded (*serunding*) chicken (4.12%) than shredded (*serunding*) beef (1.45%), chicken meat was more susceptible to lipid oxidation than beef, which was evidenced by a higher conjugated dienes (CD) content of shredded (*serunding*) chicken, 3.0-3.5 mmol CD/mol lipid than CD of shredded (*serunding*) beef (1.5-2.0 mmol CD/mol lipid) and a rapid accumulation of malondialdehyde (MDA) (week 2 to 10). Chicken *serunding* also experienced more severe protein co-oxidation than beef *serunding*, as seen from lower protein solubility (18-24 mg/mL; beef *serunding*: 28-35 mg/mL), higher protein carbonyl (chicken *serunding*: 36.63 nmol carbonyl/mg (60°C), 18.59 nmol carbonyl/mg (40°C), 6.80 nmol carbonyl/mg (25°C); beef *serunding*: 27.40 nmol carbonyl/mg (60°C), 8.68 nmol carbonyl/mg (40°C), 6.23 nmol carbonyl/mg (25°C)), higher tryptophan loss and higher Schiff base formation. The second objective of evaluating the relation of

selected parameters with lipid-protein oxidative markers by principal component analysis (PCA) confirms that meat type, storage time, and storage temperature influence changes in extracted lipid, MDA, and protein co-oxidation reaction (tryptophan loss, Schiff base, and protein carbonyl) over six months. PCA results support the general discussion about colour changes, lipid oxidation, and protein co-oxidation in beef and chicken *serunding*. Chicken *serunding* demonstrates lower lipid and protein stability and higher lipid oxidation and protein co-oxidation rates than beef *serunding*. Subsequently, in the third objective, the butylated hydroxyanisole (BHA) antioxidant was added (200 ppm based on the lipid content of sample), and different multilayer packaging was used to improve the lipid and protein stability of chicken *serunding*. The proximate composition, FAC and SEM of chicken *serunding* with BHA addition (CS-BHA) were measured directly after production, and the results were comparable to control (CS). After the completion of the preceding analyses, the storage study of six months was conducted. During storage, the simultaneous interacting effects of different storage temperatures (25, 40, and 60°C), antioxidant (BHA), and multilayer packaging materials (metallised polyethylene terephthalate (MPET) and aluminum-containing packaging) were evaluated. All lipid and protein markers elevated with increasing temperature (25 < 40 < 60°C), indicating that storage of low-moisture meat at high temperature is not feasible. BHA was effective against lipid oxidation, as indicated by the significantly lower extracted lipid content (CS-BHA: 40-44%; CS: 42-46.5%) and delayed formation of MDA, i.e. MDA peaked at week 14 in CS-BHA samples (MPET packaging) while MDA peaked at week 10 in CS samples (MPET packaging). However, BHA is not effective against protein co-oxidation, as shown by the insignificant effect on preventing tryptophan loss, protein carbonyl formation, and Schiff base accumulation. As for the packaging effect, MPET packaging with a superior light and oxygen barrier provided significant protection compared to aluminium. MPET packaging showed lower lipid oxidation, as the CD content in aluminium-packaging peaked earlier (week 2–3). Additionally, MPET packaging consistently had a significantly lower MDA level than aluminium-packaging. In terms of protein oxidation, MPET packaging recorded a tryptophan loss of −32.16% at 25 ^{0} C, −35.98% at 40 ^{0} C and −41.74% at 60 ^{0} C and packaging B a loss of −21.85% at 25 ^{0} C, −22.33% at 40 ^{0} C and −34.64% at 60 ^{0} C, respectively, showing that MPET (packaging A) induced less severe protein co-oxidation than aluminium (packaging B). Finally, PCA reveals the variations in extracted lipid, MDA and protein co-oxidation reaction (tryptophan loss, Schiff base and protein carbonyl), monitored over six months of storage, are impacted by storage temperature and packaging material but not by antioxidant. In conclusion, from the quality and economic aspects, 10 to 12 weeks of shelf-life are sufficient to reliably estimate the lipid oxidation onset of shredded (*serunding*) meat at storage temperature of 25°C and 40°C, therefore three months post-production is the recommended storage term. 25°C, with antioxidant addition in metallised barrier multilayer packaging, is considered as the ideal long-term storage condition to delay simultaneous lipid oxidation and protein co-oxidation. These findings provide insights into simultaneous lipid oxidation and protein co-oxidation in RTE low-moisture, shredded (*serunding*) meats and might be extrapolated to minimise probable harmful impacts on the quality of low-moisture, high-lipid and high-protein foods.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGOKSIDAAN LIPID DAN PENGOKSIDAAN BERSAMA PROTEIN DALAM DAGING CARIK (SERUNDING) LEMBU DAN AYAM SEDIA DIMAKAN (RTE) DAN KELEMBAPAN RENDAH

Oleh

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Produk daging sedia untuk dimakan (RTE), lembapan rendah, lemak tinggi dan tinggi protein adalah mudah dalam keadaan kecemasan seperti gempa bumi, banjir kilat dan penutupan global COVID-19. Memandangkan produk ini mempunyai masa penyimpanan yang lama, komponen lipid dan protein mungkin lebih terdedah kepada pengoksidaan, menyebabkan perubahan warna, pengurangan jangka hayat, kemerosotan kualiti pemakanan, ketengikan lipid, dan denaturasi protein. Objektif pertama penyelidikan ini bertujuan untuk menentukan kesan jenis daging, masa penyimpanan dan suhu ke atas pengoksidaan lipid dan pengoksidaan protein daging lembu dan ayam yang dicarik (serunding) selama penyimpanan enam bulan. Pengeluaran daging lembu dan ayam yang dicarik (serunding) dilakukan dengan kandungan lembapan yang dikehendaki (<7.5%) masing-masing sebanyak 6.36% dan 7.46%. Analisis segera pasca pengeluaran serunding daging lembu dan ayam termasuk komposisi proksimat, aktiviti air, kandungan lembapan, komposisi asid lemak (FAC), gula penurun, dan mikroskop elektron pengimbasan (SEM). Setelah selesai analisis, kajian penyimpanan dijalankan serta-merta untuk menilai fizikokimia, pengoksidaan lipid dan pengoksidaan protein daging lembu dan serunding ayam pada suhu 25, 40, dan 60°C. Oleh kerana kandungan asid lemak politaktepu (PUFA) yang lebih tinggi dalam serunding ayam (4.12%) daripada serunding daging lembu (1.45%), daging ayam lebih mudah terdedah kepada pengoksidaan lipid daripada daging lembu, yang dibuktikan oleh kandungan diena terkonjugasi (CD) yang lebih tinggi. serunding ayam, 3.0-3.5 mmol CD/mol lipid daripada CD serunding daging lembu (1.5-2.0 mmol CD/mol lipid) dan pengumpulan pesat malondialdehid (MDA) (minggu 2 hingga 10). Serunding ayam juga mengalami pengoksidaan protein yang lebih teruk daripada serunding daging lembu, seperti yang dilihat daripada keterlarutan protein yang lebih rendah (18-24 mg/mL; serunding daging lembu: 28-35 mg/mL), karbonil protein yang lebih tinggi (serunding ayam: 36.63 nmol karbonil/ mg

(60°C), 18.59 nmol karbonil/mg (40°C), 6.80 nmol karbonil/mg (25°C); serunding daging lembu: 27.40 nmol karbonil/mg (60°C), 8.68 nmol karbonil/mg (40 °C), 6.23 nmol karbonil/mg (25°C)), kehilangan triptofan yang lebih tinggi dan pembentukan asas Schiff yang lebih tinggi. Objektif kedua menilai hubungan parameter terpilih dengan penanda oksidatif lipid-protein oleh analisis komponen utama (PCA) mengesahkan bahawa jenis daging, masa penyimpanan, dan suhu penyimpanan mempengaruhi perubahan dalam tindak balas pengoksidaan lipid (lipid yang diestrak dan MDA), dan pengoksidaan bersama protein (kehilangan triptofan, asas Schiff, dan protein karbonil) selama enam bulan. Keputusan PCA menyokong perbincangan umum tentang perubahan warna, pengoksidaan lipid, dan pengoksidaan bersama protein dalam daging lembu dan serunding ayam. Serunding ayam menunjukkan kestabilan lipid dan protein yang lebih rendah serta kadar pengoksidaan lipid dan pengoksidaan bersama protein yang lebih tinggi daripada serunding daging lembu. Seterusnya, dalam objektif ketiga, antioksidan butilasi hidroksianisole (BHA) telah ditambah (200 ppm berdasarkan kandungan lipid sampel), dan pembungkusan pelbagai lapisan yang berbeza digunakan untuk meningkatkan kestabilan lipid dan protein serunding ayam. Komposisi proksimat, FAC dan SEM serunding ayam dengan penambahan BHA (CS-BHA) diukur secara langsung selepas pengeluaran, dan hasilnya adalah setanding dengan kawalan (CS). Selepas selesai analisis sebelumnya, kajian penyimpanan selama enam bulan telah dijalankan. Semasa penyimpanan, kesan interaksi serentak suhu penyimpanan yang berbeza (25, 40, dan 60°C), antioksidan (BHA), dan bahan pembungkusan berbilang lapisan (polietilena tereftalat berlogam (MPET) dan pembungkusan yang mengandungi aluminium) telah dinilai. Semua penanda lipid dan protein dinaikkan dengan peningkatan suhu (25 < 40 < 60°C), menunjukkan bahawa penyimpanan daging rendah lembapan pada suhu tinggi tidak boleh dilaksanakan. BHA berkesan terhadap pengoksidaan lipid, seperti yang ditunjukkan oleh kandungan lipid yang diekstrak dengan ketara lebih rendah (CS-BHA: 40-44%; CS: 42-46.5%) dan tertunda pembentukan MDA, iaitu MDA memuncak pada minggu ke-14 dalam sampel CS-BHA (pembungkusan MPET) manakala MDA memuncak pada minggu ke-10 dalam sampel CS (pembungkusan MPET). Walau bagaimanapun, BHA tidak berkesan terhadap pengoksidaan bersama protein, seperti yang ditunjukkan oleh kesan yang tidak ketara terhadap mencegah kehilangan triptofan, pembentukan karbonil protein, dan pengumpulan asas Schiff. Bagi kesan pembungkusan, pembungkusan MPET dengan penghalang cahaya dan oksigen yang unggul memberikan perlindungan yang ketara berbanding aluminium. Pembungkusan MPET menunjukkan pengoksidaan lipid yang lebih rendah, kerana kandungan CD dalam pembungkusan aluminium memuncak lebih awal (minggu 2–3). Selain itu, pembungkusan MPET secara konsisten mempunyai tahap MDA yang jauh lebih rendah daripada pembungkusan aluminium. Dari segi pengoksidaan protein, pembungkusan MPET merekodkan kehilangan triptofan sebanyak -32.16% pada 25 ^{0} C, −35.98% pada 40 ^{0} C dan −41.74% pada 60 ^{0} C dan pembungkusan B kehilangan −21.85% pada −21.85% pada 2.5□. 40 ^{0} C dan −34.64% pada 60 ^{0} C, masing-masing, menunjukkan bahawa MPET (pembungkusan A) menyebabkan pengoksidaan protein yang kurang teruk berbanding aluminium (pembungkusan B). Akhir sekali, PCA mendedahkan variasi dalam tindak balas pengoksidaan lipid, MDA dan protein yang diekstrak (kehilangan triptofan, asas Schiff dan karbonil protein), dipantau selama enam bulan penyimpanan, dipengaruhi oleh suhu penyimpanan dan

bahan pembungkus tetapi bukan oleh antioksidan. Kesimpulannya, dari aspek kualiti dan ekonomi, jangka hayat 10 hingga 12 minggu adalah mencukupi untuk menganggarkan dengan pasti permulaan pengoksidaan lipid serunding daging pada suhu penyimpanan 25°C dan 40°C, oleh itu tiga bulan pasca pengeluaran adalah tempoh penyimpanan yang disyorkan. Suhu penyimpanan pada 25°C dan juga penambahan antioksidan dalam pembungkusan berbilang lapisan penghalang logam, dianggap sebagai keadaan penyimpanan jangka panjang yang ideal untuk menangguhkan pengoksidaan lipid serentak dan pengoksidaan bersama protein. Penemuan ini memberikan gambaran tentang pengoksidaan lipid serentak dan pengoksidaan bersama protein dalam daging RTE yang lembap rendah, dicarik (serunding) dan mungkin diekstrapolasi untuk meminimumkan kemungkinan kesan berbahaya terhadap kualiti makanan rendah lembapan, lemak tinggi dan protein tinggi.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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ΔE_{0-24}	Total colour difference throughout storage
a*	Redness
a_w	Water activity
ABTS	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)
ALU	Aluminium
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
b*	Yellowness
BHA	Butylated hydroxyanisole
CD	Conjugated dienes
CS	Chicken *serunding*
CS-BHA	Chicken serunding with BHA addition
$CHCl_3$	Chloroform
DNPH	2,4-Dinitrophenyl hydrazine
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FAO	Food and Agriculture Organization of the United Nations
FRAP	Ferric Reducing Antioxidant Power
L*	Lightness
LLDPE	Linear low-density polyethylene
LO	Alkoxyl radical
LOO	Peroxide radical
LOOH	Hydroperoxide
M	Molar
MCT	Medium chain tryglycerides

MDA	Malondialdehyde
MPET	Metallised-polyethylene terephthalate
MRE	Meals Ready-to-Eat
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
MΩ	Mili-Q
P	Probability (of error)
Packaging A	PET/PE/MPET (High Barrier)/LLDPE
Packaging B	PET/PE/ALU/LLDPE
PBS	Phosphate buffered saline
PC	Protein carbonyl
PCA	Principal component analysis
PE	Polyethylene
PET	Polyethylene terephthalate
PITC	Phenyl-isothiocyanate
PUFA	Polyunsaturated fatty acids
RTE	Ready-to-eat
SEM	Scanning electron microscopy
SFA	Saturated fatty acids
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Ready-to-eat (RTE) food products need no additional preparation beyond freezing, cooking, processing before consumption, or little preparation such as boiling or reheating (Howard et al., 2012). Recent trends have observed an increase in consumer demands for convenience in meal preparation, shelf-stable foods as life have gotten more hectic. Faced with increased working hours and lengthy commutes, consumers worldwide seek to maximise their extremely limited free time (Montero et al., 2021). In response to this need, the food industry has expanded its availability of complete pre-prepared ready meal solutions, including ready-to-eat (RTE) options (Diaz, 2021).

RTE food products are a boon for crises like earthquakes, flash floods, and the current global COVID-19 lockdown (Kumar et al., 2016; Telukdarie et al., 2020). With the current pandemic, RTE food products, specifically RTE meat products, become a practical means of providing nutrient-dense sustenance. However, meat-based RTE is lipid and protein-rich, thus susceptible to oxidative deterioration during long-term storage. This study thus assessed the oxidation of such food system using *serunding*, a locally popular RTE shredded meat product that is cooked under long-hour from beef or chicken meat, known for its high-lipid (6.0–32.3%), high-protein (21.8–40.7%) and low-moisture (4.3–13.6%) (Huda et al., 2012; Knechtges, 2012), as the representative food model. *Serunding* is a popular specialty dish in Southeast Asia, with distinct flavours derived from a combination of cooked meat, coconut milk, herbs and spices. In general, *serunding* is acknowledged as shredded meat, meat floss or meat fibre. *Serunding* is accessible all year in Malaysia, particularly during festive season. *Serunding* offers convenience to be readily consumed together with staple foods like rice and bread and easy storage up to 6 months without refrigeration.

1.2 Problem statements

In the context of food science, *serunding* is categorised as high-lipid, high-protein food system which is highly susceptible to oxidation, a major problem leading to discolouration and shelf-life reduction, sensory and nutritional quality degradation, by developing off-flavour, off-odour, rancidity in lipid molecules and the production of potentially toxic compounds that could risk consumers' safety (Zahid et al., 2020; Gutiérrez-del-Río et al., 2021). The loss of quality in meat and ready-to-eat meat products due to lipid oxidation and protein co-oxidation reactions has impacted the food processing industry. It has resulted in customer rejection and economic losses. Therefore, controlling oxidative processes is crucial and a challenge for the food industry (Domínguez et al., 2019; Gutiérrez-del-Río et al., 2021; Domínguez et al., 2022).

During lipid oxidation, non-lipid substrates (majorly protein) are spontaneously attacked by highly reactive, protein bound, lipid oxidation-derived aldehyde such as malondialdehyde (MDA) (Requena et al., 1996; Vandemoortele et al., 2019). MDA, an α,β-unsaturated aldehyde, is electrophilic and attacks the nucleophilic groups on proteins, causing unfavourable alteration in the protein structure and stability (Fuentes et al., 2014; Wang et al., 2019), leading to the formation of Schiff base adducts (MDA-modified amino acid residues, predominantly MDA lysine adducts) (Traverso et al., 2004) and a subsequent occurrence of protein co-oxidation, adversely affecting the product functionality and performance. According to Wang et al. (2019), MDA could also promote protein carbonylation and loss of tryptophan fluorescence in the myofibrillar protein while Hellwig (2020) termed the reaction of protein with oxidised lipid product as “lipation”, where bulky modifying group on lipid is covalently attached to the nucleophilic site on amino acid. While the effect of protein oxidation/co-oxidation on meat quality is still being investigated in numerous studies, it is generally accepted that it results in the loss of essential amino acids, decreased protein solubility, physicochemical changes, altered protein functionality and formation of protein carbonyl derivatives (Lund et al., 2011). Previous research on the effects of lipid oxidation on protein oxidation has been done in both high-moisture raw and low-moisture cured meat (Fuentes et al., 2010; Fuentes et al., 2014; Wang et al., 2019). However, these studies concentrated on lipid-protein oxidation because of the processing conditions. Until now, no research has been done on the effect of storage conditions on lipid oxidation and protein co-oxidation in low-moisture, ready-to-eat, cooked meat. To that end, recent work merits special attention since it is a pioneer in examining how a high-lipid, high-protein, low-moisture, RTE shredded meat system analyses the simultaneous progression of lipid oxidation and protein co-oxidation by using two different shredded meats (beef and chicken), three different storage temperatures (25, 40 and 60 °C) within six months storage.

It is hypothesised that the shredded (*serunding*) chicken could have a faster rate of lipid-protein oxidative degradation than shredded (*serunding*) beef throughout long-term storage. The hypothesis is supported by the presence of higher amounts of PUFA in chicken meat than in beef, making shredded (*serunding*) chicken a more favourable substrate to initiate lipid oxidation (Angelo, 2012; Gruffat et al., 2021). Antioxidant’s addition could be added to tackle faster lipid-protein oxidative degradation issue in shredded (*serunding*) chicken meat. Currently, synthetic antioxidants are added commercially to various food such as baked goods, fried food, processed meat, dried cereal, processed potatoes, dessert mix and beverages (Movileanu et al., 2013; Shahidi, 2015). Despite the potential side effects of synthetic antioxidant, its application in the food industry is ongoing (Admassu, 2019) to prolong the shelf-life of lipid-rich food products at a low cost. The lipid-soluble (lipophilic) BHA antioxidant was chosen to evaluate the effect of antioxidant addition on lipid oxidation and protein co-oxidation in the shredded (*serunding*) meat because the shredded (*serunding*) meat has a high fat content (3.20–39.00%) (Huda et al., 2012; Sukisman et al., 2014; Sukisman et al., 2018). This facilitates the complete dissolution of BHA during the production of shredded (*serunding*) meat. In addition, the enormous sample size investigated in the present study made it difficult to quantify the effect of various antioxidants; hence a single antioxidant was selected. Aside from antioxidants, packaging with moisture and oxygen barriers are both critical for protecting RTE meat products from lipid-protein oxidative degradation. Considering that oxygen is the most prevalent and essential component for the simultaneous progression of lipid-protein oxidation, packaging that prevents or limits oxygen exposure is an effective technique for preventing and retarding oxidation (Gruffat et al., 2021).

1.3 Objectives of the study

The overall aim of this current study was to determine the physical properties and chemical characteristics of lipid-protein oxidative degradation of ready-to-eat (RTE) low-moisture shredded (*serunding*) meats during long-term storage.

Specifically, the objectives were:

1. To determine the effect of long-term storage (six months) at different temperatures (25, 40, and 60°C) on simultaneous lipid oxidation and protein co-oxidation in two different meat types of RTE low-moisture shredded (*serunding*) beef and chicken.

2. To evaluate the correlation of the different storage times and storage temperatures on simultaneous lipid oxidation and protein co-oxidation in two different meat types of RTE low-moisture shredded (*serunding*) beef and chicken by using principal component analysis (PCA).

3. To assess the interacting effect of the different storage temperatures (25, 40, and 60°C), antioxidant butylated hydroxyanisole (BHA) and multilayer packaging materials (metallised polyethene terephthalate (MPET) and aluminium containing packaging) on simultaneous lipid oxidation and protein co-oxidation in RTE low-moisture shredded (*serunding*) chicken during six months of storage.

4. To evaluate the interaction of the effect of different storage temperatures, storage time, antioxidant addition and packaging material on simultaneous lipid oxidation and protein co-oxidation in RTE low-moisture shredded (*serunding*) chicken during six months of storage by using PCA.

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